2020 Center for Produce Safety Research Symposium
Session 5
July 21, 2020

CPS held the fifth and final installment of their five-part 2020 Research Symposium on July 21, 2020. The ongoing Coronavirus pandemic meant that this year's annual event needed to be conducted virtually over the course of five consecutive weeks

During Session 1 held on June 23, 2020, we explored the use of computer-based modeling to help address two burning issues for the produce industry: understanding potential *Listeria* growth and persistence in whole produce commodities and the development of sampling strategies to support the validity of assumptions surrounding microbial testing needs and design of acceptable protocols (Key Learnings Session I). In Session 2, we expanded our knowledge base on *Listeria monocytogenes* and its persistence and growth on specific commodities and fresh-cut products and examined novel methods to control *Listeria* growth on food contact surfaces (Key Learnings Session II). In Session 3, we explored projects that took wholistic, systems approaches to solving challenges with pest intrusion into leafy greens fields, pathogen transference on co-managed farms and the impact of traits associated with concepts of soil health on pathogen persistence. We also examined *Cyclospora* presence in the irrigation canal systems in the Yuma, AZ production region (Key Learnings Session III). Session 4 featured the use of genomics and metagenomics to address challenges in identifying new or revisited indicators and index testing-targets of human viral pathogens that may ultimately be used in the produce industry, the distribution and relatedness of *Listeria* species in the U.S., and the use of that information to better understand source-risk related to facilities and product, identification of competitors of *Listeria monocytogenes* that might control that organism in composts, and build our knowledge base of bacterial pathogen persistence and rates of genetic diversification in the Yuma and Salinas vegetable production regions (Key Learnings Session IV). Session 5 featured research describing the "die-off" rates of human pathogens in agricultural water from three locations around the world, the persistence of pathogens in shade-house production environments, pathogen persistence in wash water systems and the potential role of damaged cells to contaminate washed products, the efficacy of irrigation water sanitation and the potential role of sediments in canal systems as reservoirs of human pathogens. An executive summary and the key learnings from Session 5 are described below.

Executive Summary:

- **Pathogen die-off in baby spinach and lettuce occurs in two, segmented linear phases**. The initial phase involves rapid die-off and the rate is determined by relative humidity, produce type and bacteria strain. The second phase is more prolonged, and detection of remaining bacteria can often only be accomplished by enrichment techniques.
- **Set FDA pathogen die-off rates may not be reliable in every instance**. The set 0.5 $\log_{10}$ die-off rate per day for 4 days determined by FDA during FSMA regulation development was found to be generally acceptable for *E. coli*, which is seemingly less hardy than the *Salmonella* strains used in this study. However, there was a large variation in the

bacterial counts at each time point of the die-off across experimental plots and trials. Relative humidity can slow die-off, indicating that the produce industry should be aware that the current time-to-harvest intervention does not completely eliminate the associated food safety risks.

- **Pathogens can persist and grow in certain shadehouse production environments**. Attenuated *Salmonella* and avirulent *E. coli* strains inoculated on field-grown cucumber, jalapeño pepper and Roma tomato fruits die-off quickly but are more persistent and can grow in shadehouse production environments when humid conditions are encountered.
- **Persistence and growth can lead to transference**. As has been documented in field production previously, once pathogens are present in a shade house, they can be transferred to the produce in the course of crop maintenance and harvest. Every precaution should be taken to prevent entry of pathogens into the production environment.
- **Incomplete wash water disinfection can damage pathogens so that they cannot be cultured, but they are not dead**. Experimentally, wash water sanitizer and fresh-cut produce combinations that reflect industry practices have been identified where pathogen cells are killed, some are damaged, and some are still alive. These damaged or viable but not culturable cells (VBNC) may represent a previously unaccounted for source of cross contamination in wash systems. Research is ongoing to assess the risk.
- **Irrigation water disinfection to reduce pathogens is a risk management tool but does <u>not</u> eliminate the potential hazard.** Pathogens can be reduced by irrigation water sanitation, but variability in irrigation delivery system designs, flow rates, water quality, sanitizers employed, and target organisms means that bacterial breakthrough at the last point before the water exits the system can be observed. Research continues to expand trials, fine tune disinfection parameters, and determine impact of sanitizers on soils.
- **Irrigation canal sediments may be a reservoir for pathogens**. Irrigation canal sediments can harbor pathogens that can be resuspended in irrigation water by weather or other conditions that stir up the canal bottom, representing a contamination hazard for crops irrigated with that water. Ongoing research will provide growers with tools to identify “hotspots” where pathogens accumulate and models to guide water use.

