2019 Center for Produce Safety Research Symposium

Key Research Learnings and How They Can be Used

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Introduction

The Center for Produce Safety (CPS) held its 10th Annual Research Symposium in Austin, TX in June 2019. In July 2019, 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 2019 CPS Symposium can be found on the CPS website .

As we get ready for the 2020 Research Symposium, we wanted to highlight the key learnings from the 2019 CPS Symposium to help prepare you and get your mind ready for the exciting learning opportunities that lay ahead. As a result of the ongoing coronavirus pandemic, CPS has altered the traditional Symposium to create a series of webinars where you will be able to hear about the latest research results so that you can use that knowledge to improve your produce safety programs. The first of five webinars CPS will convene starting in late June.

In this review of 2019 highlights, we will describe the key takeaways and then place them in context as to why these findings are important and how they might be implemented or used by to the produce industry (growers, packers, processors, distributors and retail/foodservice), the research community and regulators.

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Executive Summary – Twenty fully completed research reports and fifteen preliminary interim reports were presented at the CPS Symposium in Austin, Texas in June 2019. The program for the 2019 Symposium listing the projects presented can be found at www.centerforproducesafety.org. A summary of the key research learnings follows:

- A recurrent theme throughout the 2019 CPS Symposium was to warn against "one size fits all" approaches to solving produce safety challenges.
- Although disinfection of irrigation water sourced from canals in the Yuma Valley can be an important part of a strategy to reduce contamination risks associated with furrow irrigation, it does not eliminate the risks and needs to be supplemented with close attention to controlling water flow into the fields, diligent pre-harvest inspection to identify areas that may have become flooded and aggressive cleaning and sanitation programs to prevent the harvest equipment from facilitating pathogen transfer from contaminated soil or harvested product to subsequently harvested commodities.
- Animal intrusion or the use of contaminated open sourced water to mix pesticides can result in pervasive product contamination and need to be managed carefully.
- Reliance on "one size fits all" die-off rate that can be used by growers to permit use of water sources that exceed permissible standards set out in the Produce Rule is questionable. Die-off is not a linear function and data developed using attenuated *E. coli* and *Salmonella* strains on baby spinach and romaine describe die-off rates that are highly variable based on locations, species, produce type and trial.
- Microbial control in produce wash systems is dependent on the equipment or system being employed, the product(s) being washed, and the antimicrobial(s) or disinfectants used. The buildup of organic compounds in the water measured as chemical oxygen demand or COD over time results in reduced availability of active antimicrobials that can make the system itself a source of contamination. Predictive models are being developed that can help operators more effectively manage wash water to ensure effective levels of antimicrobials are reliably achieved.
- New detection methods for *Cyclospora* have made it easier to find this pathogen in water sources. Although *Cyclospora* has been found in open water sources in Arizona, no illnesses have been tied to products from Arizona. Adherence to good agricultural practices like inspection of irrigation water sources to make sure they are not compromised by effluents from sewage treatment plants and monitoring the health of workers and providing facilities and enforcing strict handwashing practices remain important tools for controlling this risk.
- When discussing testing surface waters for the presence of human pathogens, the "one size does not fit all" theme was evident. It is important to account for environmental (run-off from adjacent land), water chemistry (seasonal changes, dissolved oxygen in the water) and weather factors (rain, temperature, solar radiation) and to understand that the relationship between indicator organisms like generic *E. coli* and pathogens are variable. The industry needs to evolve to data- and risk-based sampling protocols employing sufficient volumes to account for these variables to truly be able to identify and manage contamination risks associated with surface water use.
- Systems approaches to managing defined produce safety challenges are emerging. Examples include the development of biological fences designed to prevent tree frogs from breaching the fence by using materials that leverage the biological structure of the frog's toe pad to prevent gripping on the fence, the use of raptors to control their natural prey, nuisance birds and rodents, in leafy greens fields and finally the use of layered vegetative buffer zones to control airborne pathogen dispersion from poultry houses and small dairies onto neighboring vegetable

production. These systems approaches provide growers with alternatives to manage on-farm risks.

- Whole genome sequencing tools are permitting researchers to track *Listeria monocy*togenes as it moves through production and non-production environments. On a macro level, distribution maps are being developed that identify prevalence of *Lm* throughout the U.S. Research focusing on specific production regions in California illustrates the technology permits tracking the movement of specific *Lm* strains between fruit packing facilities by season. The lament of "*Listeria* is everywhere" is proving to be true, but the emergence of whole genome sequencing gives the industry a tool that can be used proactively to discern problematic resident contaminations that can lead to public health risk and common sporadic occurrences that pose a less significant public health problem. In the end, seek and destroy strategies centered on aggressive environmental monitoring programs and cleaning and sanitation programs remain our best tools to control *Listeria* threats.
- Studies on how pathogen-supporting biofilms are formed in relationship to materials commonly used in equipment construction in packing and processing facilities has created awareness and a caution to growers that brushes used in many types of fruit packing to move, wash or apply postharvest preservation treatments can be difficult to sanitize owing to the porous surface of brush filaments. In another iteration of the dangers of "one size fits all" approaches, relying on standard sanitation practices across different types of surface materials is proving to be inadvisable. Materials being passed over equipment, the design of the equipment and the materials used, and the selection of cleaning and sanitization chemicals employed are important variables to be considered when developing sanitation strategies. An interim project report indicated that rechargeable antimicrobial and anti-fouling chemistries can be incorporated into films or used to coat harvest containers like bins, totes, RPC's and buckets to help prevent biofilm formation and ultimately enable more practical cleaning and sanitation of these items.

Key Research Learnings and How They Can Be Used

1. **Production field-level contamination risks.** Recent outbreaks associated with romaine lettuce have dominated industry produce safety discussions for the last three years. CPS has always prioritized the ability to move expeditiously when a unique research opportunity arose to address critical areas of need. And so, it happened that following the *E. coli* O157:H7 outbreak associated with romaine in the Yuma desert region in 2018 that ***Vic Smith*** of JV Farms in Yuma, Arizona, a grower of romaine and many other commodities stepped forward to offer access to romaine fields, harvest equipment and crews, commercial-scale irrigation equipment and production expertise to study potential causative factors implicated in the outbreak. CPS initiated a rapid response project and the results were shared at the 2019 CPS Symposium and subsequently in an expanded form in a CPS webinar.

   Channah Rock and her group at the University of Arizona worked in concert with the team at JV Farms to pursue three principal research objectives: (1) the exploration of chemical treatment of irrigation water, (2) the role of freeze damage and injury in romaine lettuce contamination and (3) the risks associated with transference of pathogens via commercial scale harvest equipment. The following key learnings were uncovered:

   - Disinfectant treatments (2-6 ppm peroxyacetic acid and 3-5 ppm sodium hypochlorite) were effective at reducing generic *E. coli* in irrigation water (2.7 log reduction); however, bacteria remain detectable after water treatment and persist in soil and on plant surfaces.

