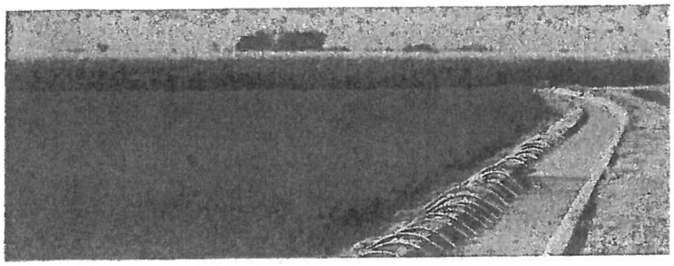
# General Session

*Asterisks denote Specialty Crop Block Grant support. Key on page 36.*



## AGRICULTURAL WATER

**Project Title**
**Identification of novel indicator organisms to determine the risks of fecal contamination of irrigation waters**

**Principal Investigator**
**Kelly Bright, University of Arizona**

**Project Term**
January 1, 2016 – December 31, 2017

**Non-Technical Summary**
The methods used to detect *E. coli* were developed for drinking water and are known to produce high levels of false-positive and false-negative results when used for irrigation waters. Therefore, growers are required to make decisions about water quality/safety based on inaccurate tests. Our project goal is to identify microorganisms which may be used as novel indicators of the presence of pathogens (not just fecal contamination) in irrigation waters to allow the produce industry to make more accurate risk-based assessments to determine when it is safe to irrigate crops. Our specific objectives are the following: 1) We will examine irrigation water to determine the levels of fecal indicator and pathogenic bacterial/viral species by existing cultural and/or molecular methods; 2) We will determine the composition (presence and relative abundance) of the entire bacterial, protozoan, and fungal communities found in irrigation water using "next-generation" sequencing; 3) We will identify groups or specific species whose presence correlate well (presence/absence and relative abundance) with the occurrence of foodborne pathogens in irrigation waters. The use of more meaningful indicator species will provide growers with more accurate information upon which to optimize their irrigation practices to minimize the risk of contamination of produce by foodborne pathogens.

**Funding**
California Department of Food and Agriculture Specialty Crop Block Grant; Center for Produce Safety**

**Acknowledgements**
Luisa Ikner, Walter Betancourt, Enue Sicairos, Patricia Gundy, Huruy Zerzghi, Sherif Abdel Maksoud, Monique Torres, Brianna Leija, Candace Garrett, Jennifer Pearce-Walker, Irma Perez, Christina Morrison

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**Project Title**
**Microbial food safety risks of reusing tail water for leafy greens production**

**Principal Investigator**
**Michael Cahn, University of California, Cooperative Extension**

**Project Term**
January 1, 2016 – December 31, 2017

**Non-Technical Summary**
The use of sprinklers and furrow irrigation frequently results in significant volumes of run-off, also referred to as tail water. Although vegetable growers have made much progress in reducing irrigation run-off by using drip lines, overhead sprinklers are needed for germinating and establishing crops, and for watering high-density leafy greens such as spinach and baby greens. Also, a significant number of acres of lettuce and other vegetables are irrigated by furrow after crop establishment. Many Central Coast ranches have infrastructure for reusing tail water for irrigating crops, including sediment basins, reservoirs, and pumping systems. Currently growers are reluctant to irrigate crops with tail water due to a lack of information on microbial food safety risks. Several options exist for reusing run-off water, which may

minimize microbial food safety risks for produce. Tail water could be used for irrigation practices that do not result in direct contact with the crop, such as pre-irrigation and germination, as well as for dust control of unpaved roads. Tail water could be reused for crops on drip. Other options include treating run-off by chlorination or other means to kill microbial pathogens, and blending tail water with a clean water source so that microbial levels meet industry or regulatory target levels for surface water. The objectives of this project will be to: 1) monitor, characterize and quantify microbial populations in run-off water from Central Coast vegetable fields; 2) evaluate the risk of using this water source for the production of lettuce and other leafy green crops by quantifying survival of microorganisms during reuse applications; and 3) evaluate economically-feasible methods to treat tail water that would minimize microbial food safety risks for a range of reuse applications (e.g., pre-irrigation, dust control, irrigation).The food safety risk of reusing run-off water needs to be evaluated in commercial vegetable fields under conventional irrigation practices. Our project would address this need, and develop information on the food safety risks associated with re-using run-off water for leafy green production

