



A Practical Guide to the Scientific Research Presented at
The Center for Produce Safety (CPS) Produce Research Symposium
June 26, 2013

Rochester, New York



November 10, 2014

Prepared by



ACKNOWLEDGEMENTS

This report was made possible by the dedicated contribution of the following individuals:

Diane Wetherington, CEO of IDS & iFood

Susan Leaman, Vice President, IDS LLC

Diane Mendez, Graphic Artist, Western Growers

Sonia Salas, Science & Technology Director, Western Growers

1.0 Background	4
2.0 Comments Regarding This Summary	4
3.0 Session I – COMPOST AND AG PRACTICES/PATHOGEN SURVIVAL	5
Project #1: Validating <i>Salmonella</i> inactivation during thermal processing of the physically heat-treated chicken litter as a soil amendment and organic fertilizer (1/1/2012 – 1/31/2014).....	6
Project #2: Validation of testing methods for the detection and quantification of <i>E. coli</i> O157:H7, <i>Salmonella</i> spp., fecal coliforms and non-pathogenic <i>E. coli</i> in compost (1/12/2012 – 12/31/2012)	8
Project #3: Investigation of <i>E. coli</i> survival on contaminated crop residue (1/1/2011 – 12/31/2012)...	10
Project #4: Glucosinolate-derived compounds as a green manure for controlling <i>E. coli</i> O157:H7 and <i>Salmonella</i> in soil (1/1/2012 – 2/28/2014).....	12
4.0 Session II – WATER QUALITY FOR IRRIGATION AND POSTHARVEST PRACTICES	15
Project #5: Assessment of <i>E. coli</i> as an indicator of microbial quality or irrigation water use for produce (1/1/2012 – 12/31/2012).....	17
Project #6: Evaluation of sampling protocol to provide science-based metrics for use in identification of <i>Salmonella</i> in irrigation water testing programs in mixed produce farms in the Suwannee River Watershed (1/1/2012 – 12/31/2013).....	19
Project #7: Enhancing the efficacy of fresh produce washing operations through establishing monitoring methods and water disinfection technologies based on a combination of filtration and UV(1/1/2011 – 12/31/2012)	21
Project #8: Irrigation regime, fruit water congestion and produce safety: parameter optimization to reduce susceptibility of tomatoes and peppers to post-harvest contamination, pathogen transfer and proliferation of <i>Salmonella</i> (1/1/2011 – 12/31/2012).....	23
5.0 Session III – PATHOGEN TRANSFERENCE: PRE-HARVEST, HARVEST AND PACKING	25
Project #9: <i>Escherichia coli</i> O157:H7 in bioaerosols from cattle production areas: evaluation of proximity and airborne transport on leafy green contamination (1/1/2011 – 12/31/2012)	27
Projects #10: The likelihood of cross-contamination of head lettuce by <i>E. coli</i> O157:H7, <i>Salmonella</i> and norovirus during hand harvest and recommendations for glove sanitizing and use (1/1/2011 – 12/31/2012).....	29
Project #11: Pathogen transfer risks associated with specific tomato harvest and packing operations (1/1/2011 – 12/31/2012).....	31
Project #12: Survival, transfer and inactivation of <i>Salmonella</i> on plastic materials used in tomato harvest (1/1/2011 – 2/28/2013).....	33
6.0 Session IV – HOT TOPICS	35
Project #13: Sanitization of soft fruits with ultraviolet (UV-C) light (1/1/2012 – 3/31/2013).....	37
Project #14: Influence of the pre-harvest environment on the physiological state of <i>Salmonella</i> and its impact on increased survival capability (1/1/2011 – 12/31/2012)	39
Project #15: Comparative assessment of field survival of <i>Salmonella enterica</i> and <i>E. coli</i> O157:H7 on cilantro (<i>Coriandrum sativum</i>) (1/1/2012 – 12/31/2012)	41
Project #16: The role of riparian zones in bacteria dispersal to produce farms (1/1/2012 – 3/31/2014)	42

I.0 BACKGROUND

This report is a compendium of the 2013 Center for Produce Safety (CPS) Symposium presentations with a discussion of the findings and their significance for industry to use.

This report is intended as a summary and layman guide to the technical sessions presented at the 2013 Center for Produce Safety Research Symposium held at Wegmans Conference Center, Rochester, New York. The Center for Produce Safety was designed and operates as collaboration among industry, government, scientific and academic entities. CPS is a leader in the delivery of science-based research for the produce industry recognized by stakeholders for linking scientists with stakeholders, and for their scientific research funding process, research project management, translation of scientific reports and industry outreach.

At the 2013 Symposium, Dr. Robert Whitaker, Chairman of CPS' Technical Committee and Chief Science and Technology Officer at the Produce Marketing Association (PMA) facilitated presentation of the final results for 16 projects in four separate sessions:

- Compost and Agricultural Practices/Pathogen Survival,
- Water Quality for Irrigation and Postharvest Practices,
- Pathogen Transference: Preharvest, Harvest and Packing, and
- Hot Topics.

Researchers presenting represented eight academic institutions in seven states and one Canadian province as well as the U.S. Department of Agriculture. As in prior years, each session consisted of a brief presentation followed by a Q&A session with a panel comprised of industry executives, regulatory leaders and academic scientists, discussing the findings. The panel discussions highlighted how the research results are being applied or could be applied to a particular commodity, product, or process. Concluding the event was a fifth session consisting of a government/industry lead discussion facilitated by Bryan Silbermann, President and CEO of PMA. There were also poster presentations for an additional 33 projects. Poster presentations and project final reports are available on the CPS website at https://cps.ucdavis.edu/poster_session.php and https://cps.ucdavis.edu/grant_opportunities_awards.php.

