CPS Symposium Executive Summary – Session 2

June 22, 2021

The second session of the 12th Annual Center for Produce Safety (CPS) Research Symposium was held on June 22, 2021. The session started with an industry panel discussion on "*How to put CPS research to work in the real world*". A recording of this important exchange can be found on the CPS website. The CPS website also contains reports from 187 research programs funded over the last 14 years that you can draw from to improve your produce safety programs. You can find these programs here. Session 2 continued with three featured research presentations from leading researchers covering the science of product sampling, the potential for induction of viable but not culturable pathogen cells (VNBC) in commercial produce washing systems and the public health risk these quiescent organisms may or may not represent and innovative approaches to the prevention of biofilm formation and a strategy for improving equipment sanitation efficacy. An executive summary of the critical learnings from CPS session 2 presentations follows.

1. **It is important to understand what preharvest product sampling can and cannot accomplish.** Matthew Stasiewicz from the University of Illinois presented the results of his project, "*Simulation analysis of in-field produce sampling for a risk-based sampling plan"* [Stasiewicz 2018]. The research team developed an interactive computer simulation model [Stasiewicz model] based on field experimentation data to test field sampling various and then validated the model with specific in-field studies. The preponderance of raw product testing has been with leafy greens. Concerns have been raised about the significance of testing a relatively small number of leaf grab samples against the enormity of an entire lot. Indeed, despite several years of raw product testing and the implementation of several patterns of sample collection by growers, academic researchers and regulators, >99-percent of the samples are negative for pathogenic *E. coli* and *Salmonella* yet outbreaks still occur. Some critical learnings from this study are:

    - ***It is easier to detect large, systematic failures than point-source contamination events***. If the extent of a contamination is systemic like might happen in a flooding event or if contaminated irrigation water is applied aerially over edible portions of a crop just prior to harvest; then there is a greater chance that current product testing practices would find the contamination compared to trying to find point source contamination events like low levels of bird droppings or minor animal intrusions into a field. With systematic contamination the mass of the event or the amount of product contaminated drives its detection.
    - ***Increasing the sample size increases the probability of pathogen detection***. Detection increases from zero to 70-percent as sample size increases and/or the number of contamination points increases, i.e., the contamination crosses into a systemic failure.
    - ***Current industry sampling patterns are not any better than randomized sampling***. Current industry sampling practices using N=60 Z-patterns or K-step sampling introduce more variability than completely randomized sampling plans seeking to detect low level and sporadic contamination events. Based on this study end users should consider reviewing their sampling protocols.
    - ***When raw product sampling is employed as a verification tool it is important to understand its limits.*** If an operation uses raw product sampling as a verification tool, it is important to understand if the hazards can reasonably be detected by the sampling plan. The potential level of contamination the hazard represents, characteristics of the growing environment, commodity characteristics, prevalence of potential pathogen vectors, and weather patterns that might result in systematic or widespread contamination of the crop may lead the operator to believe

contamination, if it occurred may or may not be detected. However, while current raw product sampling may support broad surveillance efforts to detect widespread contamination events, they are not reliable for lot acceptance applications as negative results do not necessarily connote the absence of the pathogen in the field since low level and sporadic point source contamination events are not likely to be detected.

2. **Control of wash water sanitation is critical**. Anna Allende of the Spanish Research Council (CEBAS-CSIS) shared the results of her research project titled; *"Significance of sanitizers on induction of viable but non-culturable foodborne bacteria and their survival and resuscitation in fresh produce"* [Allende 2018]. Wash water disinfectants like sodium hypochlorite (free chlorine), chlorine dioxide or PAA are used to kill cells dislodged by the wash to prevent them from reattaching to uncontaminated products as they move through the system and creating a systemic contamination event. However, there has been a concern that cells exposed to a non-lethal dose of sanitizer, may survive the stress caused by the sanitizer by slowing their metabolic activities; essentially going to sleep, only to be revived during distribution and thereby presenting a risk to those who consume the product. This state of "suspended animation" is called viable but not culturable or VNBC. Dr. Allende's work explored the potential for induction of the VNBC state when pathogens are exposed to non-lethal sanitizer doses in wash systems, subsequent attachment to fresh produce and the potential for resuscitation of VNBC in storage. Important things to consider are:

    - ***E. coli O157:H7 and Listeria monocytogenes VBNC cells can be induced by exposure to sub-lethal doses of sanitizers***. 15-20 ppm free chlorine was shown to kill a cocktail of *E. coli* O157:H7 effectively and did not generate VNBC while a 2-minute exposure to 12 ppm free chlorine induced VNBC *L. monocytogenes* cells. In these experiments and under the conditions employed, chlorine dioxide and PAA did not totally inactivate *E. coli* O157:H7 or *L. monocytogenes* resulting in VNBC indicating more research is needed to identify dose levels to better control these pathogens.
    - ***VNBC are able to attach to fresh produce (romaine lettuce, spinach, cabbage and onions) during the wash process***. Under the limited and well controlled commercial distribution and storage conditions described in these studies (7°C or 45°F for 15 days), ***VNBC can survive and be resuscitated to culturable cells***. Significantly, resuscitation of VNBC is very low indicating there is a low probability that VNBC cells pose a significant public health risk. However, much more needs to be learned as distribution and storage conditions can be highly variable (temperatures, moisture, nutrients, etc.) and potentially support VNBC resuscitation more vigorously.
    - ***It is Important to set minimum effective sanitizer concentrations and contact times for each wash system***. To eliminate the risk of VNBC cell induction, transfer and resuscitation during washing, storage and distribution of fresh produce, it is important to maximize sanitizer efficacy by closely controlling wash water chemistry (pH, COD, solids, etc.), sanitizer contact time and employ continuous monitoring of sanitizer concentration.

3. Boce Zhang from the University of Massachusetts reviewed the outcomes of his CPS-funded project "*Non-fouling food contact surfaces; prevention of biofilm and surface mediated cross contamination*" [Zhang 2018]. Surface coatings have been used on industrial equipment in many manufacturing industries to protect the equipment against corrosion. This research examined surface chemistries or coatings and topographical alterations that might mitigate against *L. monocytogenes* and biofilm deposition which makes cleaning and sanitation more difficult. These important learnings were discussed:

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- ***Dursan® can be used to treat equipment surfaces to improve cleaning and sanitation efficiency***. Dursan® is a proprietary, patented, NSF certified, and FDA compliant chemistry that can improve the durability, anti-fouling and corrosion resistance of equipment and instruments. Dursan® showed the best performance against *L. monocytogenes* biofilm deposition. Validation studies conducted in production facilities confirmed Dursan® improved sanitation efficiency.
- ***Equipment surfaces can be complex biological environments***. Other microorganisms in addition to *L. monocytogenes* can significantly impact biofilm formation and equipment sanitation efficiency.
- ***Coatings can be used selectively to mitigate Listeria***. Coatings like Dursan® may be used strategically on hard-to-access parts or surfaces that are difficult to clean and sanitize daily. This approach would help control the cost of using coatings. Dursan® may also be a robust solution for treating legacy equipment designed and constructed prior to our current knowledge of *Listeria* and biofilm significance to produce safety. However, in the end, thorough cleaning and sanitation are critically important as even the use of anti-fouling surface coatings cannot eliminate resident *Listeria* risks completely.

Acknowledgements*: The Center for Produce Safety would like to thank session 2 presenters and moderators for their work and dedication to produce safety. More detail on the research projects can be found at www.centerforproducesafety.org. This Executive Summary 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. If you have additional questions, please feel free to contact Bonnie Fernandez-Fenaroli (info@centerforproducesafety.org). Thank you.*