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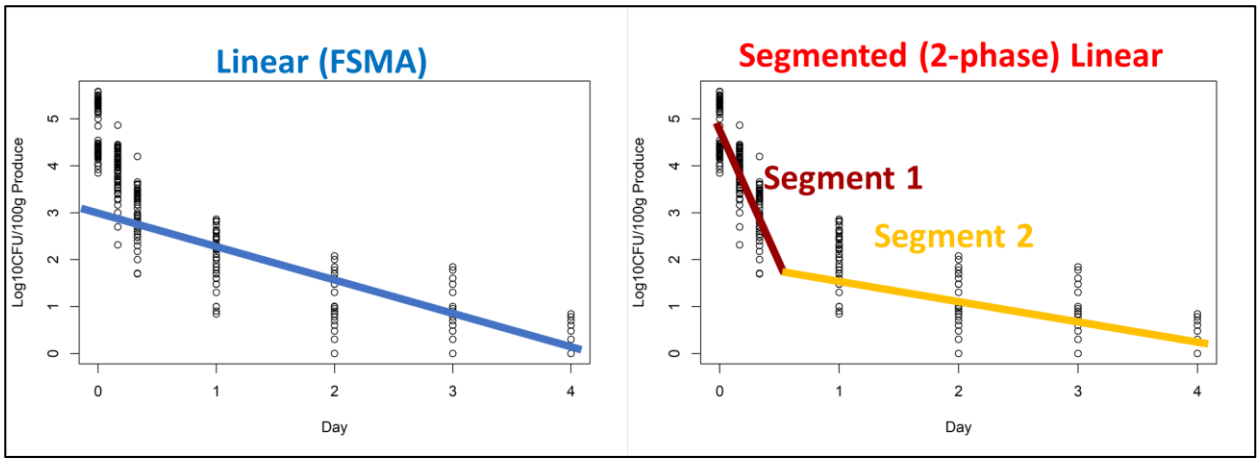
Key Learnings:

1. **Pathogen die-off rates on spinach and lettuce irrigated with contaminated water.** If irrigation water that does not meet current irrigation water microbial standards is applied to a crop, the Produce Safety Rule within the Food Safety Modernization Act stipulates growers can employ a wait period of up to 4 days maximum between application and harvest to allow for bacterial pathogen die-off. This 4-day wait period assumes a 0.5 $\log_{10}$ pathogen die-off/day rate. However, there is limited scientific evidence to support this die-off rate. ***Renata Ivanek*** from Cornell University presented her work, "*FSMA agricultural-water die-off compliance provisions benefit from condition-specific modifiers*" examining FSMA die-off rate assumptions and whether they are reasonable or need to be modified (Ivanek 2019). The research project was designed around two key objectives: (1) to estimate die-off rates of indicators and attenuated pathogens on baby spinach and baby lettuce in a replicated field trials in three different climatic regions: California, New York and Spain and (2) to develop a predictive model of pathogen die-off rates under relevant environmental conditions and industry practices and use the model to evaluate the Produce Rule/FSMA agricultural water matrix. Key learnings were:
    - ***Relative humidity, bacterial strain and produce type drives the rate in segment 1***. Over 5,000 baby spinach and lettuce samples were evaluated in the field trials and the die-off rate in the first segment is variable and appears to be associated with relative humidity, produce type, and bacteria. If the relative humidity is high (>50%) then you can expect a slow die-off. The die-off rate of *Salmonella* is always slower than *E. coli.*



"Die-off is best described as a two-phase process". Figure courtesy of Renata Ivanek, Cornell University

    - ***Rapid dew point changes signal two-phase or segmented die-off***. When the dew point temperature changes rapidly, a segmented die-off rate can be expected. The dew point is the temperature the air needs to be cooled to (at constant pressure) in order to achieve a relative humidity of 100%; at this point the air cannot hold more water in the gas form.

