2015 CPS Symposium Summary:
Key Learnings and What They Mean for You
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Overview: The sixth annual Center for Produce Safety (CPS) Research Symposium was held in Atlanta, GA in June 2015. The Symposium featured food safety research programs funded by CPS over the last few years along with discussions by industry, academic and regulatory experts regarding the implications of the research for everyday produce growing, packing and processing operations and how that research might be used to improve company food safety programs. This summary is designed to capture "key learnings" from the 2015 CPS Symposium and offer our thoughts on why these results should matter as you examine your own food safety programs; especially in light of new FSMA regulations and our industry's ongoing efforts to improve food safety performance.

Among the key findings from the 2015 CPS Research Symposium:
- Human pathogens can persist in fruit and vegetable production environments for extended periods of time. There are multiple variables that impact human pathogen survival in our production environments and these need to be considered when developing food safety plans.
- Mobile apps are currently in development to assist growers in making food safety-related decisions.
- Understanding of transference of human pathogens from various types of animals to crops has advanced significantly over the last several years. There really are no "risky" animals, just environments that bring animals into contact with human pathogens that might then be transferred to fruits or vegetables in specific instances.
- Our understanding of the organization and expression of human pathogen genomes is rapidly advancing the development of detection tools, revolutionizing public health investigations and shedding light on new strategies for future human pathogen control.
- Hazard analysis is still the most important tool in supply chain food safety program development.
- Validation of preventive controls and verification of practices is critical.
- Research is providing a better understanding of how sanitizers work biologically and this could lead to next generation disinfectants for use in produce production.

A discussion of these simple findings follows. A more detailed version of these reports can be found at pma.com.

1. ***Norovirus can survive on tomato surfaces and in water.*** The Centers for Disease Control (CDC) estimates that 40-50% of foodborne illnesses associated with produce are due to product contamination with norovirus? Melissa Jones (University of Florida) investigated norovirus survival and stability in simulated produce production environments and found that high temperatures and UV light effectively reduced norovirus levels in water but phosphate and ammonium (common constituents of

   Photo credit Dr. Melissa Jones, University of Florida

   fertilizers) increased the stability of low concentrations of norovirus in water. Additionally, preliminary experiments seem to suggest a synergistic affect between bacteria and norovirus, i.e. bacterial presence appear to enhance the stability of norovirus, most likely by attachment to

bacteria. Proper handwashing, vigilant worker management to make sure ill workers do not handle food and effective and verifiable sanitation programs for food contact surfaces can be effective preventive controls to manage the risks of norovirus cross contamination.

2. ***Soil type can impact survivability of Salmonella and STEC's***. The ability of human pathogens to survive in soils and perhaps cross contaminate subsequent plantings of fruits and vegetables has

Photo credit Dr. Keith Warriner, University of Guelph

been a point of concern for several years. Research reported by Keith Warriner (University of Guelph) suggests that the predictability of human pathogen die-off in soils is affected by soil type, manure type, cultural practices and climatic conditions. In field studies, Dr. Warriner's group demonstrated human pathogen survival and persistence was positively influenced by the depth that human pathogens are incorporated into soils and higher organic content. Additionally, loam soils supported human pathogen survival and persistence more than sandy soils and pathogens in soils did better in cooler, damp or winter/spring conditions than in hot, dry environments. Also, Robyn McConchie (University of

Sydney) presented laboratory experiments that demonstrated enhanced *Salmonella* survival in clay loam soils versus sandy soils. Growers need to be aware that soil type, environmental conditions, cultural practices and soil amendments can have dramatic impacts on pathogen survival and persistence in their soils and these risk factors must be considered when determining crop rotations and cycles.

3. ***Solarization may be a tool to reduce pathogens in soils.*** Human pathogens have been demonstrated to persist in the soil for prolonged periods of time.
Robyn McConchie (University of Sydney), in collaboration with Trevor Suslow (University of

California Davis), evaluated the use of cover crops and solarization to reduce human pathogen levels in contaminated fields. Solarization was shown to have positive effect in promoting human pathogen die-off while cover crops showed little effect.
In solarization field trials where the fields were inoculated at 10,000 (4 logs) CFU/g, *Salmonella* was not detected after 36 days in the US field trials even after samples were enriched. *Salmonella* was detected in every control sample. In the Australian field trials, the pathogen was not detected after 49