- Sample volume matters when measuring pathogen or surrogate levels in irrigation water sources. Higher sample volumes are more advantageous when trying to detect low level contaminations.
- At high concentrations, bacteria were able to survive on "freeze-damaged" romaine leaves. Variety had only minimal impact. At lower bacterial concentrations, "freeze damaged" romaine may have the ability to harbor bacteria for extended periods of time.
- Pre-harvest sampling on one-acre plots detected contamination, but <u>postharvest</u> sampling facilitated detection of pathogen surrogates more consistently. Given the logistical challenges that accompany postharvest product sampling, if product sampling is to be performed, more attention needs to be given to developing more effective preharvest sampling methods are needed to replace current S= and Z-pattern testing. CPS has funded research on product testing and initial reports will be shared in 2020.
- Contamination by furrow irrigation was detectable through harvest.
- Common harvest practices facilitate contamination transfer to subsequently harvested romaine. If romaine in the field becomes contaminated by irrigation water or if the harvest equipment picks up soil similarly contaminated during harvest, then pathogens can move from the contaminated equipment surfaces onto the product.
- Animal intrusion and aerial application contamination events result in pervasive contamination

Why is this information important for industry? Coming out of the 2018 *E. coli* O157:H7 outbreak attributed to romaine grown in the Yuma Valley, the were many questions raised by FDA. The industry was left to hypothesize on what might have been the causative factors leading to the outbreak. Setting aside the issue of cattle feedlots and pathogen transmission, there were direct knowledge gaps that needed to be addressed. Certainly, the microbial quality of irrigation water became a focal point for discussions. There were questions about how to disinfect open water sources like canals and that extended to the method of irrigation, as the predominant mode in the region is furrow irrigation. There were questions about pathogen transference due to animal intrusion, the use of contaminated water to mix pesticides that were applied aerially, the potential impact of freeze damage leading to harborage of *E. coli* O157:H7, and transference from harvest equipment. While we had research data on each of these topics already in hand, this project represented the first effort to investigate these questions simultaneously in the Yuma desert production environment. In many ways, this project corroborated or reinforced what we already knew and should build confidence in growers to develop improved strategies to manage risks. Some highlights include:

- Chemical treatment of irrigation water sourced from canals can result in a 3-log (99.9%) reduction of bacteria but not a complete reduction to zero. That tells us that treatment of irrigation water needs to be part of a multi-part strategy to reduce risks associated with canal water. The first priority must be eliminating to the extent possible the contamination of the canals in the first place. Easy to say and hard to accomplish, but limiting runoff from adjacent areas, preventing animal and human intrusion, and mandating that irrigation water is not used for mixing pesticides or as a water source for cleaning and sanitation are critically important. It is also important to seek mitigations to prevent airborne, dust mediated transmission that may require water districts to re-examine canal design and distancing from intense animal operations in the future.
- There were numerous discussions early on comparing furrow irrigation to overhead sprinkler irrigation methods with the expectation that flood irrigation might represent

less risk as the water ideally does not contact the edible portions of leaf crops. This project demonstrates that contaminated water that in turn contaminates the soil can lead to transference from soil to plants and from soil and plants to harvest equipment where it can spread more widely across the harvested product. Again, more precise channeling of water may offer a mode of risk management if water levels can be controlled so that flooding does not occur, and the irrigation water remains in the furrow and therefore does not contact the plants. Growers might also consider raising bed heights to maximize the distance between the crop and the furrow.

- The research shows that both mixing pesticides with contaminated water and animal intrusion precipitate pervasive transference of *E. coli* and should be avoided at all costs. These are issues where the scientific literature is strong and most any produce safety program already warns against. Growers should work closely with pesticide applicators to make sure there is awareness of the risks and demand written protocols and verification that pesticides are applied properly. Animal intrusion is another subject area that has been well-studied, and the risks understood. With the vast acreages involved in fruit and vegetable production, it is difficult to completely control access to both animals and humans. However, experience and research has shown that pre-harvest inspections to detect animal intrusion and buffering those areas to prevent harvest can be effective mitigations.
- This research also points to the importance of harvest equipment cleaning and sanitation. If contamination is present in a field, it can be transferred to the harvest equipment during operation and can subsequently move onto the product. Growers should be laser focused on equipment sanitation. At a minimum, sanitation should be daily; better yet would be every time the equipment moves to a new production lot, and optimally sanitation would be scheduled throughout the workday. The data from this project using commercial harvesters on commercial fields of romaine certainly supports a reassessment of sanitation frequencies to minimize and limit the scope of contamination and subsequently the risk of a major public health incident.
- When it comes to outbreak prevention, frost damage is not an issue unless pathogens are there to begin with. The focus should be on how to manage the source of the contamination from being present in the first place. Freeze damage in the form of blistering and tissue degradation does not by itself a represent a produce safety risk and only becomes so if pathogens are present in the environment and are spread to the surface of leaves through water or airborne transmission pre- or post-freezing conditions. We now know freeze damaged tissue can support *E. coli* O157:H7 for extended periods of time. That knowledge needs to be part of the risk equation for determining where leaf crops are planted, accounting for potential reservoirs of pathogens in the growing environment and whether a crop can safely be harvested following freeze damage.

Why is this information important for researchers? As was highlighted at the 2019 CPS Symposium and will be discussed throughout this document, partnership between the research community and the produce industry is vital to filling in knowledge gaps in produce safety and driving the industry to a deeper understanding of science-based preventive controls. Building relationships with the industry and establishing a willingness to seek solutions to farm, packing, processing, cooling, storage and transportation challenges permits access to facilities, authentic materials and most importantly the observations and expertise of industry professionals. It takes courage from both communities, industry, and researchers alike, but the results can be powerful and provides the only avenue for controlling contamination risks throughout the industry.

The other point to be made is that practices vary from region to region and different environments offer different challenges regarding produce safety. Certainly a great deal can be gained from a study in any region and that information can be extrapolated to another region, but there remain unlimited opportunities to specifically evaluate key variables and their impact of produce safety in important fruit and vegetable production areas.

Why is this information important for regulators? During and immediately after the 2018 outbreak centered in the Yuma Valley, FDA posed several questions for which the industry had only partial, science-based answers. This work deepens our knowledge and reinforces previously held convictions. The results of this program may not provide ultimate solutions to any one challenge, but they do provide direction for regulators and growers. As regulators pursue their research programs, this CPS-funded rapid response project lays down a foundation that can be built upon and permits the regulatory and grower communities to ask more focused questions for further study. This project also provides opportunities for marketing agreements on leafy greens and other commodities spread across the country to examine and explore how these results, taken with the body of scientific data on irrigation water, irrigation, animal intrusion and sanitation, can be used to fine tune their programs.

Images courtesy of Channah Rock, University of Arizona

2. **Pathogen Die-Off Rates** – With the FSMA Produce Safety Rule-mandated requirement to test irrigation water sources and the adoption of several commodity-based marketing agreements that feature water testing for generic *E. coli* it is important to reconcile test data with the fact that some microorganisms including human pathogens die off in the growing environment over time. FDA's Produce Safety Rule recognized this fact of biology and offered growers a safe harbor of 0.5 log per day for up to four consecutive days before harvest to be able to use water sources that exceed generic *E. coli* standards. This "one size fits all" approach was well intended but lacked sufficient data to support its universal application to the myriad of growing environments found in the industry. Indeed, a 2015 CPS proof of concept research report by Eduardo Gutierrez Rodriguez, then at North Carolina State University, on EHEC and STEC die off on cilantro leaves indicated that many factors influenced die off rates (including but not limited to expose to the sun) and the equation may not be as simple as delineating a one-size fits all linear die off target number (HERE). The 2019 CPS Symposium featured another look at this issue.

Pathogen die-off rates need to be determined based on location, environment, species and crop. ***Renata Ivanek*** from Cornell University presented on research designed to examine the FDA's Produce Safety Rule allowance of 0.5 log die off per day (HERE). Her results demonstrated:

- The assumption of a log linear die off rate is not always appropriate. Indeed, a segmented log-linear die-off rate was a closer representation for bacterial decline in this study.
- Die-off rates using attenuated *E. coli* and select *Salmonella* strains on baby spinach and romaine are highly variable based on locations, species, produce type and trial. The researchers observed everything from rapid die off on the first day to relatively stable survival rates during some of the trials.
- A two segmented die off that was rapid on day one and then much slower thereafter in the second segment was observed. The transition or the slope of the curve on the rate of die-off from segment one to two can vary by as much as 2-3 days.