- ***Die-off is not the same as elimination***. After the breakpoint when the die-off rate slows, the die-off rate is less variable; however, there is still a large variation in the counts at each time point in this segment across experimental plots and trials. This indicates the produce industry should be aware that a time-to-harvest intervention does not completely eliminate the associated food safety risks.
- ***The FSMA die-off rate may not always be reliable***. The experimental die-off for the plots investigated in the current study were compliant with the FSMA die-off rate the majority of time, and relative humidity was again identified as a predictor of whether die-off would align with the FSMA-assumed die-off rate. If the relative humidity was low and the organism in question was *E. coli*, alignment with the grace period offered by the FSMA produce rule was likely (i.e. die-off rate was faster and met the ≥2 log reduction over 4 days or 0.5 $\log_{10}$ per day). However, the use of the wait time was not always effective as an intervention strategy if high relative humidity was encountered or the hardier *Salmonella* was tested.
- ***Models can be utilized to predict pathogen die-off rates***. A predictive model based on the data collected in this project was developed. This model accounts for weather conditions (relative humidity and dew point), bacteria strains, and produce type to describe pathogen die-off. Two previous and independent experimental studies from the Salinas Valley were used to validate the predictive model, and the die-off rates aligned well. Predictive models allow consideration of weather in prediction of when the use of a wait period is likely to be effective at reducing the risks associated with harvesting produce that has been previously irrigated with water that exceeded the current Produce Rule testing standards for generic *E. coli*.

Why are these results important to the produce industry? These results are important to growers because they provide data that can help them make informed decisions on the use of irrigation water sources that do not meet current FSMA microbial water standards. When writing the regulations, FDA recognized that growers might face situations where their irrigation water source might temporarily exceed generic *E. coli* standards but that failure to irrigate might result in crop loss just prior to harvest. Knowing that generic *E. coli* is a poor indicator of pathogen presence in open water sources, that research had shown that pathogens die-off in production environments, and wanting to give growers some alternatives, FDA wrote the rules to permit growers to assume a 0.5 $\log_{10}$ die-off rate per day for a maximum of 4 days prior to harvest. In other words, if a grower uses a non-compliant irrigation water source, they can assume that if they wait 4 days from application till harvest there will be a 2 $\log_{10}$ reduction in pathogen level and they should be able to harvest within prescribed microbial water standards. The fixed die-off rate was essentially a stake in the sand; a way to move forward and really an invitation to the research and produce communities to validate the fixed rate or show how it could be improved.

The Ivanek project shows that pathogen die-off rates are variable within and between different locations. The project also demonstrates that weather conditions, target organisms and commodities impact die-off rates. The bottom line for growers is that die-off rates for pathogens that might be transmitted from irrigation water are not an absolute. In

other words, the variability brought about by weather conditions (relative humidity and dew point changes) means that "die-off" cannot be equated with elimination of the potential risk. Die-off rates are only a tool that growers can use to help them understand potential risks from irrigation water sources and that knowing their water source, having microbial testing data that characterizes each source over growing seasons, monitoring the delivery system, maintaining the irrigation equipment properly and monitoring potential hazards that could impact the water source are equally important to insure best management of irrigation water risks.

Why are these results important to the research community? This project is another call to action for the research community. The Ivanek group has developed a predictive model that growers can use to help them make decisions on when to harvest after the application of the final irrigation to minimize risks of contamination. But as we all know, models are only as good as the data used to build them. During the question and answer period following the presentation in Session 5, the need for additional experimentation in other growing regions with different weather patterns, types of irrigation water, different soil compositions and commodities being studied was discussed. The protocols used in this study are available for those interested in extending this work and contributing data to further model development. The only thing that is certain about irrigation water use is that "one size fits all" approaches are unlikely to be sufficient, so collection of broadly sourced data is crucial to creating a robust model that growers can use as a tool to help manage potential contamination risks from irrigation water.