Photo credit Dr. Robyn McConchie, University

days. While solarization has been routinely employed in agriculture to eliminate plant pathogens and pests, these initial studies by McConchie and Suslow show the potential to use this tool to reduce or eliminate human pathogens in fields as well.
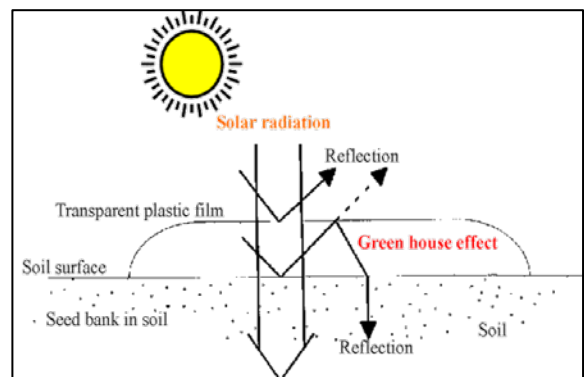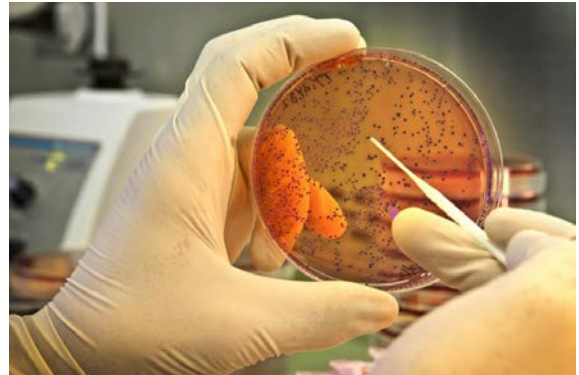
4. ***Rainfall can be a predictor of cross contamination risk***. In studies undertaken in the coastal plains of Georgia by George Vellidis (University of Georgia), rainstorm events increased the likelihood

of *Salmonella* in irrigation ponds. Before rainfall events, 33% of pond water samples tested positive and after rain events, 58% of samples tested positive for *Salmonella*. *Salmonella* concentrations were, on average, elevated by 10 fold (1.05 $\pm$ 0.38 log) MPN/100 mL after rain. Similarly, in studies conducted in New York fields, Martin Wiedmann (Cornell University) reported increased detection of *Listeria monocytogenes* (*Lm*) in soils within 24 hours of a rain event. In the first 24-hours after a rain event, they measured a 25-fold increase in positive *Lm* samples. The significance of these findings for growers is one of awareness. Rainfall is certainly an uncontrollable factor for growers and the fact that rainfall events can increase detections of pathogens in surface waters used for irrigation and in soils may create a feeling of helplessness. However, being aware of the increased likelihood of human pathogens being presence can also help growers make more informed decisions to manage risks, e.g. based on weather forecasts and the flexibility a grower might have with product maturity and markets, a grower might choose to harvest a day earlier or a few days later than planned to minimize the risk of cross contamination from soil or water that might contact the crop. Similarly, a grower might choose to hold back on a scheduled irrigation following a rain event to permit the surface water source to settle and thus reduce the likelihood of produce contamination from contaminated irrigation water.

5. ***Seasonal variations can affect pathogen persistence***. A recurrent theme in the research presented at the CPS Symposium was the effect of seasonality on human pathogen persistence in the environment. In the Arizona desert growing regions, Michele Jay-Russell (University of California Davis) demonstrated seasonal patterns for peak pathogen prevalence in birds and cattle. More birds tested positive for non-O157 STEC and *Salmonella* in the spring while more cattle tested positive in the fall. In addition, Channah Rock (University of Arizona) detected *Salmonella* in Arizona irrigation canal water samples only during the summer months and pathogen presence was correlated with water temperature. In Georgia's coastal plains, George Vellidis (University of Georgia) described a higher proportion of irrigation pond water samples tested positive for *Salmonella* during the late summer. In laboratory studies by Martin Wiedmann (Cornell University) simulating New York environmental conditions during the months of March and June, *Salmonella* and EHEC survived better on lettuce under March conditions with relative humidity (RH) of 45% than on lettuce under June conditions with 75% RH. Keith Warriner, (University of Guelph), found *Salmonella* persisted longer in soil after winter and spring manure applications than after summer applications. Once again, the value of these data is awareness; seasonal human pathogen variations may be instructive to producers and guide some decisions about crop production (e.g. employing drip irrigation versus overhead sprinklers when irrigation sources demonstrate elevated pathogen levels, applications of organic fertilizers, etc.) whereas it may also open new areas of research for scientists to develop a deeper understanding of these variations that could lead to future preventive controls.