This report discusses the 16 presentations, final reports for each project, prior research funded through CPS relevant to the current findings and considerations from the panel discussions. The authors' intent is for the produce industry to use this report as a guide in understanding the latest research and in determining how to apply the research to day-to-day operations. In addition to a review of each research project's key findings, an interpretation of what the findings mean for growing, harvesting and processing operations are presented. Several overall recommendations are also made to industry for use in applying the research and/or research methodology to food safety concerns.

2.0 COMMENTS REGARDING THIS SUMMARY

This report has been prepared as guidance for members of the produce growing, harvesting and processing communities and does not supersede any regulations nor does it constitute legal guidance. Project titles and Layman's Summaries appear as submitted by the principal investigators in their project proposals. Recommendations and statements of "what does this mean for you" are solely the interpretation of the authors of this document.

SESSION I: *Compost and Ag Practices / Pathogen Survival*



3.0 SESSION I – COMPOST AND AG PRACTICES/PATHOGEN SURVIVAL

PROJECT #1: Validating *Salmonella* inactivation during thermal processing of physically heat-treated chicken litter as soil amendment and organic fertilizer (1/1/2012 – 1/31/2014).

Principal Investigator: Xiuping Jiang, Ph.D., Clemson University

Layman's Summary (Source: CPS Program Notebook – June 26, 2013)

There is a growing demand for the physically heat-treated chicken litter being used as a soil amendment and organic fertilizer for plant growth. However, chicken litter is often contaminated with human pathogens such as *Salmonella* spp. Heat treatments have been recommended to reduce or eliminate these pathogens, but no scientific research has been reported to validate if these treatments are adequate to produce the finished product free from pathogenic microorganisms. The proposed study will determine the thermal resistance of a mixture of four *Salmonella* serotypes at temperatures recommended for heat treatment of chicken litter. Several key environmental factors such as moisture level, nutrient variation, and the freshness of chicken litter will be evaluated. Furthermore, a practical method for combining moist heat treatment with drying process will be investigated for rapidly inactivating *Salmonella* in broiler chicken litter. The results from this study will provide some practical guidelines on time-temperature combination to treat chicken litter of different properties to produce the finished products *Salmonella* free. The ultimate goal of this project is to help the produce industry to grow safe fresh produce using organic fertilizers free from human pathogens.

Technical Findings:

The objective of this project was two-fold: 1) to validate *Salmonella* inactivation during the heating processes as recommended for the physically heat-treated chicken litter by considering several factors such as type, dryness and freshness of chicken litter, and 2) to develop a two-step heat treatment for rapid pathogen inactivation. The experimental approach used four rifampicin-resistance *Salmonella* serovars — Enteritidis, Heidelberg, Senftenberg, and Typhimurium, and compared results in aged and fresh litter and broiler and egg-laying chickens. To meet study objectives, first, the efficiency of several different media for the recovery of heat-injured *Salmonella* was evaluated. Then a procedure to create desiccation-adapted (DA) *Salmonella* was developed and optimized. This was followed by experiments to test the thermal resistance of desiccation-adapted *Salmonella* in partially composted chicken litter exposed to heat treatments at 70, 75, 80, 85 and 150°C with adjusted moisture content (20, 30, 40, and 50%).

The following are key findings (obtained from the June 2013 CPS Symposium presentation, the panel discussion and the final project report):

Finding 1: Thermal resistance was enhanced in DA *Salmonella* in comparison to control non-adapted *Salmonella*. Higher numbers of desiccation-adapted *Salmonella* survived for longer as compared to control cells.

What does this mean for you?

Salmonella is able to survive harsh conditions such as low moisture levels by adapting to those conditions. The adapted pathogen tends to survive other environmental stresses as well. This stresses the importance of process validation and monitoring processes to make sure process

parameters are being met and maintained both during manufacturing and storage. In the case of thermally treated chicken litter, *Salmonella* that had been under environmental stress (i.e., desiccated) may have increased resistance to thermal treatment. In this study, DA *Salmonella* was not detectable when temperatures reached 150°C, moisture levels were > 40%, and thermal process duration exceeded 40 minutes. As a result, when lower processing temperatures (<150°C), low moisture content (<40%) and shorter processing times (<40 min.) are used to treat chicken litter, *Salmonella* can regrow particularly when applied to soil.

Finding 2: *Salmonella* serotypes had varying responses to thermal exposure.

What does this mean for you?

Salmonella Senftenberg was the most heat-resistant *Salmonella* serotype making it a potential candidate for an indicator organism to assure microbial safety of chicken litter when validating thermal processing protocols or other heat challenge studies.

Finding 3: Thermal resistance of *Salmonella* in chicken litter is influenced by moisture level, types and freshness of litter, physiological state of the pathogen (e.g., stressed), and type of heat source.

What does this mean for you?

Composting companies should understand how these various chicken litter parameters affect their treatment processes and adjust the process accordingly. Thermal treatment processes need to be understood and tested, particularly in relation to how moisture affects the treatment results — either during treatment or during subsequent storage. Compost users need to make sure compost companies have validated the effectiveness of their thermal processing procedures. Users should study how they store and apply chicken litter to minimize the possible risk of *Salmonella* regrowth.