**Funding**
California Department of Food and Agriculture Specialty Crop Block Grant; Center for Produce Safety; CA Leafy Greens Research Board **

**Acknowledgements**
Co-PIs: Linda Harris, Department of Food and Science and Technology, University of California, Davis; Steven Koike, University of California, Cooperative Extension, Monterey County

Staff: Anne-Laure Moyne, Department of Food Science and Technology, University of California, Davis and Laura Murphy, University of California, Cooperative Extension, Monterey County

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**Project Title**
**Demonstration of practical, effective and environmentally sustainable agricultural water treatments to achieve compliance with microbiological criteria**

**Principal Investigator**
**Ana Allende, CEBAS-CSIC, Spain**

**Project Term**
January 1, 2015 – December 31, 2016

**Non-Technical Summary**
Growers should be assisted in determining the risk associated with agricultural water and the best mitigation option to remove pathogens if needed. Water disinfection is one of the most recommended intervention strategies for irrigation water. The main purpose of this project is to demonstrate a practical, effective, and environmentally sustainable water disinfection treatment. Within this regard, we propose the use of stabilized chlorine dioxide ($ClO_2$) as a suitable disinfection treatment. We will try to establish if stabilized $ClO_2$ could be a suitable disinfection treatment to ensure the compliance with the established microbial limits, particularly fecal indicator bacteria such as E. coli. First, agricultural waters from different water sources will be characterized by microbiological and physicochemical parameters. Optimal operational conditions for stabilized $ClO_2$ as a suitable disinfection treatment will be established first at a pilot scale. After that, demonstration of practical, effective and environmentally sustainable agricultural water disinfection treatment will be carried out at commercial fields, where the impact of stabilized $ClO_2$ on the environment will be also evaluated. We believe that the conclusions will be very valuable for growers who will be able to integrate this technology in their water management practices.

**Funding**
Center for Produce Safety

**Acknowledgements**
Mabel Gil, Senior Researcher CEBAS-CSIC and Co-PI of the project; Pilar Truchado and Francisco López-

# General Session

Gálvez, Post-doc researchers at CEBAS-CSIC; Macarena Moreno, Silvia Andújar and Nathalie Hernandez, Technicians at CEBAS-CSIC; Trevor Suslow, Extension Research Specialist and Scientific Cooperator of the project; PRIMAFLOR SAT and STC S.L.U., Industry cooperators of the project

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**Project Title**

**Improved sampling and analytical methods for testing agricultural water for pathogens, surrogates and source tracking indicators**

**Principal Investigator**

**Vincent Hill, Centers for Disease Control and Prevention**

**Project Term**

January 1, 2015 – December 31, 2016

**Non-Technical Summary**

New rules proposed under the Food Safety Modernization Act (FSMA) establish monitoring frequencies and *Escherichia coli* (*E. coli*) concentrations for characterizing agricultural water quality. In addition to monitoring for *E. coli*, other strategies for collecting and testing irrigation water can provide farm operators with a better understanding of the quality of water used in crop production. These strategies include collecting source water samples during times of greater potential risk for contamination (e.g., after rain events) and testing for pathogens and alternative water quality surrogates. In this project, ultrafiltration will be used to collect large-volume irrigation water samples from three farms in Georgia to investigate the benefits of collecting such samples for microbial water quality testing. Baseline and precipitation-impacted samples will be collected to enhance the comparison of large- versus small-volume collection procedures. Samples will be tested for traditional indicators of fecal contamination (*E. coli* and enterococci), alternative surrogates of fecal contamination (F+ coliphages), pathogens (*Salmonella*, *Cryptosporidium* and *E. coli* O157:H7), and analytes that can be used to identify sources of fecal contamination affecting agricultural water quality. This study will result in development of sampling and testing procedures for analysis of large-volume irrigation water samples for alternative microbial water quality parameters.