6. **There is likely a mobile app for measuring food safety in your future.** In studies reported by Martin Wiedmann (Cornell University), field surveys designed to validate a predictive model for *Lm* in the production environment demonstrated a low and sporadic level of *Lm* contamination that varies by soil type and other environmental factors (e.g. rainfall, see above). However, soil samples taken from high- and low-risk fields showed correlations between positive *Lm* samples and the topography of the surrounding area. Dr. Wiedmann found that proximity to roads and water can be used to accurately predict field risk for *Lm*. They also reported that in high-risk fields, *Lm* prevalence was greatest of the edges of those fields. Importantly, in high-risk fields where *Lm* was detected on 10% of the soil samples, only 1% of the lettuce leaves also tested positive for *Lm* and in low-risk fields where *Lm* was detected in 3% of the soil samples, there were no positive leaf samples. The Wiedmann group is exploring the use of algorithms to use data like this to create predictive models that growers might use to manage *Lm* contamination risks at the field level.   Similarly, using generic *E. coli* as an indicator of irrigation water contamination, a research team headed by Channah Rock (University of Arizona) identified factors that either increase or decrease generic *E. coli* levels in irrigation water sources. Ultraviolet light exposure, irrigation methods, water residence time, and lined canals positively affected irrigation water quality. Canal maintenance activity, rain events within 5 to 50 miles of the sampling site, elevated water temperature, humidity, and unlined canals were all associated with negative consequences that can result in irrigation water quality that exceeds the current LGMA action levels. Dr. Rock reported that her team is actively developing a mobile app to permit growers to predict water quality prior to irrigation activities. In addition to the Wiedmann and Rock projects described above, Keith Warriner spoke about his program having an objective (see section 1 above) to create a mobile app to permit growers to assess the risk posed by pathogen persistence in different types of soils. It is important to remember, food safety models will only be as good as the data used to build them; so what are you doing with the data you generate today? How might that data be used to build useful tools for your operation or the industry as a whole?

7. **Pathogen transference from animals to produce: have we tested enough animals yet?** The question of whether wild animals represent a significant source of human pathogens that can be

transferred to fruits and vegetables have been a major focus for CPS-funded projects since its inception. Over the last several years a number of studies have shown that amphibians, reptiles, birds, insects and domesticated and wild animals all the potential to carry *Salmonella* spp. and/or *E. coli* O1157H:7 and non-O157 STECs. We have also learned that generally the frequency of human pathogens being associated with animals is fairly low, i.e. not all of the animals tested carried pathogens. In support of this assertion, Michele Jay-Russell (University of California, Davis) shared data from her studies examining domestic cattle, javelina, feral pigs, wild birds and wild rodents from California and Arizona desert production areas. Domestic cattle were shown to have a 17.3% rate of carrying   STEC O157, 38.4% for non-O157 STEC and 6.9% for *Salmonella* whereas fauna including wild rodents, birds, and javelina are potential reservoirs

for *Salmonella* and non-O157 STEC, but do not appear to be significant sources of *E. coli* O157H:7. Confirming previous research, cattle and feral swine feces were shown to more likely contain *E. coli* O157:H7 than that of small mammals and avian species. Bird tracking revealed these animals travel regularly between CAFOs and sites greater than 400 feet away including over produce fields, but they are rarely captured in the fields suggesting they spend most of their time either at the CAFOs or their roosting areas but not at areas between these sites. Rodents have small "home" ranges (approximately a 100 foot radius) making their intrusion into produce areas less likely when fields are removed from concentrated animal operations. Field infiltration is further hindered by substantial barriers such as roads or waterways. In the Arizona desert growing regions, seasonal patterns were associated with peek pathogen prevalence in birds and cattle. More birds tested positive for non-O157 STEC and *Salmonella* in the spring while more cattle tested positive in the fall. These data emphasize the importance of performing a hazard analysis on produce fields and packing operations with a specific focus on signs of animal presence and movement patterns, a determination of their population densities and their access to potential sources of human pathogens.

8. **The next level of pathogen detection?** In a general session presented by scientists from the Centers for Disease Control (CDC), the agency's use of whole genome sequencing (WGS) to identify and differentiate between bacterial strains was discussed. A number of key points were made:

   - The ability to isolate and sequence pathogen DNA combined with the exploding computational capacity of modern computers permits the creation of genomic sequence maps that provide researchers and public health officials the opportunity to conclusively identify pathogen strains with unsurpassed precision.

