2020 Center for Produce Safety Key Learnings
Session 1
June 23, 2020

As a result of the ongoing Coronavirus pandemic, the 11th Annual CPS Research Symposium is being conducted virtually over the course of five consecutive weeks. In the first session, the focus was on the creation of modeling tools to help the produce industry address key issues around persistence and growth of *Listeria monocytogenes* on whole produce commodities and the development of science and risk-based microbial sampling programs. An executive summary and the key learnings from these outstanding presentations and the discussions that followed are here:

Executive Summary:

- ***Computer-based modeling***. Computer-based modeling tools and simulations based off industry produce safety data can advance our understanding of key industry challenges, identify knowledge gaps and lead to the development of improved preventive controls to improve the safety of our products.
- ***Partnership***. Partnership and data sharing between researchers and industry experts is a requirement of developing models that essentially create a digital operation that simulates the real-world farms, packinghouses, processing plants and distribution facilities across our industry.
- ***Listeria growth and persistence on whole commodities***. An important application of computer-based modeling is the determination of *Listeria* growth on whole, fresh fruits and vegetables. FSMA and customer requirements have focused industry attention on the potential for *Listeria monocytogenes* growth on intact fruits and vegetables if the product should encounter temperature abuse anywhere in the supply chain. pH, physical characteristics of the commodity and time at the elevated temperature are key variables for *Listeria* growth and persistence. A model has been developed based on growth media which can be used to predict *Listeria* growth and guide decisions on the safety of products stored temporarily outside of refrigerated temperatures.
- ***Improving models with laboratory data***. Further laboratory-level investigation of *Listeria* growth and persistence on whole fruits and vegetables elucidated the impact of temperature abuse (as temperatures rise above 39°F up to 95°F *Listeria* growth rates increase), the role of relative humidity (lower relative humidity suppresses growth), and rapid changes in O_2 and CO_2 in produce storage (growth inhibiting). The surface topography also plays a role in *Listeria* persistence on whole produce with rougher surfaces supporting persistence. In general produce industry recommended storage is not a high risk for LM propagation
- ***Microbial sampling***. A second application of computer-based modeling presented at Session 1 was directed at the development of operation-specific microbial sampling strategies. Microbial testing is used across the produce supply chain to detect pathogens or their indicators in agricultural inputs like irrigation water, for sanitation efficacy verification and environmental monitoring programs and raw and finished

product acceptance. Sampling is currently problematic because we are essentially looking for a needle in a haystack; a sporadically occurring, low concentration, unevenly distributed contaminate in the vastness of our farms, facilities, and agricultural inputs. Model construction using industry produce safety data and the ability to conduct thousands of simulations where growth characteristics of pathogens, facility and equipment design parameters, production environment data, people and animal movement and other parameters can be factored in to inform development of risk-based sampling strategies.

- ***The end of "one size fits all"?*** The use of models and simulations to develop sampling strategies provides insights and creates opportunities to leverage testing results to identify gaps in historical data and focus future research efforts. It also means that "one size fits all" sampling strategies need to be supplanted with operation-specific protocols that enhance the chances of finding pathogens and providing the company with the opportunity to prevent contaminated products from entering commerce and performing root cause analysis to determine where the contamination originated and why it was there.

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Key Learnings:

1. **Produce safety data is driving next generation tools that address industry needs.** An overarching theme that infused the session was how data science is becoming a more important tool in addressing produce safety challenges. We have seen this before where CPS-funded research programs led to data driven tools. In 2017, Channah Rock presented her work based on her efforts to characterize agricultural sources in Arizona and described a mobile application that permits growers to make informed decisions on irrigation (Rock 2017). But in session 1 of the 2020 CPS Symposium, it seemed like the use of data to create simulations and use models to address important produce safety issues has moved to the next level.

   Why is this important to the produce industry? The simple answer is that it demonstrates the value of having a produce safety knowledge base. Models are only possible if data are available to guide their development. The corollary is that models are only as valuable as the quality of the data used to construct them. As we embark on the 11th CPS Research Symposium, the produce industry, CPS and other research funding agencies have been responsible for identifying, prioritizing and funding produce safety research with the result being a body of evolving knowledge and data that can be leveraged to build new tools for growers, packers, processors, shippers, distributors, retailers and foodservice entities. We often look at research programs for what their results mean in the moment, but session 1 helps us see that there are collective and cumulative benefits where the combination of a body of research can be used to create models and approaches to solve complex challenges for the industry.