Why is this information important for industry? An allowance was made in the FDA's Produce Safety Rule to help growers who find that their irrigation water source exceeds the geometric mean of 126 CFU generic E. coli per 100mL of water. The rule permits growers to assume 0.5 log die off over four consecutive days. The die-off per day allowance was proposed by FDA to give growers a safe harbor. For example if a grower used irrigation water exceeding the generic *E. coli* metric, the grower could interpret the data by considering that the concentration would diminish by 0.5 logs per day so that by managing the interval between the last application of that water and the harvest date appropriately, the grower could comply with the produce rule. The results of this research program tells us that growers cannot rely on a one size fits all die-off rate as a safe harbor unless experimental work is performed to better understand the impact of their specific growing environment, the crop and the target organisms of concern for their products.

Why is this information important for researchers? There is a tremendous opportunity to help growers develop data that they can rely on for calculating the potential risks the use of agricultural inputs like water or soil amendments on raw agricultural commodities in multiple crops in the major production regions. This is a knowledge gap that can only be filled by careful experimentation over time with multiple replications and expertise in handling human pathogens. This work is generally out of the purview of most growers but clearly needed to provide growers reliable direction.

Why is this information important for regulators? The concept of a safe harbor that could provide growers with some latitude and guidance for understanding how to apply testing data is desirable. Leveraging the fact that bacteria including bacterial pathogens do not thrive in growing environments and that they die-off has been demonstrated in previous CPS research projects going back as far as 2008 (HERE). This research can aid regulators in reconsidering how to best regulate agricultural water risks.

3. **Fruit and Vegetable Wash Water Systems** – Wash systems and their microbiological control have long been a major point of focus for CPS funded research. As our knowledge base has grown, the industry is beginning to ask more specific questions about alternative antimicrobials and we find ourselves in a position to leverage data on the chemical parameters of wash water to explore development of performance models that help operators better proactively manage their systems.

Can chitosan play a role in water sanitation? ***Anita Wright*** from the University of Florida presented her initial results from studies on applications of chitosan microparticles to eliminate human pathogens from agricultural water (HERE). Chitosan derived from shellfish processing has been touted as a biodegradable, non-toxic alternative to chlorine and other agents that can be used to treat agricultural water prior to application to field crops. The important findings reported were:

- Chitosan was shown to be effective against multiple stationary phase *Salmonella* strains (0.01 to 0.06% chitosan reduced *Salmonella* to the limit of detection in 24-hours) and other indicator organisms and viruses.
- Chitosan can be effective at low pH.
- It was estimated that the cost of chitosan treatment is about 10-times more expensive than common chlorination.

Why is this information important for industry? It is too soon to tell at this point. This was a report delivered during the first of a two-year program. Chitosan microparticles may or may not have production-related applications. The contact time of twenty-four hours to reach the limit of detection for *Salmonella* makes it difficult to see how chitosan could be used in an open irrigation water injection system unless the entire volume of the irrigation water to be applied was batch treated prior to application. The effectiveness and potential limitations of this treatment would not have been understood if this research had not occurred. The principal investigators cost estimate versus chlorine disinfectants is also troublesome for large scale uses. The true value here might lie in smaller scale uses where contact time is not critical and water volumes are smaller. For example, treating holding tanks of water that are used for mixing agricultural chemicals if municipal water is not available. Chitosan may also add value some wash or hydrocooling systems where water may be recycled over longer periods of time.

Why is this information important for researchers? The principal investigator provided key pieces of information to the industry by outlining concentrations needed, contact time and costs compared to chlorine disinfectants. There is obviously more to come from this project, but the project creates awareness for growers and researchers that one-size fits all disinfection approaches are likely not attainable nor even desirable. The properties being described for chitosan in the context of produce could spark additional thinking and new applications. The research community does not have to hit home runs in water disinfection, i.e. a universal treatment for irrigation water. Rather, by identifying disinfection strategies for smaller applications like treating recycled cooling water or water used for making ice, a piece of the challenge is solved for those looking for alternative and more effective solutions.

Why is this information important for regulators? Alternative technologies under development to provide a broader spectrum of agricultural water disinfection meshes well with addressing an important industry need and fits FDA's "smarter food safety" initiative. Chitosan is a readily available biodegradable industrial byproduct that could bring value down the road as part of a water treatment strategy for a specific segment of the industry. Understanding its benefits and limitations will help the regulatory community as it evaluates regulatory approvals and performs on-farm inspections.

Wash water control is about process control. ***Mabel Gil*** (CEBAS-CSIC, Spain) presented the latest from the ***Ana Allende*** research group on their project to establish operating standards for produce wash systems (HERE). This work is focused on helping operators gain insight on how to validate and then verify critical parameters and operational limits for wash water systems. The results thus far strongly indicate something we probably already knew; the design of the system, antimicrobial used, and products being washed impact the critical and operational limits for controlling the persistence of microorganisms in the water that can result in cross contamination of produce. This project is providing additional data points to support industry data and observations and illuminating a path for packers and processors to follow as they set their system-specific operating parameters. Important learnings are:

- When hypochlorite is the disinfectant, residual free chlorine (hypochlorous acid), pH and the organic chemistry of the water are key parameters to monitor.
- When peroxyacetic acid (PAA) is the disinfectant, the key parameter is measurement of concentration.
- Sensors for free chlorine and PAA are not all the same and need to be selected based on the characteristics of the system. High organic loads in the water can reduce the accuracy of some types of chlorine sensors and oxidative reduction potential sensors (ORP).
- Critical limits for free chlorine and PAA vary depending on the produce being washed.

Continuing with wash water control, a novel approach to wash water microbial control was presented by ***Daniel Munther*** from Cleveland State University on his project on mathematical modelling tools for practical chlorine control in produce wash water (HERE). The project aims to construct data-informed models to predict commodity-specific organic load and free chlorine decay/ quenching in produce wash systems. This project is a first step in providing a tool that could be used to more accurately permit antimicrobial dosing of recirculating wash systems to insure proper and effective levels of antimicrobials are always available to control microorganisms in the water. Key learnings thus far are:

- Percent chemical oxygen demand (% COD) increase is a reliable predictor of organic load that results in free chlorine (hypochlorous acid) decay. Calculation of %COD increase over time and measurement of free chlorine can form the foundation of algorithms that can be used to improve dosing wash systems with chlorine.
- % COD increase is a better predictor of organic load than turbidity, total dissolved solids, and COD.
- Using % COD as an input, models can be developed that describe free chlorine dynamics with several fresh-cut products (cabbage, iceberg, and carrots).

Why is this information important for industry? These projects reinforce previous CPS projects on wash water control. The Gil presentation was an interim, first-year report, and much more is to come as the project moves to completion. However, even in preliminary form, the results alert operators that each wash system can be different and therefore when validating wash water control protocols, each system, product, and antimicrobial combination must be considered when assembling validation data and setting operational and critical limits. The project also highlights the importance of choosing appropriate sensors and offers insights on how to effectively measure organic load in wash water. The Munther work can be looked at as an extension of some of the basic "how-to" concepts presented by Gil on behalf of the Allende group in Spain. While we know the understanding of organic load is important because the organic content of the water determines the efficacy of sodium hypochlorite in the water and therefore our ability to kill microorganisms in the water, the work by Munther provides a predictive measure we can use to reliably relate organic load through % COD increase to free chlorine decay. As the development of algorithms matures and are enriched by additional data, these tools can be used to validate specific wash system control practices.

Why is this information important for researchers? These projects shine a light on the importance of process validation and reminds the research community and the industry that it must work together to develop validation tools and verification models. The produce industry generally does not have the capability to perform properly controlled experiments *in situ* and certainly cannot use live pathogens in wash systems to validate wash water control strategies. It is expected that validation can be accomplished with a combination of published scientific literature on the subject, experiments conducted on representative systems by qualified research professionals and focused industry efforts to fine tune performance. Partnership between the research community and industry achieved by sharing published and raw data, providing access to common industry equipment, and translating results in non-technical terms will be required for success. Having models based on measurements and calculations operators can perform can ultimately provide user-ready tools that are reliable and specific for different types of wash systems.