**Funding**

California Department of Food and Agriculture Specialty Crop Block Grant; Center for Produce Safety *

**Acknowledgements**

Center for Produce Safety; Dr. Vincent Hill, CDC Dr. Karen Levy, Emory University; Dr. George Vellidis, University of Georgia; Dr. Moukaram Tertuliano, University of Georgia; Amy Kahler, CDC; Mia Mattioli, Emory Postdoctoral Fellow; Candace Miller, CDC

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## PACKINGHOUSE/SUPPLY CHAIN FOOD SAFETY

**Project Title**

**Factors that influence the introduction, fate and mitigation of foodborne pathogens on mangoes throughout the production chain**

**Principal Investigator**

**Michelle Danyluk, University of Florida**

**Project Term**

January 1, 2016 – December 31, 2017

**Non-Technical Summary**

Understanding the persistence and mitigation of foodborne pathogens on the surface of mangoes is

essential to the establishment of Best Management Practices for the responsible handling, packing, distributing, and importing of mangoes, and is a fundamental management prerequisite to providing customers with safe mangoes. There is inadequate science-based data to establish management standards and criteria for mangoes to meet pending requirements of the Food Safety Modernization Act. The purpose of this research project is to evaluate the persistence of foodborne pathogens on the surfaces of whole and fresh-cut mangoes, assess potential mitigation strategies for control of pathogens on mango surfaces, and appraise the ability of *Salmonella* to infiltrate mangoes under standard packinghouse conditions and then to determine the fate of the internalized cells. The research results will specifically address data gaps the National Mango Board currently faces, and will provide research-based metrics to validate mitigation strategies.

**Funding**
Center for Produce Safety

**Acknowledgements**
National Mango Board; Lorrie Friedrich, Biological Scientist; Xinyue Wang, MS Student; Vijendra Sharma, Technician

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**Project Title**
**Impact of wash water disinfectants on *Salmonella enterica* transfer and survival in mango packing facility water tank operations**

**Principal Investigator**
**Mary Anne Amalaradjou, University of Connecticut**

**Project Term**
January 1, 2015 – December 31, 2016

**Non-Technical Summary**
Foodborne outbreaks associated with consumption of raw mangoes have been traced back to the use of contaminated wash water. This highlights the critical role of wash water disinfection in mango processing, affecting its quality, and safety. While investigations on the efficacy of disinfectants to reduce pathogens on other fruits have been performed, no studies have been conducted on mangoes. Therefore, this study will investigate the efficacy of different disinfectants (chlorine, peracetic acid and FIT fruit and vegetable wash solutions) for killing *Salmonella* on mangoes and prevention of water-to-mango cross contamination. The study will be performed under conditions that simulate dump tank washing, hot water treatment and hydrocooling. Additionally, the study will investigate the efficacy of trans-cinnamaldehyde, a GRAS status antimicrobial for use as a natural, alternative disinfectant in mango wash water. It is expected that this study will provide insight into the efficacy of disinfectants to inactivate *Salmonella* in mango packing facility water operations. Furthermore, this proposal will help us understand the role of organic load in mango wash water on disinfectant efficacy. In conclusion, the proposed research is expected to help develop best practices regarding post-harvest washing and disinfection of mangoes to control *Salmonella* and other potentially pathogenic organisms.

**Funding**
National Mango Board; Center for Produce Safety

**Acknowledgements**
National Mango Board; Center for Produce Safety

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**Project Title**
**Control of cross-contamination during field-pack and retail handling of cantaloupe**

**Principal Investigator**
**Laura Strawn, Virginia Tech**

**Project Term**
January 1, 2016 – December 31, 2017

**Non-Technical Summary**

Following recent melon-associated foodborne outbreaks, California cantaloupe growers voluntarily developed and implemented commodity-specific food safety guidelines for the safe handling of cantaloupes and other netted melons. While this document details best food safety practices to reduce the risk of contamination during production, packing, and distribution, science-based metrics describing transfer coefficients for pathogen contamination onto melons during field-packing operations are needed. Cross-contamination is a known food safety risk in many environments, and is dependent on many variables, including transfer surface, commodity surface, and contamination level. Cross-contamination was highlighted by the Food and Drug Administration as a critical factor contributing to recent cantaloupe-associated outbreaks. To date, no published literature evaluating the cross-contamination potential of whole melons has been established under typical field-packing conditions. Furthermore, there is a lack of data on handling practices related to cross-contamination risks for melons in the retail distribution supply chain, as well as guidance on the safe and uniform handling of melons throughout the retail environment. Through this proposed project, potential cross-contamination points likely to increase risk will be identified, and intervention strategies targeted to reduce the occurrence of pathogen transfer events during the handling of melons at harvest and retail will be developed.