   Why is this important to the research community? Creating models and simulations can provide near-term tools to help the produce industry. But by examining those models and identifying their weakness and vulnerabilities we can also identify knowledge gaps and design experiments to fill those gaps and fortify the knowledge base. Equally important to both researchers and growers is the fact that models can be manipulated freely unlike real world production environments. With a model, a researcher collaborating with growers can change temperature, pH, humidity and other environmental factors, manipulate product flows, change the physical characteristics of a process line or adjust animal intrusion patterns in a field with a few well-place keystrokes whereas in the real world these variables are not so easily manipulated. This freedom permits researchers and growers to simulate how changes in practices or conditions can impact contamination events or permit pathogen persistence and growth. Once variables are understood, together, researchers and producers can discuss and test practical mitigation strategies to control contamination and protect the safety of the products.

   Why is this important to regulatory agencies? The importance can be summed up as awareness, participation, and extension. It is always important for regulatory bodies to be aware of emerging research and new tools being developed by the industry they regulate. FDA, CDC, and others work diligently to say current on research findings and new approaches to data analysis and that benefits the produce industry. The regulatory agencies are also active researchers and collect data as part of their own internal research efforts as well as in investigations, audits, and inspections. So, in that light, by their participation, regulatory agencies can help strengthen efforts to build models and use simulations to test hypotheses. Lastly, models and simulations are not new to FDA and CDC across the spectrum of their total activities. One would encourage them to examine emerging models being developed around produce safety and extend them to tackling the issue of pathogen contamination and actual risk to public health. We know today that pathogens exist in our production environments, humans that can spread pathogens are actively interacting with our products from farm to fork, our sampling strategies are not capable of one hundred percent detection and the bulk of our products are served fresh with no preparatory steps that can kill pathogens if they are present. Yet illness outbreaks associated with produce items are still a relatively rare occurrence even though our outbreak surveillance efforts have advanced immensely in the last decade. It is a complex challenge with multiple and diverse factors contributing to why and how products cause illness but perhaps we are now entering a phase where the knowledge base and foundational data are intersecting with data collecting and analytical advancements and expertise to finally push through and address the challenge.

2. **Predictive models can be used to assess Listeria monocytogenes persistence and growth on whole or intact produce commodities.** While there is considerable data on *Listeria monocytogenes* (Lm) growth and persistence is fresh-cut fruit and vegetable products, very little has been published on Lm survivability on whole, intact produce commodities. Indeed, a 2012 effort by FDA at constructing predictive Lm growth models on whole fruits and vegetables was thwarted by a lack of data. Yet, FSMA's Preventive Controls and Sanitary Transportation Rules require a more thorough understanding of how Lm might grow in produce items under various temperature fluctuations that may occur postharvest and during distribution. In parallel, concern by foodservice and retail operators over the potential threat posed by Lm growth if refrigerated transport trailer temperatures exceed parameters during shipment or unloaded pallets be left under ambient conditions prior to storage in cooled distribution centers or point of sale coolers and display case temperatures rise above prescribed levels have been a constant source of discussion on whether the products involved represent a risk to public health should they be sold and consumed. Operators find themselves without sufficient information to make informed judgments on product safety and salability.

   In session 1, ***Don Schaffner***, Rutgers University and ***Laura Strawn***, Virginia Tech presented the results of their separate, but mutually supporting research projects addressing this produce industry challenge. Schaffner's project *"Managing Listeria in Fresh Produce Using Predictive Models"* (Schaffner Link) focused on comparisons of relative risk of Lm growth under different time and temperature conditions to develop a guidance tool that can be used to form science-based risk management decisions. Lm was chosen as the organism to model as it is an important pathogen from a public health perspective, and it can grow under the temperature conditions generally encountered by produce items postharvest. The following learnings were shared at the Symposium:

- Intuitively, we know that produce held at 1°F above optimal temperature for one minute is at less risk of significant Lm growth than produce held at 10°F above ideal temperature for ten minutes. The problem is that the data do not exist for most commodities to permit quantitative assessments to drive produce safety decisions. Computer modeling can be used to bridge the data gap and provide tools that can be used right now to guide decisions.
- pH and time at a specific temperature are important variables in supporting Lm growth. A model for Lm growth developed by Schaffner makes assumptions on pH and temperature based on the known pH of several whole commodities and the temperatures produce might be exposed to in commerce.
- Using an "off the shelf" computer model called ComBase a spreadsheet tool was developed that examines predicted Lm growth at different pH, time, and temperature scenarios. The model is highly conservative as the growth rates used for Lm were based on growth on nutrient-rich media as opposed to the much less nutrient dense surface of a fruit or vegetable. The model also assumes high water activity and no growth lag-phase. Given these assumptions the model should pose a 'worst case scenario"; in other words, the growth rate predicted by the model should be much higher than what would occur on the surface of a fruit or vegetable postharvest.
- A temperature equivalence calculator that is really a working Excel spreadsheet was created to permit users to quantify Lm growth on produce. The user only needs to input the time and temperature in grey box at the top and read the results in colored boxes that represent the known pH of the commodity of concern. For example, using this spreadsheet, holding a commodity having a pH of 7.0 for 4 hours at 55°F is equivalent from a Lm growth perspective as holding the same commodity for 18.1 hours at 40°F. In other words, the elevated temperature exposure would not necessarily mean the product should be disposed of, but that the risk of Lm growth (if Lm were present) might dictate a reduced product life.

Working Excel Spreadsheet – Temperature Equivalence

Inputs			
What was the undesirable temperature the product experienced?	55 °F		(only 33.8 °F to 55 °F)
How many hours was the product at that temperature?	4 hours		
Outputs			
pH 7			
This is eqivalent to a Listeria risk from storing product at:			
	25.0 hours	at	38 °F
	18.1 hours	at	40 °F
	15.6 hours	at	41 °F
pH 6			
This is eqivalent to a Listeria risk from storing product at:			
	25.5 hours	at	38 °F
	18.3 hours	at	40 °F
	15.8 hours	at	41 °F
pH 5			
This is eqivalent to a Listeria risk from storing product at:			
	26.2 hours	at	38 °F
	18.7 hours	at	40 °F
	16.1 hours	at	41 °F
pH 4.5			
This is eqivalent to a Listeria risk from storing product at:			
	25.3 hours	at	38 °F
	18.2 hours	at	40 °F
	15.8 hours	at	41 °F

Spreadsheets reproduce here courtesy Don Schaffner, Rutgers University

While the Schaffner project constructed a predictive model, the industry can use to meet current challenges without exhaustive Lm growth data on hundreds of whole produce commodities, Laura Strawn's research project *"A Systematic Review of Listeria Growth and Survival on Fruit and Vegetable Surfaces: Responding to a Critical Knowledge Gap"* (Strawn Link) sought to fill some of those data gaps by mining the existing research literature on Lm growth on intact produce commodities, generating Lm growth curves and persistence on selected commodities and leveraging these data to fine tune the Schaffner growth models. Key findings from the Strawn project include:

- Lm growth and/or survival on intact produce differed by commodity. Produce surface conditions affected Lm growth and/or survival with increasing roughness supporting Lm growth/survival.
- Naturally occurring microflora on commodity surfaces can represent an increased competitive background which limits Lm growth/survival.
- Produce storage conditions affected Lm growth or survival. Intact produce held at 72°F and 95°F had the highest Lm growth rates. In general, Lm growth rates increased as temperature increased from 39°F to 95°F. At cooler storage temperatures (≤54°F), relative humidity influenced growth and survival with low relative humidity limiting survival.
- Large shifts in CO_2 and O_2 concentrations within storage containers may suppress the growth and survival of Lm on produce surfaces.
- Carrying capacity or the maximum number of bacteria that can be sustained on the surface of a fruit or vegetable based on available nutrients, water and

habitat characteristics is crucial to characterizing growth and survival patterns of Lm.

