2020 Center for Produce Safety Key Learnings
Session 2
June 30, 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 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 microbial testing needs (Session I Key Learnings). The second session conducted on June 30, 2020, helps expand our knowledge base on *Listeria monocytogenes* and its persistence and growth on specific commodities and fresh-cut products and examines novel methods to control *Listeria* growth on food contact surfaces. An executive summary and the key learnings from these outstanding presentations and the discussions that followed are described below.

Executive Summary:

- ***Always check new products for ability to support Lm growth***. When new combinations of produce commodities are used in a new product offering, it is important to understand if they represent more or less of a *Listeria monocytogenes* (Lm) growth risk if subjected to temperature abuse.
- ***Lm growth supported by novel salad ingredients***. Salad ingredients like Brussels sprouts, beet greens, broccoli stalks and kale can support growth of Lm if temperature abused, and while this observation is consistent with the Lm computer-based growth model described in Session 1 of the CPS Symposium, in some items the growth rates are slower than what might have been expected likely owing to the presence of a naturally occurring component of crucifer crops known as glucosinolates.
- ***Listeria innocua as a Lm surrogate for growth studies***. A potential surrogate for Lm is *Listeria innocua*. Growth studies on salad ingredients indicate that its growth rates very closely mimic those of Lm. *L. innocua* has been proposed as a surrogate for Lm in other validation scenarios and it seems that it may also have use in crucifer crop studies.
- ***pH and temperature are the primary determinates of Lm growth on produce***. As discussed in Session 1 and elaborated on during Session 2 of the CPS Symposium, pH and temperature are key factors in supporting Lm growth on whole, fresh-cut, and juiced produce items. Generally, pH values near neutral (7.0) and elevated temperatures (above 39°F) support elevated growth.
- ***Mixed species biofilms protect Lm***. Biofilms containing multiple species of bacteria plus Lm are more difficult to eliminate than biofilms containing only Lm. Strains that are efficient biofilm producers can be found consistently in packinghouses, indicating they are adapted well to packing environments. Mixed species biofilms are more resistant to sanitation efforts effectively "protecting" Lm cells that are resident in the biofilm.
- ***Rough surfaces are more conducive to biofilm stability***. Worn equipment with rough surfaces provide niches for biofilm production which can protect Lm from cleaning and sanitation efforts.

- ***Different sanitation strategies may be needed for different equipment.*** Equipment made from different materials and in different states of wear may require different cleaning and sanitation strategies to manage the risks of biofilm formation that may result in product cross contamination. Combinations of sanitizers that have different modes of action can be effective tools in developing sanitation strategies to minimize contamination risks.
- ***The challenge of cleaning and sanitation of reusable totes and bins may be addressed by using plastics infused with antifouling and antimicrobial chemistries.*** Produce containers, liners and spray-on biocoatings developed with N-halamine chemistry that delivers free chlorine to control biofilms and kill bacteria have been tested and shown to be effective. These products are rechargeable; meaning they can be refreshed multiple times by simple exposure to 1-percent bleach for 15 minutes. Additional antifouling and antimicrobial materials are also being evaluated in an ongoing CPS project.
- ***Collaboration remains an integral piece of successful produce safety research***. The research projects in Session 2 were all supported by industry collaborators that provide researchers access to facilities and products and share knowledge and data to shape the subsequent research priorities and inform interpretation of the results.

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Key Learnings:

1. **<u>Listeria persistence and growth on produce</u>**. In Session 1 of the 2020 CPS Symposium series we learned about computer-based modeling and laboratory level studies designed to provide data and tools to guide the produce industry on the potential risks involved with temperature abuse and *Listeria monocytogenes* (Lm) growth. As we opened our second session, ***Amanda Lathrop*** of the California Polytechnic State University shared the final results of her project, "*The effects of storage conditions and the microbiome of non-traditional salad ingredients on the fate of Listeria monocytogenes*" (Lathrop 2019). It is important to understand if or when foods support growth of Lm and it is especially important to recognize that the array of products within the produce industry are changing and that these changes mean we have to examine their potential to support Lm growth. Lathrop's work assessed four fresh salad components: broccoli stalks (shredded), Brussels sprouts (sliced), kale leaves (chopped) and beet greens (chopped) for their ability to support Lm growth, survival, or die-off. Additionally, the project explored the growth characteristics of *Listeria innocua*, a relative of Lm on the same fresh salad ingredients to determine if it might be a non-pathogenic surrogate for future studies on Lm. Some key learnings from this project include:

- Lm can grow in kale, Brussels sprouts, broccoli stalk, and beet greens even under ideal storage conditions at 40°F and then at the abuse temperatures of 54, 72, and 95°F. However, the rate of growth was dependent on the commodity, with kale and beet greens showing approximately 1-log growth around day 17 (typical end of product shelf life) and broccoli stalk at day 10. Brussels sprouts were least supportive of Lm growth, requiring 25 days to reach comparable Lm levels.
- These data can be used to help assess the potential for in Lm growth. Lm growth was dependent on product type and a potential explanation for this observation is the presence of natural antimicrobials commonly found in crucifer crops like Brussels sprouts, kale, beet greens and broccoli stalk. The precursor to these antimicrobial chemicals are generally known as glucosinolates and in particular allyl isothiocyanate and has been targeted for future study by the Lathrop team. Additionally, there was no significant difference between physically abused and non-abused products in supporting Lm growth indicating risk did not increase beyond what was observed as a result of chopping, slicing or shredding.
- *L. innocua* can be used as a surrogate for Lm in kale, Brussels sprouts, broccoli stalk, and beet greens studies. As with Lm, simulated physical abuse during storage and distribution of the tested products did not increase the rate of *L. innocua* growth.

Continuing the theme of defining Lm growth on fruits and vegetables, ***Elliot Ryser***, Michigan State University shared an interim report on his new project "*Fate of different Listeria monocytogenes strains on different whole apple varieties during long-term simulated commercial storage*". This project is in its second year and is focused on assessing the impact of apple variety, production region, growing season, and storage atmosphere on the survival of different Lm strains on unwaxed apples. Ryser's group will also characterize the impact of three different industry-relevant, apple-waxing scenarios on survival of Lm on Granny Smith, Gala, or

Honeycrisp apples during atmospheric storage. Studies in the first year revealed these learnings:

- Type of cell growth (within a biofilm or planktonic), apple variety, and growing region may have an impact on the overall survival of Lm on apples. However, after two months of apple storage *Listeria* populations generally remained stable.
- Biofilm strains survive longer on apples than planktonic (single cell) strains.
- Eight Lm strains have been developed with chromosomal genetic labels to track their persistence, growth, or die-off to be studied in extended storage studies. The genetic labels did not alter growth characteristics, diminish virulence, or affect their abilities to form biofilms.

In another interim report, ***Xiangwu Nou*** at the USDA ARS Beltsville Agricultural Research Center shared his initial findings on his project, "*Listeria monocytogenes growth potential, kinetics, and factors affecting its persistence on a broad range of fresh produce*". This project will continue to help build the research knowledge base on Lm growth and persistence on various types of whole and fresh-cut produce under normal and abuse storage and retail display conditions. The research team plans to examine the impact of variable produce nutritional and physiochemical characteristics on Lm growth as well as evaluate the impact of naturally occurring, resident microorganisms on Lm growth. Though an interim report, the following learnings were presented:

- Lm growth was demonstrated on three fresh-cut products: cantaloupe, cauliflower and celery held in storage over one to three weeks. In general, increased temperatures resulted in increased Lm growth.
- Juices were prepared from several fruits or vegetables and evaluated for ability to support Lm growth. pH is a primary determinant for growth with those commodities having a pH of <4 (apple, blueberry, grape, pineapple, peach, mango and tomato) failing to support growth and those with near neutral pH (cantaloupe, carrot and celery) supporting growth of Lm.
- Maturity and varietal differences can impact pH and ability to support Lm growth.

Why are these results important for the produce industry? These results of these projects are essentially "more bricks in the wall". Looking at existing literature on Lm growth and persistence on produce items and the projects of Schaffner and Strawn (2020 CPS Research Symposium Session I – page 5) these projects add, or will add when complete, depth to our knowledge base and provide valuable guidance to the industry. Importantly, the results reported by Lathrop are in line with those predicted using Schaffner's modeling spreadsheet presented in Session 1 (Schaffner Calculator 2019).