**Funding**

California Department of Food and Agriculture Specialty Crop Block Grant; Center for Produce Safety **

**Acknowledgements**

Michelle Danyluk, Co-PI, University of Florida; Lorrie Friedrich, Biological Scientist, University of Florida; Ben Chapman, Co-PI, North Carolina State University; Chris Rupert, MS Student, North Carolina State University; Rachel Pfuntner, Research Scientist, Virginia Tech; Laura Truitt, Research Scientist, Virginia Tech

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**Project Title**

**Validation of chlorine level in sanitation systems to avoid cross-contamination**

**Principal Investigator**

**Qin Wang, University of Maryland**

**Project Term**

January 1, 2015 – December 31, 2016

**Non-Technical Summary**

Scientifically validated data are needed by regulators and commercial fresh-cut processors to determine the minimum chlorine concentration required to prevent pathogen cross-contamination in produce wash waters. This project will address this issue by development of a microfluidic mixer that simulates cross-contamination and pathogen survival scenarios in chlorinated produce wash water. The microfluidic mixer has the unique capability of manipulating solutions at a miniaturized scale within instantaneous response times. Thus, the mixer will provide a technological solution to the limitations encountered in macroscale testing. This project will investigate the relationship between contact time (0.1 second – 5 minutes) and chlorine level (0.125 to 50 ppm) to prevent cross-contamination in wash water. Minimal contact times for given chlorine levels will be determined for planktonic and biofilms of bacterial cells. Results will be further validated using industrial produce wash water to investigate the effects of operational variables (pH, temperature, organic load) on the contact time requirement at different chlorine levels. We anticipate the proposed study will provide insight on the relationship between minimal contact time and chlorine level, and therefore predict the processing requirement at given variables to effectively prevent cross-contamination in wash water.

**Funding**

Center for Produce Safety; University of Maryland

**Acknowledgements**

Dr. Yaguang (Sunny Luo), Research Food Technologist, USDA Agricultural Research Service; Dr. Patricia Millner, Research Microbiologist, USDA Agricultural Research Service; Dr. Boce Zhang, ORISE Fellow,

USDA Agricultural Research Service; Dr. Bin Zhou, Research Microbiologist, USDA Agricultural Research Service; Dr. Zi Teng, Post-doctoral Research Associate, University of Maryland, University of Maryland NanoCenter and Fabrication Lab

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## VALIDATION

**Project Title**
**Comparative genomics analysis and physiological assessment of the avirulent *Salmonella* surrogate relevant to food safety**

**Principal Investigator**
**Julie Meyer, University of Florida**

**Project Term**
January 1, 2016 – December 31, 2017

**Non-Technical Summary**
Coliforms and generic *E. coli* are poor predictors of the behavior of human pathogens (like *Salmonella*, pathogenic *E. coli* and *Listeria)* in the crop production environment. Mounting evidence suggests that accurate models of *Salmonella* behavior in the production environment will have to be built based on the experiments conducted with *Salmonella*, and not based on data from distantly related surrogates like generic *E. coli*. This, however, necessitates availability and careful characterization of "disarmed" strains of *Salmonella* that could be used for on-site research. Upon completion of this study we will have developed robust tools for modeling behavior of these outbreak strains in the pre- and post-harvest production environments. The purpose of this project is to carry out comparative genomic and physiological characterization of the outbreak strains under production conditions and to compare them with the nonvirulent strain of *Salmonella* that we have developed. We will also have tested two key hypotheses aimed at understanding why only a dozen out of over 2,500 *Salmonella* serovars are associated with produce-linked outbreaks of illness. With previous CPS funding we engineered and verified the first nonvirulent, nontransgenic strain of *Salmonella* suitable for on-site studies as an indicator organism.