Conclusions/Recommendations/Next Steps:

Studies have demonstrated the presence of *Salmonella* in chicken litter. Since chicken litter is used as an organic soil amendment and fertilizer, care needs to be taken to ensure *Salmonella* does not become a contamination source for the associated crop. This may require a re-examination and/or changes in current thermal treatment and storage practices. However, it appears as if a validated two-step process using moist heat treatment for one hour at 65°C followed by dry heat treatment for one hour at 85°C is needed to obtain a >5-log reduction by stabilizing the chicken litter and overcoming the potential for *Salmonella* resistance to heat treatment through water replacement. During storage and application, suppliers need to minimize the potential for recontamination. Considerations should be given to the how the heat treated chicken litter is being used (i.e., application to planting/harvest intervals) and an evaluation made of the potential risks associated with thermal treatment and storage practices (i.e., protocols that minimize recontamination).

PROJECT #2: Validation of testing methods for the detection and quantification of *E. coli* O157:H7, *Salmonella* spp., fecal coliforms and non-pathogenic *E. coli* in compost (1/12/2012 – 12/31/2012)

Principal Investigator: Manan Sharma, Ph.D., Agricultural Research Service, USDA

Layman's Summary (Source: CPS Program Notebook – June 26, 2013)

Compost is a valuable soil amendment used by organic and conventional growers to improve the physical, chemical and biological properties of soil. Compost is produced from a variety of feedstocks that are sources of potentially pathogenic microbes, e.g. landscape trimmings, animal manure, food residuals, and biosolids. Thermophilic compost production processes are designed to achieve significant reductions in fecal coliforms and *salmonellae*; however, recent surveys of various 'point of sale' compost products have raised food-safety concerns that compost may be integrating pathogens into the farm environment. Many states are now requiring 'point of sale' compost to be tested for fecal coliforms, *Salmonella*, and *E. coli* O157:H7. These microbiological testing methods have not been evaluated for their effectiveness in detecting *E. coli* O157:H7 and *Salmonella* spp. in the wide variety of composts available for on-farm usage. This proposal will evaluate microbiological testing methods that are currently recommended by the Environmental Protection Agency (USEPA) and the United States Composting Council (USCC) for accuracy in detecting pathogens across a wide variety of 'point of sale' composts. The results from this study will determine the most practical and sensitive microbiological testing methods to ensure the safety of compost for use in the produce industry.

Technical Findings

The objectives of this project were to compare the U.S. Composting Council Test Methods for the Examination of Composting and Compost (TMECC) with the U.S. EPA method to determine their relative effectiveness in recovering fecal coliforms, *Salmonella* spp. and *E. coli* O157:H7. In conducting the research, 29 finished compost samples from 19 states were inoculated with 10-100 CFU/g of non-pathogenic *E. coli*, *Salmonella* spp. and *E. coli* O157:H7 strains and then homogenized for uniform distribution throughout the compost. Inoculated samples were analyzed using U.S. EPA and TMECC methods. In addition, two rapid microbial detection methods utilizing immunomagnetic separation techniques were evaluated in their ability to detect low levels of *E. coli* O157:H7 in point-of-sale compost. A secondary objective was to assess whether soluble carbon profiles can accurately predict the potential for *E. coli* O157:H7 or *Salmonella* regrowth in finished products. *Salmonella* and *E. coli* O157:H7 regrowth was evaluated after compost was stored for three days at 25°C.

The following are key findings (obtained from the June 2013 CPS Symposium presentation, the panel discussion and the final project report):

Finding 1: The U.S. EPA methods recovered significantly higher fecal coliform and *E. coli* levels than the TMECC methods. Both U.S. EPA and TMECC methods recovered equivalent *Salmonella* levels. (The one exception was in compost with a biosolid feedstock where the U.S. EPA method detected higher *Salmonella* levels.) Differences in pathogen recovery levels between the U.S. EPA and TMECC methods may be the result of starting sample volume differences (i.e., 30 g in EPA method versus 25 g in TMECC method).

What does this mean for you?

Test results are affected by the methods and instrumentation used to handle and prepare samples and detect/measure microorganisms. The more knowledge and understanding a compost user has about these methods and instrumentation, the better informed he will be to make decisions about compost use. Compost users should understand their compost supplier's testing programs including details on the sampling methods and sample sizes. Compost suppliers and users should be well acquainted with the laboratory conducting the tests including the methods and instrumentation used for testing. Testing laboratories should educate compost suppliers on test methods and instrumentation being used and associated level of detection. Compost suppliers and users need to become familiar with and understand how detection limits affect test results and confidence in those results, e.g. lower levels of detection imply tests are more sensitive.

Finding 2: Both immunomagnetic separation techniques were equally effective in recovering low population of *E. coli* O157:H7 from inoculated compost samples.

What does this mean for you?

Separating a microorganism of interest from all the other sample components is one of the most difficult challenges in analyzing complex heterogeneous environmental samples like compost. Use of rapid detection methods such as immunomagnetic separation techniques would increase the efficiency and reliability of compost test methods improving confidence in the results for all stakeholders.

Finding 3: No correlation was found between pathogen regrowth and any of the physicochemical properties (total organic carbon, carbon-nitrogen ratios, compost maturity, pH, electrical conductivity, percentage moisture, and percentage organic matter) measured.

What does this mean for you?

There is no simple test to determine if regrowth has occurred or the potential for regrowth. Compost users need not only to be knowledgeable about and confident in the compost production process, but they must do all they can to ensure preventative measures are in place in their own operations to protect against pathogen regrowth.