Why is this information important for regulators? The regulatory community needs to stay current with the challenges the produce industry faces in validating wash systems to "fine tune" critical limits, and understand the approaches being used and the research data behind the validation strategies. This will ultimately assist auditors and inspectors as they visit facilities and interpret the methods used to monitor wash water quality and understand the basis each operation used to ensure their protocols and processes adequately control microorganisms in wash water, and the verification strategy being employed.

Linda Harris from the University of California at Davis shared results from her project on characterization and mitigation of bacterial risks associated with washing fresh market citrus (HERE). Soda ash or sodium bicarbonate is used in some, but not all, wash systems for lemon processing. Imazalil is also used in some lemon processing wash systems to control postharvest fungal pathogens that accelerate decay. The question that needs to be answered is whether these mitigations would also offer some level of control for human pathogens like *Listeria monocytogenes* and *Salmonella*? Some key learnings from this project follow:

- Cross contamination with *L. monocytogenes* in wash systems was observed under common lemon wash conditions using soda ash. However, using 3-percent soda ash at 60°C for 1-minutes results in >5-log reduction. Increased heat, not soda ash, is the critical factor in elevating the antibacterial response.
- In contrast, much less severe conditions are needed to control *Salmonella*. 3-percent soda ash at 40°C for 3 minutes results in a 5-log reduction of *Salmonella*.
- Imazalil had almost no impact on *Salmonella* but caused a slight reduction when tested against *L. monocytogenes* (3-log reduction over 24 hours). When >20 ppm PAA was used in combination with Imazalil at 1-3 minutes exposure with heat (104°F), a 5-log reduction of both pathogens was achieved.

Why is this information important for industry? In many ways, this project answers a question the citrus industry needed answers to: do the additives they current use to control fungal pathogen also provide protection to human bacterial pathogens? The answer came back that they do not as commonly used, but conditions can be defined that permit the use of soda ash and/or Imazalil in combination with increased heat and contact times, and addition of PAA with Imazalil to achieve >5-log reductions with *Salmonella* and *L. monocytogenes*. The project is another great example of the research community working in partnership with growers to address a very defined produce safety question.

This research also highlights how different pathogens likely require different control strategies. Conditions which control *Salmonella* had very little impact on *L. monocytogenes*. There are several factors that contribute to an organism's susceptibility to antimicrobial treatments that are beyond the scope of this analysis. However, when appropriate, the concept of differential approaches depending on the target pathogen and finding successful multi-target combination antimicrobial strategies is important to consider for our operations.

Why is this information important for researchers? This is another example of a very specific industry operational need being addressed by a researcher who has cultivated industry relationships and put her team in position to work in partnership with the industry to find workable, practical solutions. Additionally, as growers, packers and processors look to validate that their wash water control programs are effective, this project lays out operational parameters that those in the citrus industry can use at least as a starting point if they regularly employ soda ash and Imazalil.

Why is this information important for regulators? These results are another awareness opportunity for regulators. It is important for the regulatory community to understand varying wash water control strategies being implemented across the industry among different crops and systems. This information will aid them as they audit, inspect, and advise the industry moving forward.

Photos courtesy of Daniel Munther (Cleveland State), Linda Harris (UC Davis) and Ana Allende (CFBAS-CSIC)

4. **Irrigation Water** – Irrigation water remains an important research topic for the produce industry. Indeed, since the formation of CPS, the industry has invested in 36 research projects on irrigation water exploring everything from potential pathogen contamination risks to mitigation strategies and tools to sampling methods and irrigation water delivery systems. The 2019 Symposium featured survey work on the presence of *Cyclospora* in water sources in the Yuma Valley and a timely report on factors to consider when sampling irrigation water sources for the presence of pathogens and the possibilities of building predictive models growers could employ to help them make data-driven decisions.

   Cyclospora* can be found in irrigation waters in the Southwest**. ***Gerardo Lopez from the University of Arizona presented his work exploring potential reservoirs of *Cyclospora cayetanensis* (HERE). The objectives of this program were to look at both irrigation water and influent (raw sewage)/effluent (treated sewage) samples from the Yuma Valley in Arizona for occurrence of *Cyclospora*. The key findings were:

- *Cyclospora* was found in 15/119 (13%) of irrigation water samples in 2017-2018 and 6/196 (3%) of irrigation water samples in 2018-2019. The two-year cumulative average was 7% (21/315).
- Of the 6 *Cyclospora* samples in 2018-2019, 4 were recovered from unlined irrigation canals.
- All positive samples were recovered in the months of December, January, and February. No other month yielded a positive sample.
- The occurrence of *Cyclospora* in irrigation water is not correlated to *E. coli* or coliform bacteria.

Why is this information important for industry? It is important to note that *Cyclospora* is not just a Texas border, Yuma Valley or Mexico production issue or a tropical contaminate as previously thought, but *Cyclospora* contaminations have also been reported over the last two years in coastal California and as far north as Oregon. This research is a good news/bad news story. The bad news is that these results clearly indicate that *Cyclospora* was found in irrigation water in the Yuma Valley over the last two growing seasons. The good news is that the positive findings were limited to December, January, and February only and no *Cyclospora* illnesses were linked to produce during this timeframe even though this corresponds to the peak season of fruit and vegetable production in that region. This study creates awareness that *Cyclospora* is a very real but represents a low risk to the safety of produce from this region. The viability/ infectivity of the pathogen is difficult to determine, so detection does not necessarily correspond to public health risk. Growers should continue to aggressively pursue Good Agricultural Practices and inspect and maintain irrigation canals for any possible contamination routes where human sewage containing *Cyclospora* oocysts or animals acting as vectors might compromise the water quality. They should also continue to monitor agricultural workers to make sure they follow good hygiene practices as humans are known to transmit oocysts to their environments.

Why is this information important to researchers? The introduction of the FDA BAM 19b quantitative PCR-based test for *Cyclospora* opens new opportunities to study *Cyclospora* movement in the growing environment and transfer to fruits and vegetables. We know that *Cyclospora* can be found regionally in sewage treatment influents and effluents and that people can transmit oocysts. We also know that nearly every year, *Cyclospora* infections are found in the U.S. (usually in the months of May, June, and July) and indeed *Cyclospora* only trails *Salmonella* as the cause for gastrointestinal disease. So, in addition to understanding its biology, movement in the environment and transference to crops, what might be done to eliminate it from ag water or to develop equipment sanitation treatments that are effective against *Cyclospora*? How can we ascertain the public health risk associated with detection of *Cyclospora* DNA? We are in our infancy in terms of understanding this pathogen and the role of fresh produce in causing illness and hope that this and similar research encourage scientists to pursue much-needed studies on this parasite.

Why is this information important to regulators? This research project has the same impact for regulators as it does from growers and researchers. *Cyclospora* can be in the growing environment and represents a public health risk, therefore we need to learn how to manage the risk more effectively. An important question arising from this research is why is it only the months of December, January and February that yield a low level of positive samples when outbreaks from produce seem confined to May, June, and July? What is different about the Yuma Valley where no *Cyclospora*-related illnesses were detected as a result of consumption of products originating there when other regions were implicated in illness outbreaks over the last two years? Is it simply a failure to detect small increases above the *Cyclospora* illness background or is there a more important transfer vector than water for produce contamination? This research program also reported findings of *Cyclospora* on both influent and effluent from sewage treatment plants. Clearly more is to be learned by developing an understanding if this inability to eliminate *Cyclospora* from treated sewage represent a direct or indirect threat to produce production and how to better treat public sewage treatment to better control the risk.