The Lathrop results also point to the importance of testing newly developed product types for Lm growth, persistence, or die-off. As produce companies create new products combining various whole or fresh-cut products in packages, it is advisable to examine the potential for these combinations to support Lm growth under varying conditions. Certainly, computer-based modeling and laboratory testing are important tools to determine the risk represented by Lm in new product combinations, but we are reminded by the work presented here that commodity specific microbiological or chemical characteristics sometimes yield unexpected results. For example, using crucifer ingredients in fresh-cut salads introduced the potential impact of the antimicrobial properties of glucosinolates diminishing Lm growth below what might be expected. In the end, produce products are essentially biological systems where the fate of an intruding pathogen may be determined by the complexities of that system.

Along the same lines, Nou reported that carrot juice, but not fresh-cut carrots, supports Lm growth, while Strawn's presentation (Strawn 2019) during Session 1 specifically called out whole carrots as failing to support Lm growth. It is evident that different forms of the same basic constituents can dramatically affect susceptibility to support Lm growth.

The data on *L. innocua* also provides useful information to the produce industry. *L. innocua* has been proposed as a suitable surrogate for pathogenic Lm in validation studies for wash systems and sanitation protocols. Previous CPS-funded work by Suslow (Suslow 2019, Suslow 2014) pointed to *L. innocua* as a suitable surrogate for Lm in shade-house production systems and for pasteurization of netted melon surfaces, respectively, and Lathrop's results demonstrate nearly identical growth characteristics on the four salad ingredients. Clearly the research knowledge base is gaining more depth on the utility of *L. innocua* as a surrogate for Lm to permit validation studies and environmental testing alternatives for growers, packers, and processors.

Lastly, in what is a recurrent theme in every CPS key learnings report, each of the three programs included in this section was immensely benefited by the participation of industry partners. Access to facilities, obtaining raw product samples at source and industry experience in handling commodities and proprietary product knowledge result in more meaningful results with increased applicability to real-world challenges.

Why are these results important to the research community? These projects are "another brick in the wall" for the research community as well. The Lathrop project adds more understanding to Lm growth on fresh-cut salad ingredients under ideal and abuse conditions and the Ryser and Nou projects are sure to add to that knowledge base as they are completed this year using other commodities and forms of produce. The affirmation of the importance of temperature, water activity, pH, and other variables in Lm growth and persistence studies provide a roadmap for future experimentation on other, less well understood commodities and fresh-cut products. The data represented by these studies can also be used to strengthen computer-based models moving forward.

The data also raise the possibility that natural constituents like glucosinolates may play a role in controlling Lm growth. Cooksey's research also implicates naturally occurring antagonistic microorganisms can play a role in controlling Lm growth. Indeed, previously funded CPS research has raised the potential for glucosinolates in controlling *E. coli* O157:H7 and *Salmonella* in soils (Patel 2013), and the yield benefits of rotating broccoli with lettuce are well known in the industry for controlling plant pathogens. These elements reinforce the systems biology considerations and a holistic approach to produce safety challenges and solutions.

Why are these results important to regulators? Lm growth and persistence on various produce items remains an important consideration for regulators and the produce industry. Understanding which products might be more vulnerable to supporting growth under abuse conditions and the factors that contribute to growth can help inform decisions for the industry and the regulatory bodies. It was encouraging to see that the Lathrop results and conclusions line up well with the predictive models described by Schaffner and should provide FDA with confidence that computer-based models have value as they are supplemented with emerging data in the future.