Conclusions/Recommendations/Next Steps:

While compost is beneficial for both organic and conventional farming operations, care must be taken to ensure foodborne pathogens are adequately controlled during compost production, storage and later application. Not all detection and enumeration methods are validated or equal in their ability to detect/enumerate target pathogens. Compost users need to understand the issues and ramifications related to current testing methods and instrumentation being used to test compost. Some states and audit programs require specific testing methods; however, these research findings demonstrate the need for users to fully explore current requirements, compost supplier composting methods and sampling plans and then work with compost suppliers to address any potential concerns and upgrade detection methods as improved methods become available.

PROJECT #3: Investigation of *E. coli* survival on contaminated crop residue (1/1/2011 – 12/31/2012)

Principal Investigator: Steven Koike, University of California, Cooperative Extension, Monterey County

Layman's Summary (Source: CPS Program Notebook – June 26, 2013)

Our research team has conducted several in-field studies in the Salinas Valley and found that if leafy green crops were exposed to *E. coli* either prior to emergence from soil or early in their growth cycle, the plants at harvest were rarely contaminated and *E. coli* was rarely recovered from soil or water. However, we found that if a mature spinach crop was contaminated with *E. coli* and then disked into the field, we could recover *E. coli* from soil for a much longer period of time (over 85 days). We propose to investigate the dynamics of *E. coli* survival on lettuce residue incorporated into soil. We will establish experimental plots in commercial field settings and inoculate plants prior to disking. As treatments, we will employ commercial ground preparation practices (multiple disking, addition of irrigation water) and evaluate their effects on survival and decline of *E. coli* in soil. Such experiments should provide the grower with practical information on the duration of bacterial survival in soil and the role of post-harvest field practices in reducing bacteria carryover. Because the research will be conducted in the Salinas Valley, our results should reflect real world dynamics of the production environment in coastal California.

Technical Findings

The in-field research objective was to study *E. coli* and *Salmonella* survival on contaminated crop residues after incorporation into soil. Mature crops were inoculated with *E. coli* and *Salmonella* and left undisturbed for 48 hours after which they received no treatment or one of two treatments – mowed (shredding) or ring-roll (breaking up). After treatment, crops were disked either the same day or a week later. Plot irrigation levels were also altered with some plot areas receiving extra irrigation after one week and other areas no extra irrigation. After sampling soil and crop residues for *E. coli* and *Salmonella* over a period of time, a second lettuce crop was planted to determine whether a second crop would become contaminated from the previous crop residue. Plants from the second crop were collected and tested 27 days after planting for possible transference of *E. coli* and *Salmonella* from the contaminated soil to the new plants.

The following are key findings (obtained from the June 2013 CPS Symposium presentation, the panel discussion and the final project report):

Finding 1: *Salmonella* appears to be more persistent in the field than *E. coli*.

The enrichment results demonstrate fewer positive samples for *E. coli* than *Salmonella*, whether waiting one week for disking or disking the same day. In the second planting, *Salmonella* persisted longer than *E. coli*. At 27 days post-planting, no *E. coli* was recovered from plant samples while *Salmonella* persisted. At 35 days post-planting, *Salmonella* was not recovered.

What does this mean for you? This type of research conducted in commercial fields using typical cultivation/remediation practices is valuable in helping growers develop and/or modify practices to reduce pathogens in the field after a contamination event. For example, no recovery of *E. coli* at 27 days post-planting and die-off of *Salmonella* in plants after 35 days can be

helpful in determining actions growers should take following positive product samples to avoid contaminating subsequent plantings.

Finding 2: Post-harvest practices may enhance *E. coli* and *Salmonella* survival.

Salmonella was detected at every sampling in sub-plots that received extra irrigation one week after crop residue incorporation. Irrigation water may affect pathogen survival and recovery.

What does this mean for you?

When crops test positive for *Salmonella*, soil should be monitored for some time after test results are negative. Rain or postharvest irrigation may enhance *Salmonella* survival in a contaminated field. In these experiments soil amendments were not added to the studied plots. Future research investigating the incorporation of soil amendments to contaminated soil would add valuable information on pathogen die-off.

Conclusions/Recommendations/Next Steps:

Post-harvest field practices affect not only soil nutrients but also pathogen die-off, in cases where there is pathogen contamination. While further work is needed to determine how specific practices affect pathogen contamination in the field, this research demonstrates that following a contamination event, field cultivation practices need to be carefully considered.

PROJECT #4: Glucosinolate-derived compounds as a green manure for controlling *E. coli* O157:H7 and *Salmonella* in soil (1/1/2012 – 2/28/2014)

Principal Investigator: Jitendra Patel, Ph.D., Agricultural Research Service, USDA

Layman's Summary (Source: CPS Program Notebook – June 26, 2013)

There have been a series of produce-related outbreaks involving *E. coli* O157:H7 and *Salmonella* in the last 20 years. It is critical to have produce free from pathogens as most produce is consumed raw or minimally processed. Good Agricultural Practices and Good Manufacturing Practices at the pre-harvest and postharvest settings, respectively, have been implemented in recent years to minimize pathogen contamination. However, even occasional transfer of pathogens to fresh produce during pre-harvest can result in outbreaks, necessitating massive produce recalls. This project proposes to evaluate the role of cover crop systems in reducing the risk of pathogens in the pre-harvest environment; specifically, glucosinolate-derived compounds from *Brassica* spp. as a green manure to control *E. coli* O157:H7 and *Salmonella* in soil. Broccoli will be grown in high tunnels and, after harvest of florets, remnant crops will be tilled over in the soil. The persistence of *E. coli* and *Salmonella* strains inoculated in soil will be monitored over a period of time. Results will determine the efficacy of green manure as intervention to control enteric pathogens in soil and on fresh produce.