- Ten different whole, intact commodities were studied and all (except carrots) supported Lm growth at 95, 72, and 53°F but not at 36°F. Produce commodities held at refrigeration temperature (36°F) had little to no Lm growth.
- In the growth and survival studies, inoculum concentration, produce microbial carrying capacity, and temperature significantly impact the estimated Lm growth rate according to models.
- The ComBase model enabled at pH 5 was generally fail safe for Lm on all produce items (validated based on laboratory-generated data), except for tomatoes stored at 95°F. Therefore, the produce industry should feel confident using the model to predict Lm growth.

Why are these results important to the produce industry? First and foremost, these projects are a great example of where an industry need to understand Lm growth and survival on whole produce was identified, prioritized and research subsequently funded to find a solution in a timely fashion. As a result of the Schaffner and Strawn research projects, the produce industry now has a working model to predict Lm growth or survival on whole, intact fruit and vegetable commodities. Schaffner has created a computer model that can be used to predict Lm growth on produce that leverages key variables like pH, time and temperature so that suppliers and receivers can use the information generated to make informed decisions on the fate of produce that might have received temperature abuse at some point in commerce. Strawn's work has generated laboratory data on a group of selected commodities that fill data gaps, validate and fine tune the ComBase model. In the end, the industry now has a model that lays the foundation for understanding Lm growth and survival on produce items and a rationale for further discussions with FDA as they seek to define "high risk" commodities.

These results also remind us that anything we can do at the field, packing and processing level to control temperature, reduce the presence of pathogens in the production environment and be mindful of variable product characteristics relative to vulnerability to support pathogen growth and persistence is important.

Why are these results important to the research community? These projects are instructive for the industry and the research community in that they demonstrate how important it is for industry to cooperate with the research community. Both projects relied on input from industry on the critical nature of the challenge to better understand Lm growth on commodities and then further, once undertaken, on current industry practices as commodities are shipped and stored in the supply chain. These projects also demonstrate how modeling can be used to identify data or knowledge gaps to fuel future research. The Schaffner work on the initial model using existent data was enhanced by the Strawn work that provided lab data to validate the model. Both are continuing to work together to further refine the model. Additionally, the research in the Strawn lab comparing wet or aqueous inoculation (used in the majority of published research on Lm growth) where 1-log growth was observed with 24-hours of inoculation versus a dry (sand) inoculation method where little or no growth was measured merits further examination as future studies are planned. Inoculation methods and the physiological state of the bacteria and their impact on research outcomes have been previously identified in other CPS funded programs [Harris 2012] and [Wiedmann 2015] [Wiedmann 2017]

Why are these results important to regulatory agencies? Generally, these projects represent an evolutionary step where leveraging even limited, existent data to develop a model supported by novel laboratory research can help industry and the agencies create a way to deal with a complex challenge. More specifically, this work on the use of predictive models for Lm growth on whole produce commodities is a beginning leading to a fuller understanding of Lm and whole produce. To the extent that this work is further supported by additional research, regulatory and industry will have better tools to evaluate true public health risk and mitigations to control those risks.

3. **Modeling tools to guide creation of operational sampling plans.** Microbial testing has been widely debated around the produce industry for two decades. Of course, there are several types of testing commonly practices in the industry every day: agricultural input test (water, soil amendments, etc.), raw and finished product testing, and verification testing as part of sanitation and environmental monitoring programs (EMP). Each type of testing represents its own challenges and opportunities but as the science around detection assays has improved the focus remains solidly on sampling. In session 1 we heard from three leading researchers present their work to help elucidate sampling protocols that can help the industry manage contamination and public health risks most efficiently and cost effectively.

   Emma Harnett, Risk Sciences International, led off the discussions on microbial testing with her project, *"Exploring the Relationship Between Product Testing and Risk"* (Hartnett 2019). This project seeks to (1) develop a sampling-risk model that quantifies the relationship between product testing, lot rejection rates, and the public health risk which is directly related to prevalence of the pathogen and the concentration level in the consumed product, (2) analyze the relationship between product sampling variables that drive risk and (3) explore of risk management options and facilitate selection of actionable sampling strategies that have the biggest impact on risk reduction. Some key learnings from this program include:

   - This project employed computer-based models to assess risks of contamination and drive decisions. In effect, the model facilitates work on a digital operation where variables can be adjusted to determine impact on sampling strategies. The model can be developed to look at different pathogens important to the produce industry, e.g. *Salmonella*, *Listeria monocytogenes*, and EHEC/STEC. Growth characteristics, true pathogen prevalence, predicted dose levels in finished products can all be built into the model to permit assessment of public health risks.