Why are these results important to regulators? Irrigation water quality has certainly become a major focus area for FDA since the 4-day, 0.5 $\log_{10}$ die-off per day safe harbor was put forward. Outbreaks thought to be the result of the use of contaminated irrigation water have captured headlines over the last 3-4 years and FDA has embarked on internal research programs and partnered with academia to help our understanding of this risk. FDA participation at CPS during the symposium and in other forums provides a view to the research going on around pathogen die-off rates. The development of predictive models informed by key variables that significantly affect pathogen die-off rates may warrant reconsideration of the current regulations or at least create an opportunity to educate regulatory auditors on the types of tools growers are using to guide irrigation decisions as these models are fine-tuned and become more widely used.

2. **Pathogen persistence in shadehouse production.** Earlier in the 2020 CPS Research Symposium we discussed persistence of *Listeria monocytogenes* on fruits and vegetables and in packing environments (Key Learnings Session I) and persistence in soils in Yuma and Salinas Valley field production (Key Learnings Session IV). Taking a different approach, ***Trevor Suslow*** of the University of California, Davis, and now with the Produce Marketing Association, presented his project, "*Scientifically valid corrective actions for multiple harvest shade-house production systems*" where pathogen persistence was explored in shadehouse production (Suslow 2019). From 1996 to 2018 there have been eight illness outbreaks associated with cucumber consumption. Seven of these outbreaks were attributed to

Salmonella and one to *E. coli* O157:H7, with most originating from protected culture systems, like shadehouses. But there has been relatively little research done in shade houses to provide insights into human pathogen persistence and spread in shadehouse production environments. The Suslow project was organized into three objectives: (1) determine the die-off of *E. coli* and *Salmonella* on cucumbers, Roma-style tomatoes and jalapeño peppers grown in shade houses and in open fields, (2) determine if purposely contaminated products can facilitate transfer of attenuated *Salmonella* and avirulent *E. coli* during harvest activities, and (3) evaluate the potential for corrective actions to minimize survival of bacterial pathogens within a standing crop to allow continued harvest. Key learnings from this project include:

- ***Attenuated Salmonella and avirulent E. coli strains used in this study die off quickly in the field but persist at very low levels***. Attenuated *Salmonella* or avirulent *E. coli* recovery from crops growing in open-field plots showed a rapid decline within the first day after inoculation; however surviving bacteria can establish and persist at very low cell numbers that require enrichment for their detection. Bacterial survival on fruits is variable and heterogeneous on produce, sometimes even dependent on whether sunlight contacts it.
- ***Attenuated Salmonella and avirulent E. coli strains used in this study can survive and even grow under certain conditions in a shadehouse.*** *E. coli* and *Salmonella* inoculated onto cucumbers, tomato and jalapeño plants in hoop houses cannot only survive but grow on the crops, under the right temperatures and high humidity where condensation may occur in the enclosed environment.
- ***If pathogens are present in a shadehouse, they can be transferred by common production activities***. Transfer of *E. coli* and *Salmonella* from utensils (bins and clippers) and human hands (gloves) to the fruit was observed frequently on inoculation day; however, in experiments conducted within hoop houses the transference was observed until 8 days post inoculation. While the risk of contamination in commercial shadehouses will likely be reduced as compared to this study, these findings show the potential for pathogens to survive/transfer in protected environments.
- ***Foliar preharvest treatments with sanitizers can diminish pathogen presence***. Three different preharvest sanitizer treatments were evaluated as part of the effort to identify potential corrective actions to minimize contaminant persistence and transference in the preharvest environment on tomato and jalapeño plants. Foliar treatment with 1% OxiDate® 5.0 for 24-h contact time resulted in significantly lower numbers of viable bacteria on the treated plants.
- ***Postharvest handling can enhance Salmonella survival and growth***. *Salmonella* contamination surviving to postharvest phases can be increased by waxing cucumbers and holding in modified atmosphere packaging bags. Wash-line injuries (brushed cucumbers) were shown to have the potential to enhance *Salmonella* survival and growth on non-waxed and non-MAP bagged cucumbers.