2. **Listeria biofilms and equipment sanitation**. In her presentation, "*Preventive sanitation measures for the elimination of Listeria monocytogenes biofilms in critical postharvest sites*" (Cooksey 2019), ***Kay Cooksey*** from Clemson University drew our focus to control of Lm in packinghouse facilities. Our industry experience and previous research has shown that microenvironments in packing and processing facilities that are hard to access or equipment surfaces that wear down and become difficult to clean and sanitize can facilitate biofilm formation and harbor pathogens. This project leveraged laboratory testing beds constructed from materials used in equipment fabrication in the produce industry to replicate real-world conditions found in stone fruit packinghouses to determine effective cleaning and sanitizing procedures for Lm and Lm plus resident microflora biofilms (mixed biofilms). The key learnings from this final report were:
    - Mixed species biofilms were protective of Lm or more resistant to sanitizer treatments than were Lm single-strain biofilms. Mixed species biofilms showed less than 3.2-log reduction after 10-minute exposure to 200 ppm chlorine, whereas single species Lm biofilms showed approximately 5.1-log reductions. *Pseudomonas* and *Burkholderia* are opportunistic human pathogens and strong biofilm-forming microorganisms that can help protect Lm against cleaning and sanitation treatments. These protective microorganisms can repeatedly be found in fruit packinghouses and their isolation from multiple facilities and locations suggests that these background non-pathogenic strains are well-adapted in the stone fruit packinghouse environment.
    - Brushes can be porous or even hollow and are routinely found to harbor Lm. The level of contamination uncovered is dependent on time of day when sampling occurred and/or how much product had passed over the line when sampling occurred.
    - Surfaces with increased roughness protected biofilms against sanitizers. For example, exposure to 200 ppm chlorine for 15 minutes reduced Lm populations in biofilms by 5.9 log on control (smooth) coupons with 0.2-µm surface roughness, or by 4.5 log on a surface with approximately 7-µm roughness. These results indicate that mixed species biofilms and surface imperfections require optimal sanitizer concentration and longer exposure time to avoid survival of residual microorganisms.
    - Benzalkonium chloride and chlorine were the most efficient sanitizers in reducing Lm biofilms. In particular, benzalkonium chloride was most effective against mature Lm biofilms especially on rough surfaces. These sanitizers have different properties (e.g.,

mechanisms of action, diffusion coefficient, and reactivity) and their physiochemical differences were reflected in cumulative effects against biofilms.
- Single and mixed species biofilms are also more resistant to peracetic acid treatments.

Why are these results important for the produce industry? This project is a stark reminder for growers, packers, and processors that hard-to-access niches on equipment and rough or worn surfaces represent potential harborage sites for Lm and Lm-containing biofilms that can contaminate products as they pass over the equipment. With this knowledge we can survey our equipment, look at our sanitation efforts and find those areas that present challenges. Chances are if they represent sanitation challenges, then that is the best place to look for biofilms and Lm in our environmental monitoring programs. It is even better when we use that knowledge and understand that extra effort and different sanitation strategies are needed to control biofilms and Lm. Process lines constructed of different types of materials in different states of wear require cleaning and sanitation strategies that account for these characteristics. Different detergents, sanitizer combinations, contact times, concentrations and cleaning protocols need to be combined with a reassessment of equipment designs and construction materials to ensure efficacious cleaning and sanitizing. In other words, generalized or "one size fits all" sanitation programs with the same approaches for every piece of equipment are not optimal and can lead to biofilm deposition and persistence.

This project also gives us greater insights for why biofilms can be so difficult to control but also offers a strategy to control them. The data demonstrate that mixed strain biofilms are harder to mitigate than pure or single strain Lm biofilms, consistent with research by the Joerger lab funded by CPS in 2017 (Joerger 2017). Strains that are prodigious biofilm producers basically partner with Lm to protect it from sanitizers, permitting survival and growth. This is just another example of the complexity of the biological system existing in a stone fruit or any packinghouse and the synergy between microorganisms that can exist. However, the sanitation method identified by this project to counter enhanced biofilms is also a synergistic; a combination of sanitizer chemistries, benzalkonium chloride and chlorine that synergistically degrade biofilms. The research provides produce industry operators with further evidence that "just any old sanitizer will do" philosophies will not work and that sanitation chemicals and their application need to be carefully considered and their efficacy measured.

Lastly, the Cooksey program is an example of how collaboration between a segment of the industry, in this case stone fruit producers and a research team can lead to solutions for industry challenges. The Cooksey team visited eleven packing locations in California and South Carolina to observe, gain a better understanding of packinghouse operations, examine the surfaces and materials used in equipment construction, and isolate microorganisms' resident in these environments. Using this knowledge, they were able to create test beds to permit research that could not have been conducted in a working packinghouse.