Evaluation of surface waters is not a "one size fits all" exercise. ***Martin Wiedmann*** from Cornell University shared the latest results from his work on using hydrological, landscape and weather data to predict the quality of surface waters used for crop production (HERE). Agricultural water and specifically the use of surface waters has been literally under the microscope in recent times and implicated as a causative factor in several high-profile illness outbreaks. This project focused on three basic objectives: (1) to assess the association between generic *E. coli* levels and foodborne pathogen presence in surface water used for produce production, (2) identify and rank factors associated with pathogen detection in surface water, and (3) develop models to predict when and where surface water sources are likely to be contaminated by pathogens. Key learnings from this project are:

- "One size doesn't fit all" approaches to testing surface waters for the presence of human pathogens do not work. It is important to account for environmental, water chemistry and weather factors and to understand that the relationship between indicator organisms and pathogens are variable. We need to develop alternative data-based approaches that account for these variables to truly be able to identify and manage contamination risks associated with surface water use.
- By evaluating 2,361 grab samples (10-L water samples) and 362 Moore swabs (a collection device placed in a water source) taken from 60 Arizona and 68 New York waterways, the Wiedmann group concludes that choice of sampling method is an important consideration when testing water sources. The use of 24-h Moore swabs was better than 10-L grab samples for enabling detection of pathogenic *E. coli* and *Salmonella*, while grab samples were better for detecting *Listeria*. These data indicate that different water sampling methods may need to be selected based on the organism of concern
- The project included an examination of the utility of using generic *E. coli* as an indicator of ag water microbial quality. Previous research has demonstrated that the use of *E. coli* as an indicator of the produce safety risks associated with preharvest water appears to be pathogen-specific and region-specific. To determine if meeting *E. coli*-based agricultural water standards was associated with a reduced likelihood of pathogen presence in surface water, Wiedemann's group simulated water sampling using bootstrapping (N=10,000 20-sample subsets). The results suggest that meeting these standards is (1) largely a function of when the samples that comprise a subset were collected, and (2) a poor approximation of microbial water quality at the time of water use. Therefore, alternative approaches are needed to improve growers' ability to identify produce safety risks associated with preharvest surface water use in real-time.
- An important variable in the measurement of agricultural water quality is average air temperature. An average air temperature of approximately 27°C up to 3-days before sample collection (or water use) was associated with a higher likelihood of EHEC contamination in Arizona canals sampled as part of the study reported.
- In this study, microbial water quality varied over space and time. For example, PCR-based EHEC detection in Arizona was substantially higher in 2017 (51%; 43/83) than in 2018 (22%; 39/178).

- Microbial water quality is associated with environmental heterogeneity (i.e., changes in and interactions between environmental factors). Weather and water quality factors (e.g., *E. coli* levels and rainfall, dissolved oxygen and solar radiation) interacted to affect the likelihood of detecting pathogens in surface water. Understanding the nature of these interactions is difficult due to correlation between weather and water quality factors so the produce safety risks associated with preharvest surface water use appear to be dependent on environmental conditions at the time of water use.
- Although the results indicate that interactions between environmental factors were associated with microbial water quality, these findings also indicate that some factors (weather, water quality, spatial factors) may be useful as supplemental indicators of microbial water quality. Indeed, one model targeted at predicting EHEC in Arizona canal water employed quantification of generic *E. coli* combined with average solar radiation to be useful in determining higher or lower likelihood of the sample testing positive for EHEC.

Why is this information important to the industry? There is a great deal of information contained in this research program. The overarching learning is that a "one size fits all" approach is not appropriate for measuring microbial water quality or correlating the likelihood of pathogen presence in surface waters intended for agricultural use. In other words, the days of simply taking one-liter grab samples and measuring generic *E. coli* are likely over for many growers if they truly want to manage contamination risks to their crops. It is time to leverage the science that has been accumulating and develop source, location, target pathogen and use strategies for measuring and managing surface water microbial quality. We have seen via numerous CPS-funded projects that generic *E. coli* was not a satisfactory indicator of pathogens in surface water. We also have data that demonstrate increased volume for water samples is more likely to detect low levels of contaminants and that weather events like heavy rains that cause run-off into surface water can impact water quality. The Wiedmann work fills in some gaps and points to the relationship between sampling and the efficacy of finding specific pathogens. The use of 24-hour Moore swabs can capture low concentration pathogens like *E. coli* O157:H7 or *Salmonella* by essentially filtering large volumes of flowing water over an extended 24-hour time while 10-L grab samples seem to be the best method for sampling for *Listeria*. The project also identifies contributing factors like air temperature prior to sampling, solar radiation, dissolved oxygen in the water, weather, and seasonality that need to be part of our sampling and testing strategies for surface water and calculated into our interpretation of the results. So, what was a simple grab sample and test for an indicator needs to evolve into a more science-based approach that gives growers a better understanding of their surface water quality and helps them make better, data-informed decisions on its use.

Why is this information important to researchers? The case has been made above that growers need to evolve their approach for measuring the microbial quality of surface waters used in fruit and vegetable agriculture. And that is where the opportunities lie for the research community to step in to help. If we are going to tailor sampling to pathogen targets, then growers need to know what is the best way to deploy and handle a Moore swab? Early pathogen survey research has basically led us to the conclusion that pathogens can be present in any surface source for irrigation water. Since we know that there are contributing environmental and water chemistry related factors associated with pathogen presence, we need to understand these factors, have a rationale for their consideration in water source risk assessments and meaningful management strategies to reduce crop contamination risks. Whether management strategies include irrigation application modifications or broad use of antimicrobial treatments on source water, we will need the science to help growers make decisions that improve safety and are operational and sustainable going forward.

Why is this information important to regulators? FDA is still in the process of re-thinking agricultural water requirements per the produce rule. Since the initial regulations came out, the world has changed, and we know more science now. The initial requirements were developed with what we knew ten years ago; the next iteration must reflect what we know now. Though it is tempting to try to find a simple, "one size fits all approach" the industry is best served by setting up a framework that is based on hazard analysis and risk assessment for each water source on a farm. It is an aggressive approach and one that will require growers to engage in the process and not just take a sample on a prescribed basis and check the box when it is done. They will have to live the program every day. The regulatory and research communities are going to have to provide the science and do so in a way that is practical and useful to growers who will have to implement the programs and then use the data to drive their decisions. This is a mindset change for everyone across the industry and it will not be easy, but it is important to listen to what the science is telling us and we have to be open to change or we will continue to see outbreaks where the use of agricultural water is called into question.

Photos courtesy of Gerado Lopez (University of Arizona), Martin Wiedmann (Cornell) and Bob Whitaker (Whitaker Consulting, LLC)

5. **Focused research approaches can provide practical solutions.** There was a collection of projects presented at the symposium that addressed very specific operational questions and leveraged "biological" mitigations to control a produce safety challenge. The results of these programs are presented below and their applications to the industry will be addressed collectively.

Biological fences. There were several projects presented at the 2019 Symposium that were highly focused on a well-defined objective. Interestingly, this collection of projects shares common themes: the importance of incorporating systems biology into a solution, placing a priority on partnership with growers and the need to understand that solutions need to be tailored to specific environments. One of these was a program conducted by ***Michelle Green***, University of South Florida St. Petersburg who presented her work on engineering and ecological approaches to reduce Pacific tree frog intrusion into leafy greens fields (HERE). We have all seen the videos of consumers opening a bag of salad only to find frogs staring back at us. It has a certain yuk factor and represents a potential produce safety risk as frogs and other amphibians have been shown to carry *Salmonella*. The following results were reported:

- Frogs can climb mesh netting and other similar materials but cannot traverse rough surfaces or fences built with a top lip. The biology of the frog's "toe-pads" prevents them from being able to grip rough surfaces or perpendicular lips on a fence so they fall back to the ground.

- The research team is working with growers to test practical "biological" fence designs and agronomic practices for use in fields at risk from natural tree frog intrusion.