Why are these results important to the produce industry? The results of this project clearly

show that, under the right conditions (temperature and humidity), shadehouses can not only support pathogen survival but also promote growth. What that should tell shadehouse or protected agriculture growers is that it is important to assess the hazards that may exist around your facilities and aggressively manage the contamination risks to prevent pathogen introduction into your production environment. That approach is really no different than what an open-field grower needs to do.

It is important to note that humidity plays an important role in pathogen survival and growth in this study (and others focused on pathogen persistence in multiple environments). High humidity should serve as an alert to shadehouse or hoop house growers that extra care might be needed to control pathogen introduction or persistence. For example, increased diligence around handwashing and proper use of gloves, more frequent cleaning and sanitation of equipment, use of foot-dip stations to prevent external soil or debris from being brought into the house, or even product testing.

One point that came up in the discussion after the presentation is that if a grower should choose product testing, using larger sample sizes can provide greater confidence that negative samples are truly negative. Each production system and facility is different, and growers need to be aware of those differences and assess hazards and risks for their operation and develop effective preventive controls to best protect the products. Vigilance, whether growing in shadehouses or open fields, is critically important.

The use of OxiDate® or other sanitizers to reduce pathogen contamination if it is present shows promise in concept, and additional work to determine optimum application rates and contact times would seem like the next step. However, reduction of contamination especially near harvest is not elimination, and in speaking with growers, the research team found that many would choose to remove the crop entirely and not harvest.

Why are these results important to the research community? The role of humidity in pathogen persistence and growth, the potential for pathogen transference in the production environment, and the need to protect against the initial contamination event as opposed to trying to control it after it happens are common themes that emerge in this project as well as several other research efforts that have emerged in the last few years. The value of this project for the research community is the focus on a specific production model and the path the research takes from crop growth through to harvest and postharvest. Though the project had to overcome obstacles that nearly all real-world programs do in the course of conducting experiments in an uncontrolled environment, the research team was able to deliver actionable information to a growing segment of the produce industry. As we have observed throughout the 2020 virtual symposium, collaboration with industry partners can lead to insights that impact experimental design or help researchers put their results into context. An example of this was the portion of the project that looked at remediation. While a level of remediation was possible and perhaps could be fine-tuned to an even better level, the growers' bottom line is that it is not elimination of the risk and therefore not a likely course of action for them.

Why are these results important to regulators? The presentation of this project started with a rationale for the project that reminded us all that there have been outbreaks of illness associated with shadehouse production of fruits and vegetables. This project sheds light on conditions that can support pathogen persistence and growth in shadehouse production. This information increases our knowledge base in this sector of produce and helps inform FDA so that routine inspections can be adjusted to the uniqueness of shadehouse production. Similarly, inspections for cause can be more informed and directed.

3. **Interim reports on pathogen persistence offer exciting prospects.** Session 5 offered a sneak preview of research projects that have completed one year of experimentation. These interim reports share the theme of pathogen persistence but in different parts of the production landscape.

   The first interim report was by ***Ana Allende*** from CEBAS-CSIC (Spain) on her project, "*Significance of sanitizers on induction viable but non-cultivable (VBNC) foodborne bacteria and their survival and resuscitation in fresh produce*". The objectives of this project are to: (1) determine the anti-microbial effectiveness of commercial sanitizers and explore if their effectiveness is overestimated by conventional plate count methods, (2) examine process wash water to determine if the presence of damaged bacteria (viable but not culturable, or VBNC) increase the food safety risk of fresh-cut products, (3) understand if damaged bacteria survive and recover to the culturable state on the product during storage, and (4) provide guidance on whether stressed bacteria should be considered when establishing operational limits for commercial produce wash operations. The project focuses on lettuce, cabbage, onion, and spinach wash systems testing sodium hypochlorite, PAA and chlorine dioxide. Some preliminary key learnings include:

   - ***Bacteria cells have three fates when subjected to sanitizers***. When produce harboring bacteria is washed in a commercial system, the bacteria that are released into the water have three fates: 1) they die and no longer represent a safety risk; 2) they become damaged but do not die, termed viable but not culturable or VBNC, and their relevance to produce safety is currently unknown; or 3) they emerge unscathed and remain a contamination risk.
   - ***Sodium hypochlorite killed all Lm and E. coli cells in this system***. At 20 ppm sodium hypochlorite, VBNC and live or culturable *L. monocytogenes* (Lm) and *E. coli* O157:H7 cells in produce wash water were not found, independent of the commodity being washed. This result indicates that 20 ppm of sodium hypochlorite is effective inactivating Lm and *E. coli* O157:H7 cells in wash water.
   - ***PAA was less effective than sodium hypochlorite in these studies and VBNC cells were recovered***. When wash water for cabbage, lettuce, spinach, and onion were treated with 80 ppm of PAA, VBNC and culturable cells of Lm and *E. coli* O157:H7 were detected in the wash water. These results demonstrate that PAA significantly decreased the levels of culturable Lm and *E. coli* O157:H7 in inoculated produce wash water, but the PAA treatment also induced the VBNC state. In this system,

PAA was not as effective as sodium hypochlorite at wash water disinfection in killing pathogenic bacteria cells.

- ***Chlorine dioxide treatments generated VBNC cells***. Treatment with chlorine dioxide at 2 ppm showed that Lm was less tolerant compared to *E. coli* O157:H7. Also, significant differences were observed between the different types of produce in the wash water systems. In shredded lettuce wash water inoculated with Lm, 2 ppm chlorine dioxide killed Lm but also induced the VBNC state. Chlorine dioxide was much more effective at killing Lm cells in onion wash water, but that may be because onions contain antimicrobials that could be released in the water to kill Lm.
- ***Resuscitation of VBNC cells has been accomplished under optimal conditions***. Resuscitation of VBNC cells present in the wash waters from shredded lettuce and diced onions was possible under optimal conditions after treatment with chlorine dioxide. These results confirmed that injured cells present in produce wash water could be resuscitated when subjected to optimal conditions, however more research is needed as this project continues to determine the resuscitation capacity of VBNC cells under commercial conditions.

While the Allende project is examining pathogen persistence in produce wash water, ***Channah Rock*** from the University of Arizona presented initial findings in her work on irrigation water, titled "*Agriculture water treatment – Southwest region*". The objectives of this work are: (1) define optimal dose for calcium hypochlorite and PAA sanitizer chemistries since physical and chemical parameters of the canal irrigation water could shift dose determinations performed in the lab, (2) examine in-field validation of sanitation systems, (3) evaluate the frequency and timing of system monitoring, and (4) determine the impact of irrigation water sanitation on soil microbiomes in crop lands. Some initial learnings are:

- ***Movement of the sanitizer through the delivery system is variable***. Initial data indicate that residual sanitizer is detected fairly quickly at the first sprinkler head after system stabilization (full pressure). However, these systems are dynamic and for all sanitizers evaluated, time from system startup to residual disinfectant detection at the last sprinkler head is highly variable and can range from 25 to 35 minutes after system stabilization.

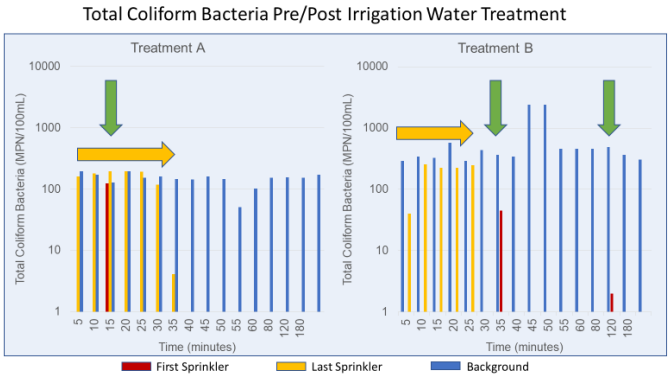


Diagram demonstrating system variability in achieving stabilization. Courtesy of Channah Rock, University of Arizona.