- Sampling strategies exist to manage or control contamination risks and ultimately illness risks for the consuming public. The location where samples are taken can matter in terms of achieving the best public health risk reduction possible from a sampling strategy. The farm to fork chain and the characteristics of the production process (environmental, equipment, people, etc.) can impact microbial survival and growth and plays an important role in developing a sampling strategy.
- While microbial sampling is often conducted in the field, there are other opportunities available to take samples, e.g. raw product receiving, cooling packing, etc. When choosing an appropriate location or production step for sampling the aim is to identify a location that can offer the best risk reduction opportunities coupled with the most practical strategy (cost, time, labor, etc.). If the sampling location does not represent risk reduction benefits, then it may be appropriate to sample earlier or later in the supply chain to offer the best options to identify contamination and avoid supply chain disruptions like recalls and consumer advisories.
- To develop an effective sampling strategy, it is important to know your production system. "One size fits all" strategies for sampling are not likely to be effective.

Renata Ivanek from Cornell University described her program, *"Modeling tools for design of science-based Listeria environmental monitoring programs and corrective action strategies*". This project uses computer models, developed to represent four different actual produce facilities; two fresh-cut and two packinghouse facilities. The four models permit the research team to simulate *Listeria* dynamics using the digital operations and measure the impacts of variables like employee movements, water quality, design and ease of equipment cleaning and sanitation, and product flow. The models were validated by using operation-specific sampling data and industry data where applicable. The use of these models permits the evaluation of differential corrective actions in response to *Listeria* spp. and development of environmental monitoring plans using the modeled fresh produce processing facilities. Key learnings are:

- The models can simulate *Listeria* dynamics in produce operations permitting researchers and operators to explore sampling plans and corrective actions to control *Listeria* in the digital world and transfer these learnings to the real world. This model can be used to simulate other environmental pathogens like *Salmonella* as well.
- When true prevalence (the number of positive tests compared to the total tests executed) is low, all evaluated sampling plans are similar. A "random" sampling plan is closest to the truth whereas sampling "zone 3" (areas where there is no

food contact, e.g. floors, walls, equipment framing, drains, etc.) is best at identifying contamination presence.

- Sampling performance is time dependent. In other words, sampling performance can change within the same facility over the course of time, even within different portions of the same season. Sampling can also be facility specific as different facilities have different design, equipment and other variable factors that impact sampling. These site-specific attributes justify different EMP strategies for different facilities. However, the order of plans with respect their performance remains the same over time and among facilities. The development of a sampling plan needs to be customized to the facility. "One size fit all" approaches need to set aside.
- The construction of computer-based models is significantly improved when collaboration between industry and researchers is realized. Gaining insights into operations and access to historical data sharpen the models and make the simulations more useful and thus the learnings and potential corrective actions that emerge are more relevant to the real world

Matthew Stasiewicz from the University of Illinois built on the preceding sampling presentations with an interim report on his project; *"Simulation Analysis of In-Field Produce Sampling for Risk-Based Sampling Plan Development*". It is important to examine field-level sampling options as raw product testing has been likened to "searching for a needle in a haystack" owing to the vast amount of plant material resident in any production field and the variability one finds in potential exposures to sources of contamination even within a single field. Over time, growers who do pre-harvest product testing, have settled on sample numbers of 30 to 60, 50 to 100-gram samples per acre taken in a "Z" pattern or other variations. Unfortunately, the reality is that despite these efforts, we still have illness outbreaks often associated in fields or regions where testing is employed. Though early in the execution of this research project, the following learnings were shared:

- Once again, this project is employing computer-based models where simulations can be used to test sampling strategies. As before, the advantage of this approach is that digital fields can be sampled in many iterations of sample number, sample size and sample patterns with a few keystrokes and analyzed for efficacy in pathogen detection.
- These models also permit examination of many different pathogens and factor in their types of contamination events, e.g. single point source contaminations like the presence of animal fecal material or widespread or systematic contamination events like flooding. In the case of point source contamination events, randomized sampling is the preferred sampling regimen while with systematic contamination events, pattern is not as important as sample mass or the amount of tissue used for the test.
- This work is moving toward the development of an app that can be employed to customize in-field sampling strategies based on specific fields, environments,

and hazards resident in the area. This represents a move away from generic sampling plans to situation-specific strategies.