Technical Findings:

In this research, the antimicrobial activity of the glucosinolate-derived compounds (GSL) found in *Brassica* spp. (cabbage, cauliflower, broccoli, brussels sprouts) was evaluated for effectiveness against *E. coli* O157:H7 and *Salmonella*. First the GSL compounds and their derivatives were tested for effectiveness against five strains of *E. coli* O157:H7, five *Salmonella enterica* serovars associated with produce outbreaks, five nonpathogenic *E. coli* strains, and three *E. coli* O157:H7 mutants using a disk diffusion assay (zone inhibition). Benzyl isothiocyanate (BITC) exhibited a significantly higher zone of inhibition than the other compounds. Following this *in vitro* analysis, field experiments were conducted at a Pennsylvania farm. Twenty-five square feet sub-plots were treated with GSL-derived compounds (acetic acid, cinnamaldehyde, sporan, and BIT) and spray inoculated with 107 CFU/ml *E. coli* O157:H12 30 minutes post-treatment. For the first four weeks, the attenuated pathogen persisted in both treated and untreated soils. After the seventh week, there was a significant reduction in *E. coli* O157:H12 between treated and untreated soil. At 8 weeks, the pathogen was only recovered in untreated soils.

Broccoli cultivars were tested for content of GSL-derived compounds. There were significant differences among the cultivars tested. In high tunnel experiments, Green magic and Imperial cultivars exhibited greater inhibition toward *E. coli* O157:H12 in soil than Arcadia or Belstar.

The following are key findings (obtained from the June 2013 CPS Symposium presentation, the panel discussion and the final project report):

Finding 1: *GSL-derived compounds in broccoli inhibit Salmonella and E. coli populations.*

What does this mean for you?

Many plants contain naturally occurring antimicrobial compounds that evolved as defense mechanisms against plant pathogens. These compounds may also be useful in controlling human pathogen populations in the production environment when used as a rotational crop.

Finding 2: Attenuated *E. coli* populations were reduced to non-detect levels by day 56 in soil tilled over with post-harvest remnant broccoli crop while populations of 3 logs were still present in soils not tilled over. *E. coli* O157:H12 was inactivated by 21 days when soil was treated with a 0.039% BITC solution.

What does this mean for you?

After flooding or other contamination event, GSL-derived compounds can be a potential risk mitigation step in reducing *Salmonella* and *E. coli* populations in agricultural soil. While further research is needed to validate this process, planting a broccoli crop as an active field treatment appears to enhance the reduction of *Salmonella* and pathogenic *E. coli* in soil. In addition, pathogen inactivation rate can be increased by spraying BITC at a low concentration (0.039%) in soil.

Finding 3: There were significant differences in GSL content among broccoli cultivars tested with further variation in the plant fraction.

What does this mean for you?

Not all broccoli cultivars are equal when it comes to GSL content. For this reason, if broccoli is used as an intervention strategy to reduce enteric pathogens such as *Salmonella* and/or pathogenic *E. coli* in the field, careful consideration should be given to the cultivar used. Cultivars with high GSL levels will produce the best results as these compounds are responsible for broccoli's antimicrobial activity. Since florets have the minimal GSL content, harvesting florets before tilling over does not reduce the plant's antimicrobial effectiveness.

Conclusions/Recommendations:

This study demonstrates the effectiveness of GSL-derived compounds in broccoli to reduce *Salmonella* and *E. coli* O157 populations in soil. Use of a produce commodity containing GSL-derived compounds (e.g., broccoli) as a rotational crop may provide a cost effective preventive control for minimizing the presence of human pathogens in soil. In addition, further exploration is needed on the use of GSL-derived compounds such as BITC for remediation of pathogen contaminated soils.

SESSION II: *Water Quality for Irrigation and Postharvest Practices*



4.0 SESSION II – WATER QUALITY FOR IRRIGATION AND POSTHARVEST PRACTICES

PROJECT #5: Assessment of *E. coli* as an indicator of microbial quality or irrigation water use for produce (1/1/2012 – 12/31/2012)

Principal Investigator: Channah Rock, Ph.D., University of Arizona

Layman's Summary (Source: CPS Program Notebook – June 26, 2013)

The goals of this project are to evaluate currently available detection methods for the accurate assessment of *Escherichia coli* contamination in irrigation waters and provide guidance for interpretation of results through a revised risk based *E. coli* standard. Currently, there is concern that the false positive rate of *E. coli* detection may be high in these waters giving false indications of the level of risk from enteric pathogens. This may result in unnecessary costly interventions as well as inaccurate perception of risk among consumers. We propose evaluating three different commercial systems for *E. coli* detection in irrigation waters and assessing false positive rates by use of molecular technologies. As a secondary objective to evaluating *E. coli* as a reliable indicator of food safety risk using data collected in the first stage of this project coupled with existing information found in scientific literature. Ultimately this work will offer recommendations towards the most reliable methods to be used by the produce industry to assess irrigation water contamination as well as a scientific risk-based *E. coli* guideline that growers can use to protect public health.