- ***Sanitizers can achieve a 2-log bacterial reduction***. All sanitizers evaluated were able to easily achieve 2-log reduction in naturally occurring total coliform bacteria at the first sprinkler head, with similar results at the last sprinkler head once sanitizer residual was detected.
- ***Sanitizers did <u>not</u> kill bacteria instantaneously***. The rate of inactivation depends on the pathogens or indicator targets, sanitizer concentration, contact time with the sanitizer, temperature of the water, and pH of the water.
- ***For all disinfectants tested to date, there was bacterial breakthrough once systems had been stabilized***. Breakthrough of microbial targets was detected for all sanitizers evaluated at the first and last sprinkler heads during treatment over time.

Certainly, irrigation water has been a priority research target in the Yuma growing region and the final report from the Rock program will be highly anticipated at the 2021 CPS Research Symposium. While the Rock program aims at canal water disinfection, ***Charles Gerba*** from the University of Arizona is focused on the sediments in irrigation canals and their role as reservoirs for human pathogens. An interim report on his project, "*Development of a model to predict the impact of sediments on microbial irrigation water quality*" was presented by his colleague ***Kelly Bright***, also at the University of Arizona. This project is built on the hypothesis that canal sediments can act as reservoirs for pathogens. Indeed, previous results suggest that 10 to 10,000 times greater concentrations of *E. coli* can be found in canal sediments than in the overlaying water. If weather or other conditions result in resuspension of sediments, the microbial water quality of the irrigation water could become compromised and present a potential safety risk to the crops that are subsequently irrigated with the water. Therefore, the project objectives are: (1) to identify factors (e.g., rainfall events, wind) that would result in the resuspension of sediment-bound bacteria (*E. coli* or Lm) or viruses (MS2 and phiX174 viruses, surrogates for human enteric viruses) in irrigation canals, and (2) quantify the impact of resuspension of different levels of these bacteria and viruses on the quality of the overlaying water, and (3) suggest guidelines for growers to minimize the occurrence of pathogenic bacteria and viruses in the irrigation water. Interim key learnings are:

- A model was developed for estimating virus impact on irrigation water. The degree of viral resuspension depends on virus and sediment type. Rainfall events will create opportunities for resuspension.
- "Hotspots" with high concentrations of *E. coli* and *Listeria* exist in artificial canal systems. It is unclear why or how this happens, but the team is starting to work on this and doing field sampling of sediments.
- Flume tank sediment experiments were begun to study the effects of flow rates, water velocity, and sediment composition on the resuspension of *E. coli*.
- Additional sampling of irrigation canals was conducted for the detection of *E. coli* and *Listeria* species. A total of 50 samples were collected during the current growing season. As expected, *E. coli* concentrations are 10 to 100-fold greater in the sediment than in the overlaying water.

Why are the interim results of these projects important to the produce industry? It is important to note that the full data from these projects are not yet available for consideration. However, it is fair to reflect on some of the interim findings and the preliminary conclusions the research teams have reached.

The early results from the Rock project on irrigation water disinfection make a very clear statement: *Irrigation water treatment is a pathogen risk reduction activity and not a pathogen elimination activity*. Canal water can be variable in terms of microbial and chemical quality, and delivery systems can also vary in length the water travels and pressurization, so it is important for growers to know their irrigation systems. The project also indicates that different disinfectants have different efficacies, and growers need to understand how different disinfectants work in their operations. An understanding of your baseline microbial water quality, recent weather events, the design of the system and the procedures your farm personnel use, how they operate and troubleshoot the systems and their training, are critical in creating confidence that your disinfection system is indeed reducing contamination risks. It is also important to conduct microbial testing to routinely verify system performance.