Survivability of *Listeria* in emerging salad ingredients*.* *Amanda Lathrop* from California Polytechnic State University at San Luis Obispo presented her highly focused work on determining survivability of *Listeria monocytogenes* in non-traditional salad ingredients (HERE). As consumers seek different flavor profiles, we have seen kale, Brussels sprouts, beet greens and broccoli stalks move to the pre-cut forefront. This project seeks to find out if *L. monocytogenes* will grow, survive, or die off if present on these types of ingredients. Initial results from this study include:

- Preliminary experiments indicate that *Listeria innocua*, a non-human pathogenic relative of *L. monocytogenes* behaves very similarly to the pathogen in these matrices.
- At 4°C or 39°F, inoculated beet greens, cut broccoli stalks, Brussels sprouts and kale demonstrated log increase growth of 1.8, 2.6, 0.6 and 2.4, respectively. In other words, *L. monocytogenes* can grow on these products if present, under refrigerated conditions.
- Interestingly, shredded Brussels sprouts supported the least growth leaving one to consider the possibility that natural antimicrobial constituents (e.g. glucosinolates) of Brussels sprouts might be suppressing *Listeria* growth.
- The research team is now moving their efforts to looking at packaging solutions and natural populations of competing microorganisms in the plants biome that might control or limit *Listeria* growth.

Biological Control of Nuisance Birds and Rodents in Leafy Greens Fields. Previous CPS-funded research (HERE) has shown that wild birds and rodents are potential vectors for human pathogens as they can move from pathogen reservoirs like animal feeding operations or dairies into fruit and vegetable production environments. ***Paula Rivadeneira*** from the University of Arizona described her project on the use of raptors to prevent nuisance birds and rodent intrusion into produce fields (HERE). Along with being potential vectors for human pathogens, nuisance birds also cause considerable crop damage and loss of yields for fruit and vegetable farmers. The key learnings at the end of this two-year study are:

- During a three month, in-field observation activation that saw 659 bird count observations and the 102 releases of trained raptors for abatement purposes (wild birds observed in the field or suspected to be in the field), the team achieved a 97-percent success rate at deterring nuisance birds from fields.
- Both hawks and peregrine falcons were deployed in various flights. It was noted that some species of birds were unaffected by the presence of these raptors while others immediately vacated the area.
- The research teams notes that the use of raptors can be expensive ($650-1,000 per day) and suggest that raptors might be most effective from a cost and efficiency perspective if deployed like one would an integrated pest management (IPM) program that might include the use of drones, screamers and other noise makers. The researchers also noted that nuisance birds are more likely to be present in the early morning and in the evenings so mitigations might be more effectively used if timed to when the targets are present.
- Each nuisance bird species will likely require a different approach and combination of deterrents; in other words, one size will not fit all. The biology of the raptor and the target animal must be calculated into the response and strategies employed.

The use of Vegetative Buffer Zones to Control Pathogen Movement*.* ***Sid Thakur*** from North Carolina State University (HERE) tackled the issue of vegetative buffer zones (VBZ) with his project looking at the establishment of VBZ to reduce the risk of STEC and *Salmonella* transmission from animal operations to fresh produce on co-managed farms. The research was conducted on a farm with poultry operations and another with cattle (150 head) production with both having vegetable production downwind from the animal farming. The poultry houses feature large fans venting air outside toward the vegetable production plots. Using staggered plantings of fast-growing hardwood trees (poplar), pine, shrubs, rye grass, and pollinator plants, layered 30-foot VBZ's were established to buffer animal production from vegetable production. Important observations include:

- There were lower recovery rates of *Salmonella* and STEC from soil, air and produce samples closest to the VBZ.
- The number of positive environmental samples increases in the late summer to fall season.
- There is a possibility that the VBZ's could become a "sink" for pathogens as they are collected from the animal operations.

Why is this information important for industry? There are obvious takeaways from this collection of projects: (1) the Green project points to the use of biological fences designed so that frogs cannot physically climb them and enter leafy greens fields, (2) the Lathrop program warns us that *L. monocytogenes* can indeed grow in chopped salads supplemented with kale, broccoli, Brussels sprouts and beet greens and that *L. innocua* looks to be a reasonable surrogate for *L. monocytogenes* for future studies, (3) the Rivadeneira project detailed the use of raptors to control nuisance birds and rodents in produce fields and advises that each species of nuisance bird may require a different strategy to achieve control including employee drones in addition to or in place of a predatory bird and (4) Thakur's work indicates that vegetative buffer zones can be an effective tool in mitigating airborne fecal contamination from poultry and dairy operations adjacent to vegetable production acreage.

These projects also share a systems biology theme. The solutions they suggest to each challenge are at least partially reliant on the biology of the pest itself or an antagonist in the environment. Hence, tree frogs can be defeated by a fence designed so that the frog's toe pads cannot grip the fence, *Listeria* control might be gained by either antagonistic microorganisms present in the salad or by chopping theses Cole crop ingredients to release antimicrobial chemicals they contain, nuisance birds can be controlled by raptors and fecal contaminants can be managed by the use of buffer zones composed of a diverse array of plants layered to block prevailing wind or air current. It is important to recognize these systems approaches to pathogen control and to be open to leveraging these opportunities when appropriate and data exist to validate their use.

Why is this information important to researchers? These systems biology approaches may provide the industry with options to address produce safety challenges while co-existing with potential contamination hazards that are part of their growing environment. Though not always the case, historically if a growers crop was threatened by tree frogs, a response might be to kill the frogs in the immediate area thus eliminating the threat to the use of the crop but also eliminating an important element of the regional environment. Similarly, the use of vegetative buffer zones may provide a mitigation tool to permit smaller scale animal operations to exist side by side with produce production without taking large tracts of land out of production. The key for the research community is to be able to work with the industry to identify these opportunities and then perform the research to elucidate the efficacy of a systems approach and define the cost ramifications so that growers can comfortably implement these types of solutions.

Why is this information important to regulators? This work is important to the regulatory community not only because it offers potential solutions to potential contamination issues but also to create awareness that mitigation strategies can be developed using principals of systems biology. There is a need to be open to these approaches and support the research needed to prove efficacy. To the extent that FDA and USDA can work with the industry and industry groups like CPS to identify and fund research needs to explore systems approaches, the more flexibility growers will have as they seek solutions that fit their operation-specific needs. This will also result in greater willingness to pursue research to demonstrate efficacy by researchers.

Photos courtesy of Michelle Green (University of Illinois), Sid Thakur (North Carolina State) and Paula Rivadeneira (University of Arizona)

6. ***Listeria* Control** – Control of *Listeria monocytogenes* on harvest equipment, in packinghouses, cooling operations and processing facilities remains a priority for the produce industry. CPS has featured research at previous Symposia that pointed to the need for industry adoption of "seek and destroy" strategies to control *Lm*. There have also been 23 CPS funded studies on formation and destruction of *Lm*-containing biofilms, the efficacy of specific disinfectants on *Lm* and surveys and environmental persistence studies and presentations to help inform operation-specific control strategies (CPS website). Each Lm research project funded by CPS builds our knowledge base, deepens it and provides insights for the development of mitigation strategies. The 2019 Symposium follows that progression of thought and enhancement of our knowledge base.

   Listeria* must be controlled by aggressive cleaning and sanitation.** ***Trevor Suslow, now at the Produce Marketing Association, was at the University of California at Davis while conducting his work on postharvest harborage sites for *Listeria* in citrus packing sheds (HERE). This project focused on partnership with industry packinghouse operators to map the presence of *Listeria* within packing environments including equipment surfaces with a view to improving the design of environmental monitoring programs and ultimately more effective sanitation programs. Among the key learnings from this project are:

   - A high prevalence of *Listeria* can be found on non-food contact surfaces in packinghouses. Sampling 10 facilities from 2016-2018 and using 1,475 swabs, 31% of the samples from zones 2 and 3 in the packing facilities were molecular positives and 30% culture confirmed.

- Seasonality seems to play a role in prevalence and location within facilities. For example, the range of positive samples varied from 3 to 93% percent for *Listeria* species, while the range for *Listeria monocytogenes* was 3 to 28% depending on the date of the sampling.
- Hot spots for positive samples included bin dumping, washing and product storage areas where moisture could be observed. Zone 1 samples also tested positive across some facilities.
- Using methods that permit genetic analysis of *Listeria* isolates (cgMLST cluster analysis), the research demonstrated that certain isolates could be found in different facilities indicating that these *Listeria* were moved or transported between facilities. Additionally, the same methods revealed that certain isolates appeared over the course of several years indicating they were persistent in the packing environment or resident strains in that facility.