Why are these results important for the research community? When the Cooksey project was presented in its interim form in 2019, one of the key learnings identified the value of interdisciplinary approaches to find solutions to key produce safety challenges. Microbiology, sanitizer chemistry, food science, engineering and materials science all play a role in developing effective cleaning and sanitation strategies. The research community has a history of inclusion to solve difficult research objectives and these projects serve to remind us that broad expertise is needed to develop practical solutions to many produce safety issues.

Why are these results important for regulators? FDA inspectors routinely interact with growers, packers and processors, the research community, and developers of new cleaning and sanitation chemistries as they petition for use in food production. As FDA transitions to a "smarter FDA" in their drive to create awareness around and the adoption of new technologies across the entire food industry, they can play an important role for the produce industry as a connector and enabler. As FDA encounters emerging technologies or cleaning and sanitation approaches applied to other segments of the food industry, they can connect those resources to produce industry leaders to initiate further innovation.

3. **Rechargeable treatments to control surface fouling and contamination with Listeria.**
Sanitation of reusable plastic containers (RPCs) can be a significant logistical challenge for growers, packers and processors as these containers are cycled from field to facility repeatedly over short periods of time or from distribution to foodservice or retail around the country in a matter of days. Yet we know that inadequate sanitation can lead to cross-contamination of fresh produce with pathogens. ***Nitin Nitin*** from the University of California, Davis, shared his final project results addressing this issue titled: "*Rechargeable antimicrobial and antifouling plastics for improved cleaning and sanitation of plastic bins and totes*" (Nitin 2019). This project focused on developing rechargeable antimicrobial and antifouling plastic material(s) and coatings and evaluating these materials to measure control of *Listeria* growth on treated RPC surfaces and reducing biofilm formation. The antimicrobial properties of the developed materials can be recharged by simply using a diluted chlorine (bleach) solution. This novel chemistry can be used to make rechargeable container liners attached to existing RPCs and/or to develop next generation RPCs with the antimicrobial chemistry incorporated into the plastic material. Key learnings are:
   - Rechargeable antimicrobial halamine (contains chlorine) films were developed that demonstrated a 3-log reduction of *Listeria* on the surface with a 20-minute contact time. N-halamine is stable and can be recharged by simply treating with a 1-percent chlorine solution for 15 minutes.
   - Demonstrated rapid inactivation (>5 log) of *Listeria* on RPCs in the presence of organic debris.
   - Enhanced prevention of cross-contamination of fresh produce was observed with more than 2-log reduction of *Listeria* compared to controls.
   - Fiber-based materials with increased surface area, and bio-coatings with localized concentration of chlorine sanitizer and enhanced stability, demonstrated superior antimicrobial efficacy. As opposed to halamine-incorporated films or plastics, biocoatings that might be used on processing equipment to control cross contamination would need to be applied on a regular basis perhaps after cleaning and sanitation.

- Rechargeable antimicrobial films with antifouling function reduced attachment of bacteria by >1 log and improved efficiency of bacterial removal from the microbial films.
- No significant changes in product quality (color and texture) as a result of the use of these antimicrobial treatments.
- Simulated production testing using fresh, inoculated tomatoes packed in trays with halamine film indicated a reduction in the level of product contamination and decrease in the number of total contaminated packages.

An interim report by ***Boce Zhang*** of the University of Massachusetts titled, "*Non-fouling food contact surfaces – prevention of biofilm and surface-mediated cross contamination*" takes a slightly different approach to the same problem of cleanability and sanitation of equipment surfaces. This project seeks to evaluate the baseline non-fouling properties of FDA-approved food contact substances (FCS) under fresh produce processing conditions. Zhang's group is using materials that already have FDA approval for use with food production to see if they can limit or prevent *Listeria* biofilm formation and improve sanitization efficacy. Some preliminary learnings include:

- Surface topography has limited impact on Lm biofouling unless at sub-micron level
- Surface chemistry and coating have a significant impact on Lm biofouling
- Both hydrophilic and hydrophobic coatings showed non-fouling properties
- Dursan used in meat and poultry packing for coating surfaces appears to have the best non-fouling properties so far