**Funding**
California Department of Food and Agriculture Specialty Crop Block Grant; Center for Produce Safety **

**Acknowledgements**
Dr. Max Teplitski, University of Florida and Dr. Marcos de Moraes, University of Florida

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**Project Title**
**Establishing die-off rates of surrogate and virulent EHEC/STEC strains from strawberry and cilantro surfaces: time, inoculum dose and chemical intervention**

**Principal Investigator**
**Eduardo Gutierrez-Rodriguez, North Carolina State University**

**Project Term**
January 1, 2016 – December 31, 2016

**Non-Technical Summary**
The fresh produce industry is facing major changes in production practices due to the implementation of the FDA Food Safety Modernization Act (FSMA). Within the new rules the standards associated with water quality are among the most contested by

industry associations. Current guidelines require water that will be in direct contact with the crop, to meet specific microbiological thresholds based on the 2012-EPA recreational water standards. Alternative provisions to comply with these rules have also been allowed by FDA when water cannot meet these standards. One of these options considers a microbial die-off rate of 0.5 log per day that may occur naturally between irrigation and harvest events as a safe alternative practice. Despite this potentially useful provision, there needs to be science-based information supporting this option, especially on cilantro and strawberry for which few or no further disinfection steps are commercially available after harvest and where large volumes of surface or well water are used for frost protection (strawberry) and overhead irrigation (cilantro). This research focuses on optimizing the existing knowledge in microbial die-off of avirulent and pathogenic EHEC- STEC microorganisms to determine whether the proposed microbial die-off rate is a safe farm practice to follow when using water that cannot meet the EPA microbial standards.

**Funding**
Center for Produce Safety

**Acknowledgements**
Co-PIs: Chris Gunter, North Carolina State University; Sid Thakur, North Carolina State University; Carole Saravitz, North Carolina State University, Dr. Victoria Albarracin

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**Project Title**
**Validating a physically heat-treated process for poultry litter in industry settings using the avirulent *Salmonella* surrogates or indicator microorganisms**

**Principal Investigator**
**Xiuping Jiang, Clemson University**

**Project Term**
January 1, 2016 – December 31, 2017

**Non-Technical Summary**
Poultry litter is an excellent source of nutrients for the growth of agricultural crops. To reduce the microbiological risks associated with the use of raw poultry litter as a soil amendment or organic fertilizer, heat treatment is recommended to reduce or eliminate potential pathogenic microorganisms. Our recent studies have demonstrated that thermal resistance of *Salmonella* in chicken litter is increased significantly when cells are adapted to desiccation or when aged chicken litter with low moisture content is heat treated. By increasing the moisture level in chicken litter or applying a two-step heat treatment (wet heat followed by dry heat), *Salmonella* can be inactivated more rapidly. Our preliminary results indicate a good correlation in thermal inactivation rates between desiccation-adapted *Salmonella* and indigenous enterococci in chicken litter, suggesting enterococci as a potential indicator for heat process validation. We will collaborate with two large poultry litter processors to validate their heat-treatment processes in industrial settings by using *Salmonella* surrogate and indicator microorganisms identified in this study. Results from this research will provide some valid guidelines and tools for the fertilizer industry to produce *Salmonella*-free heat-treated poultry litter, thereby ensuring safe production of fresh produce.

**Funding**
California Department of Food and Agriculture Specialty Crop Block Grant; Center for Produce Safety **

**Acknowledgements**
Dr. Annel Greene (Co-PI), Maple Wang and Jack Chen (graduate students), and our industry collaborators

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**Project Title**
**Improving pasteurization validation methods for pistachio processing**

**Principal Investigator**
**Bradley Marks, Michigan State University**

**Project Term**
January 1, 2015 – December 31, 2016

**Non-Technical Summary**
Microbial safety of low-moisture foods is a particularly difficult challenge, as reflected in recent outbreaks and/or recalls associated with *Salmonella*-contaminated nuts and other low-moisture products. Therefore, processing interventions are an emerging imperative to reduce the risk of *Salmonella* in low-moisture products, including pistachios. The Food Safety Modernization Act (FSMA) proposed Preventive Controls rule will mandate that the low-moisture food industry implement and validate interventions against identified hazards, such as *Salmonella*. Although a number of pathogen-reduction technologies are available to the pistachio industry (e.g., dry heat, steam, radio-frequency), there are several significant problems: (1) No single technology will be universally applicable, so that product-specific/scalable solutions are needed; (2) The cost of stand-alone pasteurization technologies is an impediment to small processors; and (3) Robust validation protocols have not been widely tested or disseminated. Therefore, the overall goal is to improve the methods for validating pathogen-reduction processes for pistachios, with particular attention to improving existing processes and enabling any processor to reliably validate those processes. The work plan includes laboratory- and pilot-scale experiments with *Salmonella*-inoculated pistachios, and a process validation demonstration at the commercial-scale. A key project outcome will be a guidelines document for methods to validate preventive control measures for pistachios.