Technical Findings:

In the first phase of the multi-year project, work was conducted to evaluate existing *E. coli* test methods used with irrigation water for their ease of use and test reliability and sensitivity (number of positives). Since many of the U.S. EPA approved *E. coli* test methods were developed for testing clean drinking water in temperate climates and not for irrigation water, the tests have limitations when applied to agricultural water. Often tests for *E. coli* in agricultural water result in a number of false positives, particularly in surface water systems. In conducting the research, 450 one liter water samples were collected between March and November 2012 from locations in Yuma and Maricopa in Arizona and the Imperial Valley in California. Samples were analyzed using three different commercial systems for detecting and measuring *E. coli* in water — MI agar (a chromogenic/fluorogenic medium), m-ColiBlue24[®] broth, and IDEXX Colilert Quanti-Tray[®]. To analyze false positive and false negative occurrences, a subset of the samples was randomly selected and assessed through DNA sequencing. Further analysis was conducted to determine whether factors such as salinity, turbidity and pH potentially contribute to false positive readings. Next, a human health risk assessment was performed based on irrigation methods, crops irrigated, pathogen transfer rates and survival post irrigation along with collected water data and a review of scientific literature to determine a minimum risk level.

The following are key findings (obtained from the June 2013 CPS Symposium presentation, the panel discussion and the final project report):

Finding 1: Test results for *E. coli* in agricultural water varied depending on which test method was used to analyze the water sample.

What does this mean for you?

This study analyzed water samples using three different commercially available test methods — MI agar (a chromogenic/fluorogenic medium), m-ColiBlue24[®] broth, and IDEXX Colilert Quanti-Tray[®]. Study authors concluded that, of the three methods evaluated, IDEXX Colilert Quanti-Tray[®] provided the best option with results that were the most easily interpreted and the highest accuracy (49%) in identifying a true positive. In contrast, m-ColiBlue24[®] was the least accurate (29%) with a 71% false positive rate. Growers need to be aware of which test methods their laboratory uses to analyze their water samples. Growers should feel confident that their laboratory understands the variety of testing methods available and the benefits and limitations of each method.

Finding 2: Each of the three test methods produced a number of false positives for *E. coli* ranging from 53% – 71%.

What does this mean for you?

Test methods developed for testing relatively clean, treated drinking water have limitations when used for microbial testing of agricultural water most notably surface water. The number of false positives obtained from the three commercially available methods calls into question the reliability of these tests for use with surface water used in agricultural settings. When agricultural water tests positive for the presence of a human pathogen or exceeds a predetermined indicator organism standard, growers should consider whether or not the crop is safe to consume and if any corrective actions are necessary. If the positive test results are false positives, growers may take actions that are unnecessary, including retesting. Inaccurate test results increases costs for the grower, the supply chain, and ultimately the consumer without any improvement in food safety. To minimize the risk of false positives or false negatives when testing agricultural water, test methods used to analyze agricultural water samples need to be validated specifically for testing the type of water commonly used in agricultural environments (e.g., surface water).

Finding 3: Even with a diversity of results given differences in methods used to analyze samples, in the vast majority of samples obtained from all sampling locations, *E. coli* counts were below the current U.S. recreational water standard of 126 MPN/100mL.

What does this mean for you?

For purposes of the quantitative microbial risk assessment (QMRA), Rock assumed *E. coli* counts were at the level of the U.S. recreational water standard of 126 MPN/100mL and then demonstrated the related potential adverse health effects. If agricultural water *E. coli* counts are 126 MPN/100mL, then the relative risk of gastrointestinal (GI) illness from the one-time consumption of irrigated lettuce is 1 case in 300,000 regardless of irrigation method. When considering irrigation type, the relative risk of GI illness is 9 cases in 100,000,000 for subsurface, 1.1 cases in 100,000 for furrow, and 1.1 cases in 1,000 for sprinkler irrigation indicating the highest risk of GI illness with sprinkler irrigation. Since actual test results are below 126 MPN/100mL, Rock concluded that the relative risk of illness from a one-time consumption of irrigated lettuce should be low. Based on these results, the agricultural water sampled in this project was determined to be relatively safe. Using this methodology a determination can be made as to the relative safety of agriculture water used for leafy green irrigation in other regions. Studies like this are invaluable when guidance is being developed or, for example, when alternative approaches to the testing requirements specified in the FDA's Proposed Produce Rule are sought.

Conclusions/Recommendations/Next Steps:

The industry would benefit from a representative survey of the test methods used for testing agricultural water as well as a comprehensive review of testing limitations particularly as they could affect a grower's operations. If microbial water quality is questionable in a particular area or if past results have demonstrated a potential microbial water quality issue, growers may want to have their water tested using two or more methods to determine how the results vary for a particular water source. Working with commodity-specific and other industry associations growers could compare their test results and water issues to similar companies in the industry. These same associations could also provide guidance on appropriate test methods based on their commodity, local environmental conditions, water sources, etc.

PROJECT #6: Evaluation of sampling protocol to provide science-based metrics for use in identification of *Salmonella* in irrigation water testing programs in mixed produce farms in the Suwannee River Watershed (1/1/2012 – 12/31/2013)

Principal Investigator: George Vellidis, Ph.D., University of Georgia

Layman's Summary (Source: CPS Program Notebook – June 26, 2013)

Irrigation water has been linked to outbreaks of foodborne illness and death associated with bacterial contamination of produce. In 2014, the FDA set forth a proposed rule to allow for inspections of produce production systems, minimal standards to be derived for on-farm processes and resources such as microbial quality of irrigation water. The proposed rule also mandated documentation of actions conducted to minimize the risks of produce contamination. Requirements to document the microbial quality of water are based on indicator bacteria and currently vary from no mandate for testing irrigation water quality to a presence/absence test to a single enumerated test to a 5-day geometric mean. There are no science-based metrics comparing the utility of these methods for detecting pathogenic bacteria in irrigation water sources. The proposed research will provide guidance for growers on water sampling methods to maximize the ability to detect bacterial contamination in surface water irrigation sources. In addition, the water sampling protocol developed in this study will provide a science-based method for collecting samples that can be documented as part of a water quality program to minimize the risks of produce contamination.