Why are these results important for the produce industry? The Nitin and Zhang projects clearly show that antifouling and antimicrobial materials incorporated into plastics or used as coatings can significantly reduce the risk of cross-contamination by reducing total microbial count, preventing biofilm formation and limiting transference from containers/ materials to produce. Conducting reliable cleaning and sanitizing on recyclable produce containers has been problematic for the industry. The use of rechargeable materials offers a solution for growers and packers that could address this challenge. Extending beyond RPCs and other containers, biocoatings may be an important approach for harvesters, packers, and processors as treatments for food contact surfaces to prolong sanitary status during the production day. Indeed, biocoatings could be important further along the supply chain extending into transportation trailers and perhaps to display cases at retail and work surfaces in restaurant kitchens where control over cross contamination and biofilm deposition are equally important.

One of the features of these projects is that multiple approaches to rechargeable films and materials are possible to suit different situations. Growers and packers can have considerable inventories of bins, totes, and bins on hand that are used over a period of several years. Indeed, in some regions and with some crops, it almost seems like there is an industry "pool" of totes and bins that move from one operation to another over the course of the season. If an operation had to replace bins and totes to have access to new rechargeable plastics technology the capital investment may give pause to many operations and simply be outside the reach of others. The use of container liners equipped with antimicrobial chemistries or the use of spray-on coatings provides an immediate solution to permit growers and packers to use current container inventories while replacing worn containers over time as they do now with containers made from plastics already constructed with antimicrobial/antifouling chemistries.

The next step for the Nitin project is to move from concept, proof of concept and pilot-scale testing described in Session 2 to commercial testing and this is where industry can play a valuable role. Ultimately the acceptance of N-halamine technology or the alternative chemistries being evaluated by Zhang will be dependent on ease of use and cost per unit. Commercial testing requires industry collaborators in order to gain a real-world assessment of cost and benefit. Research suggests the halamine chemistry can be recharged with exposure to a 1-percent chlorine solution for 15 minutes and this process can be repeated 40-50 times (though the upper limit has not been defined). It is also important to note that applications to liners, plastics in totes or spray-on coatings all have different cost factors and would need to be tested and cost-evaluated for specific uses and applications.

Why are these results important for the research community? We already touched on the value of multidisciplinary approaches to key produce industry challenges in our discussion on *Listeria* in packinghouses. These projects on creating antifouling and antimicrobial films and plastics are also examples of projects where broad expertise in chemistry, materials science, food science and microbiology has been brought to bear to address an opportunity to improve sanitation of surfaces that contact fresh produce.

During the group discussion of the Nitin and Zhang projects, the audience brought up an opportunity for Zhang as that project gets underway to examine proficient biofilm forming strains in addition to Lm strains associated with previous listeriosis outbreaks to assess mixed and single strain biofilm deposition against the antimicrobial materials being evaluated in the project. This interaction is a hallmark of CPS; coming together as the produce industry with the research and regulatory communities to discuss emerging results, how we can build on them and reduce them to practice. CPS is an important connector for the industry and a great resource for the research community as projects are formed and input is needed to make them supportable and ultimately successful.

Why are these results important for regulators? N-halamines are not new to the food industry as there are applications for their use in the poultry industry, in health care facilities and water treatment. But halamines are new for the produce industry and therefore there is a need for consultation with FDA (and EPA?) on the applications anticipated and to develop a clearer understanding of regulatory status. Regulatory input and guidance are an important part of the development process and commercialization of the approach.

Join us for Session 3 of the 2020 CPS Research Symposium on July 7, 2020, at 1:00 PM Eastern and 10:00 AM Pacific time. The program will feature three final research reports and one interim report covering an interesting range of topics dealing with on-farm risks from tree-frog intrusions to buffer zone designations on mixed-farms and *Cyclospora* prevalence in agricultural water. For information on how to register for Session 3, see the CPS website: www.centerforproducesafety.org.

Acknowledgements*: The Center for Produce Safety would like to thank the researchers who made presentations during Session 2 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 - Bonnie@centerforproducesafety.org. Thank you.*