Why are these results important to the produce industry? The three research reports presented in session 1 on sampling all reached the same conclusion: one size fits all approaches do not serve the industry well if your objective for sampling and testing is to identify contaminated products, remove them from moving into the marketplace and working back to find out the cause of the contamination. No two processing or packing facilities are exactly the same just as no two production fields are exactly the same. Indeed, even within a single facility or farm site there are seasonal variations and environmental factors (rain, wind, transient wild animal movements, domesticated animal operations, equipment change outs and employee changes) that impact pathogen presence, persistence or growth and therefore sampling strategies may need to be adjusted to successfully detect pathogens. For growers, packers, and processors this means engagement. Going forward, using a generic sampling program, and checking for a “yes” or “no” the pathogen is there or no its not will need to change. It is critically important to stay connected to the evolution of model development and how they can be used to improve your produce safety efforts. In the end, whether sampling agricultural inputs like irrigation water, doing environmental sampling to monitor *Listeria* or doing some form of product testing, as a producer you are investing significant resources in the endeavor and that investment has to mean more than just checking the box to show you did the testing for a customer or you met a FSMA requirement. Designing custom sampling strategies that help you to identify vulnerabilities for contamination and guide creation of improved preventive controls enhance the value of your sampling efforts and bring value to the organization.

These projects all employ computer-based modeling because of the flexibility it provides when studying complex, multi-variable problems. It is impractical for companies in the produce industry to test various sampling scenarios and mitigating corrective actions and identify optimal strategies. Modeling represents a time and cost-effective way to accomplish the task. Models also offer an advantage in that they are built sequentially so that as researchers build and test models they can take what works and build upon those learnings to continue to perfect the model and better simulate real world conditions. The closer the researchers get to real world simulations the better they serve the producer’s needs. The best mechanism to ensure that models and simulations converge on the real world of our farms and packing facilities is to collaborate. Each of the principal investigator that presented in session 1 pointed out that their projects could not have happened without collaboration from the industry. Your knowledge of industry and your company’s best practices and your historical testing data are vital to building useful models that maximize your testing benefits.

Why are these results important to the research community? These projects demonstrate the potential for engaging the produce industry to develop relationships with produce companies that enable research collaborations. While these modeling projects are largely computer-based, it is the company data and the knowledge of industry and corporate practices that permits validation of the models and informs the simulations.

It is also important to note that the trend toward developing customized sampling strategies based on operational characteristics or this move away from “one size fits all” approaches is important. It opens the door for the research community to work with the industry to develop practical, easy to use tools that help the industry meet this challenge. It also presents an opportunity for the research community to partner with the industry in the development of more effective, science-based preventive controls and validating their efficacy.

Why are these results important to regulatory agencies? These initial steps to using models to develop more effective sampling strategies should alert regulators to these emerging tools and perhaps inform their own sampling strategies as they sample fields and facilities during inspections and investigations. There may also be the potential for partnership as FDA collects data at all points in the supply chain that can help fine tune simulations and create greater insights on the public health risks associated with positive test results. The Harnett project spoke to this aspect of sampling and the concept of quantitative microbial risk assessment or QMRA where survival or persistence of pathogens on the product in the supply chain, the dose on the consumed product, the amount of product typically consumed, the virulence of the pathogen and susceptibility of the average consumer to infection are among the factors to consider when assessing actual public health risk.

Acknowledgements*: The Center for Produce Safety would like to thank the researchers who made presentations during session 1 of the 2020 Research Symposium. Their 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 (insert link to e-mail). Thank you.*