Interestingly, the Allende project on wash water disinfection and the potential for VBNC to impact the safety of washed produce can be seen in a similar vein as the Rock program on irrigation water. Once again, we can view wash water disinfection as a risk management undertaking but perhaps not a hazard elimination activity in some wash systems. The generation of VBNC and the differential kill of Lm and *E. coli* O157:H7 depending on sanitizer choice and commodity being washed are not necessarily surprising based on existing research, but this project is a reminder to packinghouse and fresh-cut processors that wash system validation and verification are critically important in making sure cross contamination risks are controlled properly. Much is still to be learned about the fate of VBNC and whether they can attach to fresh-cut products in the wash system and their ability to cause disease if they do.

Lastly, the Gerba program reminds growers once again that knowing their irrigation water delivery system, in this case canals, is important in controlling microbial water quality. The canal type (lined or unlined), types of sediments accumulated in the canal, flow rates, and vulnerability to weather conditions and run-off from the surrounding landscape are important factors to consider when assessing risk potential. The reaffirmation that the sediments in Yuma area canals have significantly higher levels of *E. coli* than the overflowing water strongly points to the sediment as being a potential reservoir for pathogens that can resuspend and present a direct or indirect contamination risk for crops. This speaks to working closely with irrigation districts to understand canal maintenance and upkeep and also managing contamination risks by making informed choices on when water is delivered and understanding the impact of weather on water quality, how the water is delivered to the field and avoiding flooding, ensuring water disinfection systems operate properly and executing equipment cleaning and sanitation programs optimally.

Why are the interim results of these projects important to the research community? These projects all have impressive lists of industry collaborators. Throughout the 2020 CPS

Symposium we have witnessed the value of this attribute. The access to on-farm resources or commercial equipment is important to the work and its acceptance and integration into best practices.

These projects (and many of the projects highlighted by the 2020 Symposium) also bring into focus the conundrum that producers face and the research community can help address. Whether growers and processors are dealing with irrigation or wash water, we are starting to see that remediation steps once pathogen contamination has occurred are currently less than totally effective. That puts growers and others in the supply chain in the multi-hurdle approach of pathogen control and trying to prevent contamination from happening from seed to consumer.

Just within the narrow scope of two of these projects we see that irrigation water disinfection is risk control but not an elimination activity, and yet the canal sediments are a likely reservoir for pathogens that can be resuspended and spread to the soil and/or the crop. We learn that under some conditions VBNC can be generated in subsequent washing operations thereby further complicating the situation. So while our knowledge base is expanding and it is exciting to gain these insights, much is yet to be learned about VBNC cells and pathogen physiology, persistence and virulence, improving remediation protocols and finding next generation sanitizer chemistries or treatments, and the development and use of models that can be leveraged against wash and irrigation water systems to assist processors and growers in making decisions that impact the safety of their products.

Why are the interim results of these projects important to regulators? Since these are interim reports, the key value for the regulatory community is awareness. FDA and state regulatory participation at CPS from the Technical Committee to the Board is vital in creating communications between the industry and the regulatory community about key produce safety research needs and the learnings from the subsequent research projects. Irrigation water in general and specifically from open water sources is a priority for the produce industry and for the regulatory community, and the promise of these new projects for expanding our joint knowledge is important to share. Similarly, as wash water research continues to advance, it is desirable to share research results with regulators and witness industry reaction to the science and thoughts on how the emerging data can be leveraged to improve practices.

Acknowledgements: *The Center for Produce Safety would like to thank the researchers who made presentations during Session 5 of the 2020 Research Symposium. We would also like to thank all of the researchers and session moderators who made all five sessions a success. The presentation of research results and their discussion of what that research might mean to the produce industry certainly informs the content of this paper. More detail on these research projects can be found at www.centerforproducesafety.org. This discussion of key learnings contained here 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 Bonnie@centerforproducesafety.org). Thank you.