**Funding**
California Department of Food and Agriculture Specialty Crop Block Grant; Center for Produce Safety
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**Acknowledgements**
Co-PIs: Linda J. Harris, University of California, Davis; Elliot T. Ryser, Michigan State University

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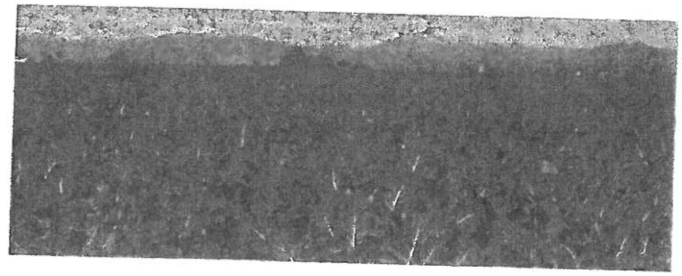

## HOT TOPICS

Understanding what a positive STEC result means, determining effective ways to control Lm, quantifying the importance of parasites as a produce safety issue, and gaining insights into the role the physiological state of a pathogen may play in conducting validation experiments are at the cutting edge of food safety challenges. The programs in this final session will provide emerging data on these topics and commentary on how the industry can best apply this information.

**Project Title**
**Rapid tests to specifically differentiate clinically significant from environmental STEC towards reducing unnecessary crop destruction**

**Principal Investigator**
**Trevor Suslow, University of California, Davis**

**Project Term**
January 1, 2015 – December 31, 2016

**Non-Technical Summary**
Though exceptionally rare events, relative to the scale of production and consumption, there is ample evidence to know that produce samples sometimes contain pathogens of serious potential human health consequences. Shiga toxin-producing *E. coli* (STEC) from diverse fresh produce were recovered from multi-year sampling programs conducted by the UDSA, largely at wholesale distribution centers. Leafy greens, herbs, and specifically spinach were singled out for concern due to a STEC prevalence rate exceeding 50% of the total isolates recovered. Product testing is used by many but not all producers to pre-screen leafy greens for bacterial pathogens, including STEC. Unfortunately, not all testing platforms rapidly distinguish STEC likely

to cause human illness from those that lack the genetic traits necessary for infection. Due to the high perishability of these commodities, testing can lead to destruction of a field due to false association with dangerous STEC. The combined objectives of protecting consumers, reducing food waste, and improving sustainability can be enhanced by applying new advancements proposed in this research in specific detection of clinically relevant STEC to risk management decisions and better defining the role of wildlife as vectors of preharvest contamination.

**Funding**
California Department of Food and Agriculture Specialty Crop Block Grant; Center for Produce Safety *

**Acknowledgements**
UCD - Adrian Sbodio, Janneth Pinzon, Jeremy Roland, Lee Ann Richmond; WCFS - Michele Jay Russell, Nora Navarro Gonzalez, Peiman Aminabadi;
Roka BioScience; Cooperating Grower-Shipper-Processors

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**Project Title**
**Evaluation of the efficacy of antimicrobial agents to prevent the transfer of *Listeria monocytogenes* from existing biofilms to produce or processing surfaces**

**Principal Investigator**
**Rolf Joerger, University of Delaware**

**Project Term**
January 1, 2016 – December 31, 2017

**Non-Technical Summary**
*Listeria monocytogenes (Lm)* is a foodborne pathogenic bacterium that can cause serious illness and even death in susceptible individuals. Outbreaks involving this pathogen have been associated with fruits, sprouts and vegetable row crops. Like most other bacteria, *Lm* can form biofilms or became part of biofilms with other bacteria on produce surfaces and surfaces in produce harvesting and processing environments. Once established in a biofilm, *Lm* has highly diminished susceptibility to antimicrobial agents and is difficult to eradicate. Cells surviving in such biofilms can detach and be carried to new surfaces where they can start the formation of a new biofilm or become part of an existing biofilm. It is therefore extremely important to prevent the transfer of cells from existing biofilms to previously uncontaminated surfaces on produce or processing equipment. The proposed study will examine the efficacy of antimicrobial agents to inactivate *Lm* released from existing biofilms and prevent the formation of new *Lm*-containing biofilms on produce and equipment surfaces.