Expanding on this idea of movement of *Listeria* in the environment, ***Martin Wiedmann*** from Cornell University described the initial results from his project on using *Listeria* whole genome sequencing reference sets to permit improved determinations of *Listeria* persistence in the U.S. and develop a baseline to permit source tracking in the event of listeriosis outbreaks (HERE). Whole genome sequencing is currently being used to identify pathogens in illness outbreaks. Specifically, whole genome sequencing has been a useful tool in exploring outbreaks of listeriosis and in helping researchers and produce operators determine resident versus transient strains of *Listeria monocytogenes* in their operations. We also know that *Listeria* strains are seemingly associated with specific geographies. Therefore, if one could survey *Listeria* species and *Listeria monocytogenes* strains using whole genome sequencing across the U.S. during outbreak investigations, that data set might be used to help identify the source of the outbreak, or at least the original source of the pathogen, more quickly. The key learnings from this research program thus far include:

- Soil sample collection in non-ag environments around the U.S. is proceeding. The country was divided into 40 equally sized areas and a team of 60 volunteer samplers was used to collect samples 20-miles apart. Out of 1,000-samples, approximately 60% have been analyzed by whole genome sequencing thus far. 31% are positive for *Listeria* covering 14 different species. 12% were positive for *L. monocytogenes*.
- The preponderance of *L. monocytogenes* positives were isolated along the eastern half of the U.S. and as the sampling moved westward, fewer were recovered; different *Listeria* species had different patterns of distribution across the country.

From the first two *Listeria* projects we generally know that the pathogen is in the environment and it can be moved into production environments. But it is not just soil transfer that can facilitate movement into our operations; *Listeria* can also enter facilities and present a public health risk if it is on the crop. ***Meijun Zhu*** from Washington State University presented their final report on control of *Listeria monocytogenes* on apples using antimicrobials applied via a manifold spray system (HERE). This project was born out of a listeriosis outbreak tracked to candied apples a few years ago and subsequent studies that indicated *L. monocytogenes* could be found on the surfaces of fresh apples. The goal here was to identify agents and a delivery system that could control *L. monocytogenes* on apples. Key learnings include:

- PAA was more effective in controlling *L. monocytogenes* than sodium hypochlorite, experimental mineral oxychlorides, or electrolyzed water even at 100 ppm free chlorine in these experiments.

- PAA at 80-ppm with a contact time of 30-seconds resulted in a 1.5-2.0 log reduction at elevated temperatures (up to 46°C). PAA efficacy is not affected by the hardness of the water or pH.
- PAA was more effective at elevated temperatures. Application at 43-46°C for 30 and 60-seconds resulted in 2.3 and 2.6 log CFU/apple reductions, respectively.
- The use of PAA was validated using a pilot-level spray bar brush bed wash system and again on brush bed wash systems in three commercial packing operations. *L. innocua* and *E. faecium* were employed as non-pathogenic surrogates in these studies. Washing apples at 80 ppm PAA with exposure times of 30 seconds and 2 minutes at 43-46°C resulted in 1.5 and 1.6 log CFU/apple reductions, respectively.
- PAA did not prevent cross-contamination in these wash systems but it did reduce the cross-contamination rates. Dirty brush beds increased cross contamination rates. Additionally, brush bed speed, sanitation efficacy and nozzle and bar arrangements also impacted bacterial transfer rates.

Why is this information important to industry? There are two overarching features in these three research programs: (1) the important role of grower/research partnerships to permit investigations into *Listeria* control by researchers who are granted access to farm and packinghouse facilities to validate lab-scale research and bring it to the commercial operational level, and (2) the growing use of whole genome sequencing and other typing technologies to identify and differentiate *Listeria* and *L. monocytogenes* strains and further identify their geographic origins and track their movements within production environments.

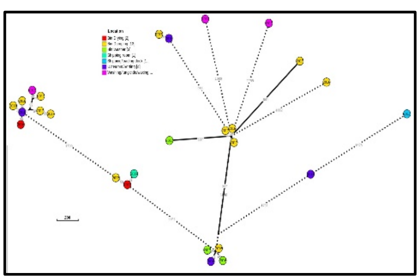
The Wiedmann project clearly demonstrates that *Listeria* species and, *L. monocytogenes* are part of the environmental landscape as they have sampled non-ag locations across the country. While Wiedmann found a lower prevalence for *Listeria* in non-ag environments out west, Suslow's work clearly shows *Listeria* can be prevalent in agricultural production environments. But it is important to consider this data carefully. Wiedmann was careful to point out during the discussion at the CPS Symposium that *Listeria* is a hazard growers, packers and processors need to consider but it is not necessarily a risk provided potentially contaminated soils are not permitted to enter packing and processing operations. Indeed, Suslow's results derived from studying packinghouse operations demonstrate that locations within the operation where soil and moisture are likely to be found offer the greatest chance for *Listeria* detection. Zhu's project reinforces the concept that preventing contamination is a sounder strategy than trying to eliminate it after it has occurred. While Zhu's results with PAA at elevated temperatures and brush bed wash systems for apples look to be encouraging, cross contamination could not be prevented; only the rates of transference to fruit diminished. In the end, all three projects point to the vital importance of aggressive and effective sanitation programs. We have known for some time that cleaning and sanitation are critically important to control *Listeria* risks; a lesson derived from the meat industry. The Suslow work clearly demonstrates that if these tasks are deficient then not only can *Listeria* be found in operations, but it can persist in facilities and be a chronic cross contamination risk. In our era of data-driven decision making, the data paints a clear picture: operators need to have proactive sanitation schedules driven by environmental monitoring programs because we know the hazard is out there. The good news is that you can reduce the risk with stepped up efforts in cleaning and sanitation.

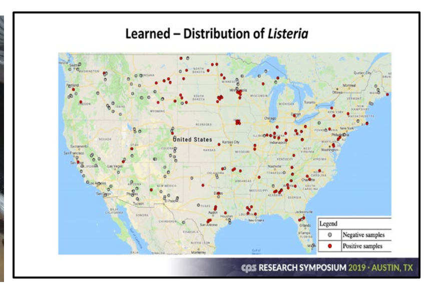
Why is this information important to researchers? Once again, the prospect of partnership is important for the research community to embrace. The Zhu and Suslow programs stand out as they engage commercial operations to address environmental and product contamination with *Listeria*.

The projects demonstrate the evolution of produce safety research whereas a decade ago this work could not have been approached owing to the wariness between food safety researchers and growers. CPS has been a catalyst in creating comfort levels for the research community and the produce industry and it is beginning to permit research efforts that might have been unthinkable in the past. Leading growers are stepping forward to seek solutions to difficult produce safety issues and researchers willing to work to develop relationships with these leaders can provide valuable contributions to the industry.

Why is this information important to regulators? FDA, CDC, and the states are increasingly using whole genome sequencing to identify pathogens in outbreaks and to assist in source tracking. While there are still knowledge gaps in how many single nucleotide polymorphisms (SNPs) can be tolerated before organisms can be said to be different or the same, understanding the geographical distribution of *Listeria* will certainly be an important contribution to improved source tracking.