   - While the emergence of WGS for pathogen identification does not obviate other tools used in epidemiology or traceback investigations, e.g. patient questionnaires and interviews, it does offer the opportunity to create databases where specific pathogen sequences might be linked to meta-data that could include information about previous outbreaks associated with that strain.

   - WGS also provides public health officials and industry professionals with a tool that can help them perform seek and destroy or route-cause analysis to track sources of specific contaminations in fields and facilities and develop mitigation measures.

   - CDC researchers appealed to the industry to provide the agency with produce-related microbiological cultures (both pathogenic and non-pathogenic) from product or environmental sampling programs to increase the agency's ability to catalogue genomic sequences and identify virulence profiles to improve their ability to alert industry to potential future illness outbreaks.

9. **Is it the genes or their expression that matters?** Perhaps as important as learning the genome sequence is increasing our understanding of its regulation and expression.   Martin Wiedmann (Cornell University) shared the results of his project examining the interaction of genotype and the environment by studying the expression of genes in *Salmonella* and EHECs when the pathogens

were exposed to leafy greens. Gene expression in the presence of lettuce was measured by examination of RNA's generated as a result of transcription from genes coded by the pathogens DNA. In effect, the measurement of RNA's is a direct method to measure the degree to which specific genes are turned "on" or "off" as a result of exposure to growth on leafy greens. Of course, RNA transcription leads to protein or enzyme production which impacts the physiological state of the microorganism. Dr. Wiedmann's research shows that the environment the pathogen exists in does indeed lead to differential gene expression (RNA synthesis) that profoundly effects the physiological state of the both EHEC's and *Salmonella*. When examining *E. coli* O157:H7 genomic expression, genes relating to oxidative stress were expressed when the organisms were grown on leafy greens and the pathogen was more resistant to the oxidative disinfectant hypochlorous acid after three days. Interestingly, *Salmonella* strains tested did not respond similarly. Specifically, the gene that was "turned on" in the *E. coli* O157:H7 experiment codes for superoxide dismutase (SOD), an enzyme that helps the pathogen cope with oxidative stress. In effect, the environmental stress these pathogens endure as a result of growth on leafy greens, pre-conditions them to be less sensitive to the oxidation potential of a chlorine sanitizer by up-regulating or "turning-on" its natural defense mechanism; SOD. Dr. Wiedmann's data would suggest that general conclusions about the efficacy of sanitizers and their effective dose ranges may be impacted by the specific pathogen (i.e. *Salmonella*, *E. coli* or *Listeria*) and by its exposure to various environmental conditions prior to harvest or during storage. This line of research certainly bears watching in the future.

10. **A deeper understanding of disinfectant efficacy may be on the way**. One theme that came out in a couple of the presentations at the 2015 Symposium was the focus on how sanitizers work and how

Photo credit Dr. Robert Whitaker

they might be improved to help the industry achieve food safety goals. Monitoring wash water disinfectant levels is an essential and soon to be mandatory preventative control in packing and processing facilities. As a contrast to address current Oxidation Reduction Potential (ORP) measurement limitations, Nitin Nitin (University of California Davis) explored bacterial response to wash water sanitizers (hydrogen peroxide and sodium hypochlorite) and found that measuring a bacterial cell's response to sanitizer exposure is a good indicator of sanitizer efficacy. For hydrogen peroxide sanitizers, a relatively inexpensive portable system was developed that measures bacterial degradation of H_2O_2 which is directly related to the reduction in bacterial load. For sodium hypochlorite efficacy, Dr. Nitin developed a label-based optical method to effectively predict the critical sodium hypochlorite concentration above which a greater than 100 fold (2 log) reduction in microbial load can be achieved. This type of research represents a new look at measuring disinfectant efficacy. The days of the "dump and pray" where operators' just added disinfectant to the water and thought the task was complete are over. In our new "FSMA-world", it is critical to measure disinfectant level and monitor a number of operating variables that can impact the efficacy of the sanitizer.

This work is meant to inform and provoke thought with an eye towards inspiring readers to examine their own food safety programs and using the research to make improvements. It is not meant as a directive on what must be done to produce safe food. As discussed in several places in this paper, food safety needs to be determined on an operation by operation basis; there are no one size fits all solutions. If you have additional questions, please feel free to contact Dr. Bob Whitaker, PMA Chief Science and technology Officer (bwhitaker@pma.com) or Dr. Jim Gorny, PMA Vice President of Food Safety and Technology (jgorny@pma.com).