**Funding**
Center for Produce Safety

**Acknowledgements**
Gordon C. Johnson, Department of Plant and Soil Sciences, University of Delaware; Arpeeta Ganguly, Visiting Scientist; Juliet Wachira, Graduate Student; Quincy Hardy, Graduate Student

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**Project Title**
**Methods for the detection of diverse parasites on packaged salads based on (viable) oocysts**

**Principal Investigator**
**Stefan Wuertz, University of California, Davis**

**Project Term**
January 1, 2016 – May 30, 2017

**Non-Technical Summary**
This project will involve an approach for managing and monitoring produce safety to reduce the risk of foodborne illness from consumption of packaged salads: detecting human parasites on the surfaces of leafy greens in a rapid, accurate, and affordable manner. A novel test will simultaneously detect four key parasites that can be associated with produce-

borne disease. Applying the newly validated tests in parallel with previously established methods for testing packaged salads will provide a realistic evaluation for the suitability of the developed assays for routine screening by the produce industry. Additional methods will determine the viability of these parasites and whether they are likely to cause disease to produce consumers if they are detected.

**Funding**
California Department of Food and Agriculture Specialty Crop Block Grant; Center for Produce Safety **

**Acknowledgements**
Karen Shapiro, assistant research faculty; Minji Kim, postdoctoral scholar; Veronica Rajal, associate professor (National University of Salta, Argentina); Beatriz Aguilar, staff research associate (SRA); Andrea Packham, SRA; Brittany Dalley, laboratory assistant; Lezlie Rueda, undergraduate assistant; Patricia Conrad, professor

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**Project Title**
**Pathogen physiological state has a greater effect on outcomes of challenge and validation studies than strain diversity**

**Principal Investigator**
**Martin Wiedmann, Cornell University**

**Project Term**
January 1, 2016 – December 31, 2017

**Non-Technical Summary**
Effective control of foodborne disease-causing microbes ("pathogens") requires science-based validation of interventions and control strategies. For example, it is important to show that a given antimicrobial treatment can reduce bacterial numbers with a certain target efficiency, regardless of the specific genetic type of organism and regardless of the conditions under which an organism was grown prior to treatment. This is important, as it has been shown that *Salmonella* exposed to dry environments can be >100 times more resistant to some treatment (e.g., heat) than *Salmonella* grown in the presence of high levels of water. This project will assemble a collection of diverse microbes that are appropriate for validation of pathogen interventions in the produce industry, and will evaluate these organisms to determine whether and how exposure to different environmental conditions will affect the ability of these organisms to survive stressful conditions and control strategies. The resulting data, along with the bacterial collection developed as part of this project, will facilitate more reliable identification of effective control strategies that can reduce the risk of foodborne illnesses and pathogen contamination.

**Funding**
California Department of Food and Agriculture Specialty Crop Block Grant; Center for Produce Safety ***

**Acknowledgements**
Center for Produce Safety. We also appreciate the contributions of the reviewers of our strain collection drafts and contributors to the strain collection (P. Gerner-Smidt, M. Danyluk, L. Harris, R. Worobo, K. Nightingale, M. de Moraes, M. Allard, P. Fratamico, T. Monson, K. Musser, P. Griffin, M. Parish, S. Assar, M. Sharma, E. Brown, R. Mandrell, B. Sauders, L. Gorski, E. Berry, R. Boyer, L. Beuchat, T. Suslow, J. Farber, F. Yiannas, J. Brennan, B. Whitaker, D. McDonald, K. Refsnider, T. Stoltenberg, G. Keller, J. Reed).

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CPS Research Symposium 2016, Seattle