Technical Findings:

This project developed and evaluated two grower-friendly sampling protocols for growers who rely on untreated surface water sources for irrigation. The protocols were designed to reflect *Salmonella* concentrations at the irrigation system intake and consisted of collecting 3 grab samples 1) from the bank near the intake, approximately 10 feet apart, and 2) distributed along the pond perimeter. For the research, five ponds used for irrigating produce were sampled from March 2012 through September 2013. For two ponds, surface runoff water and pond water was sampled before and after rainfall events between January and August 2013. *Salmonella* was tested for using MPN enumeration method with positive samples confirmed by PCR. *E. coli* was measured using the IDEXX Colilert reagent and the Quanti-tray system. Results indicate low detection levels with concentration varying by pond.

The following are key findings (obtained from the June 2013 CPS Symposium presentation, the panel discussion and the final project report):

Finding 1: Water samples taken from the ponds' banks did not consistently detect *Salmonella* when it was known to be present in water near the irrigation system intake located 10 to 20 feet from the bank at a depth of 3 to 6 feet.

What does this mean for you?

Seventy percent of water samples taken from the irrigation intake matched the *Salmonella* analytical results of samples taken on the bank. Since 30% of bank samples differed from the intake samples, bank sampling should not be used to evaluate irrigation pond water quality. Irrigation water sample location must be chosen to accurately represent the water that has contact with the crop.

Finding 2: *Salmonella* was present in various types of surface water. Forty-seven percent of all surface water samples had detectable levels of *Salmonella* (detection limit = 0.055 MPN/100 mL). More water samples taken after a rainfall were positive for *Salmonella* (58%) than water samples taken before a rainfall event (33%).

What does this mean for you?

Environmental pathogens in source water, even at low levels, need to be controlled. Growers using surface water for irrigation would benefit from understanding what pathogens are likely to be present in their source water and any factors contributing to pathogen occurrence and persistence. Does, for example, surface runoff contribute to *Salmonella* levels?

Finding 3: *Salmonella* presence in irrigation ponds and surface runoff in the Southeast was not predicted by generic *E. coli* presence at the 235 CFU/100 ml threshold proposed by the FDA.

What does this mean for you?

This study finding confirms the results of many other studies showing little correlation between generic *E. coli* levels and *Salmonella* presence in surface water used for produce irrigation. Assessing and successfully managing risk of agricultural source water requires analysis of all hazards and not just meeting standards or minimum requirements.

Conclusions/Recommendations:

Every growing environment is different. This research provides a good example of a thorough investigation of irrigation water sources in a particular growing area. Knowing what the hazards are and understanding as much as possible about their sources is beneficial for employing mitigation measures to ensure the microbial safety of produce in the production environment. This study demonstrates key considerations when developing a sampling protocol including seasonality and different environmental factors. This study also confirms the need to understand the impact of run-off in *Salmonella* loads as well as the potential for transfer through irrigation systems.

PROJECT #7: Enhancing the efficacy of fresh produce washing operations through establishing monitoring methods and water disinfection technologies based on a combination of filtration and UV(1/1/2011 – 12/31/2012)

Principal Investigator: Keith Warriner, Ph.D., University of Guelph

Layman's Summary (Source: CPS Program Notebook – June 26, 2013)

The washing of fresh produce is an important step in commercial processing to remove field-acquired contamination. In the course of commercial fresh produce processing, the microbial loading and organic loading of the water increases. Consequently, the microbial loading in the water decreases the efficacy of the wash process and increases the potential for contamination to spread through to subsequent product batches. It is common practice to partially or fully replenish tanks with fresh wash water although the timing is largely subjective as opposed to being based on a quality indicator. In the proposed project, a measurable wash water parameter(s) that can be monitored in real time will be identified to report on the microbiological quality. This will enable processors to more accurately identify when the water requires to be changed in order to maintain the efficacy of the wash process and reduce cross contamination events. In addition, a cost effective water-recycling unit will be developed based on a combination of filtration and ultraviolet light. By recycling the efficacy of the wash water process will be maintained with cost savings in resources through reduced consumption and wastewater treatment.

Technical Findings:

The research objectives were to correlate log count reductions in wash water to wash parameters, to determine if cross-contamination events are occurring during commercial washing and to develop a cost effective water recycling system. For the study, samples were collected from spinach and shredded lettuce processing facilities before, during and after the washing process. For process water treatment, facilities used either peroxyacetic acid (water was not changed) or oxidation reduction potential (ORP) controlled hypochlorite wash (water was recharged constantly). Initial microbial loads, conductivity and water temperature were measured when the samples were collected. Then using 16S rRNA-DGGE and Biolog Ecoplates methodology, microbial population counts were measured before and after wash water use.

The following are key findings (obtained from the June 2013 CPS Symposium presentation, the panel discussion and the final project report):

Finding 1: Lettuce had lower microbial loading and was more efficiently washed compared to spinach.

What does this mean for you?