These projects also reinforce the notion that "*Listeria* is everywhere". It is in agricultural and non-agricultural environments and a hazard to be considered and a contamination risk that needs to be dealt with by producers and regulators. On the regulatory side, the industry needs final guidance from FDA on *Listeria*. Specifically, the produce industry needs a program like the USDA FSIS *Listeria* program where producers can aggressively implement seek and destroy strategies to identify *Listeria* in their facilities and have an opportunity to use positive tests to make corrective actions and not have to report themselves until a second positive is identified. This "second bite at the apple" strategy has improved *Listeria* control in the meat industry and there is no reason why it would not do the same in produce. FDA's draft *Listeria* guidance goes a long way in encouraging that mindset, and the industry looks forward to final guidance that addresses the few comments offered by the fresh produce industry. The data presented in the Suslow work describe the prevalence of *Listeria* in packing environments across zones 1, 2 and 3. Producers need the freedom to strategically design environmental monitoring programs with special emphasis on high risk equipment or facility locations to validate cleaning and sanitation efforts.





Photos courtesy of Trevor Suslow (PMA), Meijun Zhu (Washington State) and Martin Wiedmann (Cornell)

7. **Emergence of New Tools to Aid Sanitation** – Understanding biofilm deposition and how to remove them from equipment surfaces more effectively remains an important objective for the produce industry. We learned from Virginia Tech's ***Laura Strawn's*** research (HERE) a year ago on biofilm formation on harvest equipment that cleaning and sanitation was best done right after operations are stopped for the day, otherwise biofilm formation can rapidly occur making effective cleaning difficult. At the 2019 CPS Symposium research was presented on the importance of designing cleaning and sanitation strategies based on the surface composition and also the use of antimicrobial/anti-fouling coatings that can be applied to surfaces on various types of harvest containers that can reduce microbial build up on those surfaces.

Different surfaces require different cleaning and sanitizing approaches. ***Kay Cooksey*** from Clemson University presented her work on preservation of stone fruits by application of antimicrobial coatings (HERE). In this report which was made after only six months from the initiation of the project, Cooksey focused her comments on biofilm production and how understanding biofilm formation and dissolution can lead to strategies for improved sanitation. Some key learnings were:

- *Listeria monocytogenes* can form biofilms on a variety of surfaces including wood. Rougher surfaces result in more cell attachment. Surfaces with crevices and pits are more vulnerable to biofilm formation and consideration should be given to increasing surface to sanitizer contact time to penetrate biofilms.
- Dirty or soiled surfaces reduce the efficacy of sanitizers. Biofilms become more resistant to sanitation in dry conditions.
- Brushes are commonly used in many types of fruit packing and certainly in stone fruits. The dilemma many operators have is how to keep them cleaned and sanitized, so they do not become transfer vectors for pathogens and spoilage microorganisms. Brush filaments can have rough surfaces, or the filaments can even be hollow, thus likely to harbor bacteria. This research showed that mineral oil coatings used to treat fruits can transfer to brushes and can increase *Listeria* attachment to brush filaments. Sanitation with 200 ppm sodium hypochlorite for 20 minutes resulted in a 3-log or 99.9% reduction but not elimination.

Looking to build antimicrobial properties into surfaces, ***Gang Sun*** and ***Nitin Nitin*** from the University of California at Davis, reported on their work examining rechargeable, antimicrobial and antifouling plastics that could reduce cleaning and sanitization efforts (HERE). This concept of developing non-fouling materials for construction of food contact surfaces is not new to CPS. Indeed, CPS funded a program in 2018 by ***Boce Zhang***, University of Massachusetts on the use of non-fouling coatings to inhibit biofilm formation (HERE). Plastics such as those used in totes, bins and RPC's often get scratched and chipped as they are used in harsh conditions and recycled constantly, sometimes over a period of years. The idea that plastic containers that are used during harvest or product shipment could act to transfer microorganisms to harvested fruit or vegetables has been raised and investigated previously (HERE). Commercial RPC suppliers have developed cleaning and sanitation protocols, but harvest bins, baskets and buckets used across the industry may only sporadically be cleaned and sanitized as they are cycled from field to packing or processing facilities to meet harvest demands. Some key learnings from this program are:

- Polymers have been developed that have antimicrobial properties that significantly reduce biofilm formation. Antifouling chemicals can be included to essentially repel and prevent attachment of microorganisms. These formulations could be used to introduce antifouling and antimicrobial functionalities in films or plastics for bins and other containers.
- Since harvest and shipping containers are re-used over long periods of time, it is important that the antimicrobial properties could be "re-charged" with some frequency to keep the antimicrobial activity sufficient to control biofilm development. Recharging can be accomplished with 500 ppm (or lower) sodium hypochlorite solutions. Re-charge takes approximately 10 minutes and would need to be done once per month depending on frequency of use. The polyvinyl ethylene film is food grade.
- There are no organoleptic issues associated with these coatings as there are very low levels of chlorine on treated surface
- The coatings are temperature and UV stable.

- Testing with inoculated bins treated with coating resulted in 3-log reduction of bacteria in 10 minutes.
- The team is currently looking at additional chemical alternatives and scale up to test bin liners that might be used for iceberg lettuce bins and will explore opportunities to prolong storage.

Why is this information important to industry? These projects are still early in their execution, but they serve to create awareness for growers, packers, and processors. Cooksey's work reinforces Strawn's report in 2018 (HERE) in that improperly cleaned or soiled surfaces reduce the efficacy of sanitizers. Cooksey also reports that brushes commonly used in stone and other types of fruit packing offer cleaning and sanitation challenges based on the porous or even hollow materials they are traditionally constructed from. This should create awareness to all fruit packers that employ brushes that they can mediate transfer of pathogens and that management through cleaning and sanitation may not be adequate to control the risk. Indeed, even rigorous treatment with 200 ppm sodium hypochlorite for 20 minutes did not eliminate *L. monocytogenes*. Cooksey suggests that fruit packers look at other alternatives to applying postharvest treatments like mineral oil or waxes; perhaps by using spray manifolds that offer less challenges for cleaning and sanitation. It is also important to point out that this current report by Cooksey is really an extension of an earlier CPS-funded project where her research team developed a flow-through device that facilitates the creation of biofilms. With that capability in hand, her team was able to pursue the current project looking at biofilm formation and removal on brushes used in stone fruit processing. The project demonstrates the iterative process of produce safety research where results from one project can be used to explore a second objective important to the industry.

The Sun and Nitin project reminds us that technology from other disciplines can offer solutions to produce industry challenges. The use of films and coatings that confer antimicrobial and anti-fouling properties on the surfaces of rapidly cycled and hard to clean and sanitize harvest totes, bins and baskets would help solve the problem facing many operations on how to control cross contamination from harvest containers to newly harvested products. The ability to re-charge the coated surfaces with simple sodium hypochlorite so that in effect the antimicrobial properties of the treated containers can be extended is also beneficial. Though much work needs to be done on alternative materials and cost control, the results are exciting.

Why is this information important to researchers? Cooksey's statement that different types of surfaces need different cleaning and sanitation strategies is sensible given what we know about sanitizers and surface materials and Sun and Nitin's work highlights how polymer chemistry can be leveraged to meet an industry need. It brings us to the reality that interdisciplinary approaches to key produce safety questions interactions. In these projects, materials science, food science, microbiology and engineering expertise combined with industry input provide the knowledge base to address cleaning and sanitation challenges growers, packers and processors face daily. The research community has a history of inclusion to solve difficult research objectives and these projects serve to remind us that broad expertise is needed to develop practical solutions to many produce safety issues.

Why is this information important to regulators? This information serves to create awareness in the regulatory community that these approaches are in development and that regulatory approval may be sought. This work also allows regulators to envision applications elsewhere within the industry. FDA inspectors are routinely in contact with growers, packers and processors and it is important that they are aware of these approaches, so they know the science and how to react when confronted with novel preventive controls to control risks. As FDA moves into the world of a "smarter FDA" in their drive to adopt new technologies across the entire food industry, they can play an important role for the produce industry as a connector. As FDA encounters emerging technologies or approaches applied to other segments of the food industry, they can connect those resources to produce industry leaders to initiate further innovation.

Photos courtesy of Kay Cooksey (Clemson University)

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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 to use 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 Bonnie Fernandez-Fenaroli (insert link to e-mail). Thank you.