Wash water systems for leafy greens should not be validated as a category. Washing system parameters need to be customized and systems validated for each type of leafy green product being processed.

Finding 2: A facility with good wash water quality (i.e., lower bacterial loading, monitored and maintained a consistent ORP level and recharged their tanks with fresh water) did not achieve a significantly higher log count reductions (LCR) on product than a facility that had high bacterial

loading in the water, did not replenish the wash water or maintain ORP levels.

What does this mean for you?

These findings add to the body of evidence demonstrating that washing is not an effective method for removing bacteria from produce. Although LCR were not significantly different between the two systems, maintaining wash water quality is important for ensuring wash water is not a source of contamination.

Finding 3: Washing can result in cross-contamination between batches of processed leafy greens, particularly lettuce.

What does this mean for you?

Warriner's research demonstrates how microbial pathogens can be transferred from pre-washed leafy greens through subsequent washings to other batches of leafy greens. Research findings confirmed that microbial load in wash water affected the carriage of coliforms on post-washed produce. This finding highlights how important it is to adequately control wash water treatment(s). Since wash water cannot remove all pathogens from produce coming in from the field, wash water control should focus not on removing pathogens coming in from the field but on preventing pathogen spread to other products. When reusing wash water, testing and treatment between uses is essential.

Conclusions/Recommendations:

Wash water systems are complex. Wash water process control should not be considered an effective process to remove pathogens from leafy greens. Instead, in many cases wash water needs to be viewed as a process that needs to be managed to reuse water safely without adding pathogens to leafy greens; this particularly important where water availability is a major concern. For pathogen control, focus is needed first on preventive controls in pre-processing environments before produce ever enters processing facilities. If microbial pathogens are introduced to produce before entering a production facility, the issue becomes one of not spreading the contamination to other products (cross-contamination) through reuse of wash water.

PROJECT #8: Irrigation regime, fruit water congestion and produce safety: parameter optimization to reduce susceptibility of tomatoes and peppers to post-harvest contamination, pathogen transfer and proliferation of *Salmonella* (1/1/2011 – 12/31/2012)

Principal Investigator: Max Teplitski, Ph.D., University of Florida

Layman's Summary (Source: CPS Program Notebook – June 26, 2013)

Recent multi-state outbreaks of vegetable-borne gastrointestinal illnesses demonstrate that human pathogens can contaminate produce at any stage of production. The fact that the outbreaks have been sporadic and the uncertainty regarding sources and routes of contamination argue for the possibility that some event(s) during the production cycle make vegetables more susceptible to contamination from environmental sources of pathogens. We hypothesize that irrigation-determined physical properties of tomatoes and peppers (fruit wetness, fruit water congestion, etc.) may make them more vulnerable to contamination from various environmental sources, including transfer from rubber gloves or wiping cloths. Fruit wetness and fruit water congestion also increase susceptibility of vegetables to plant pathogens, and the association with plant pathogens has been well-documented to promote growth of *Salmonella* in vegetables. The overall goal of this project is to test how irrigation-determined physical properties of tomatoes and peppers affect vulnerability of produce to infections by *Salmonella*. Upon completion of this project, we expect to have defined an optimal irrigation regime under which yields are maintained, yet the vulnerability of produce to contamination and proliferation of *Salmonella* are reduced. Following a successful completion of the first two production seasons, a full scale on-farm demonstration will be carried out.

Technical Findings:

The focus of this study was to determine whether irrigation directly and indirectly affects tomato and pepper susceptibility to *Salmonella* contamination post-harvest. In particular the research investigated indirect effects of cultivar selection and irrigation regimes on tomato susceptibility to *Salmonella* post-harvest and the contribution of measurable environmental variables such as precipitation, clouds, sun along with plant and *Salmonella* genotypes.

For the research three tomato cultivars and one bell pepper cultivar were grown in experimental research fields in Florida. The fields were irrigated within two weeks prior to harvest to achieve one of three soil moisture levels: Optimal, over-irrigated, or insufficient soil moisture levels. Plants were inoculated with a cocktail of outbreak-associated *Salmonella* isolates or *Salmonella enterica* serovar Typhimurium by either rubbing with a cloth or spotting into shallow surface wounds.

To investigate contamination related to water intrusion into tomato tissue (water congestion), tomato sections were floated on water to determine if water was absorbed and how contact duration was correlated with *Salmonella* proliferation.

The following are key findings (obtained from the June 2013 CPS Symposium presentation, the panel discussion and the final project report):

Finding 1: If tomatoes become contaminated, plant ripeness affects *Salmonella* growth. Mature (stage 6) tomatoes were more susceptible to *Salmonella* proliferation than immature (stages 1-5) tomatoes.

What does this mean for you?

Consideration needs to be given to harvest timing. If tomatoes are harvested when they are ripe, extra measures should be taken to ensure tomatoes are not contaminated during harvesting and handling including additional training for harvest personnel about the increased contamination risk related their handling practices (i.e., use of cloths for wiping, hand-washing, glove use, etc.).

Finding 2: Irrigating with a drip system within two weeks prior to harvest did not significantly affect *Salmonella's* ability to grow in the fruit.

What does this mean for you?

Drip irrigation with optimal, over-irrigated, or insufficient volumes of water had no effect on bell pepper and tomato post-harvest susceptibility to *Salmonella*.

Conclusions/Recommendations:

This study demonstrated that *Salmonella* proliferation was strongly affected by fruit ripeness and seasonal variability. This study also confirmed that tomato cultivar and *Salmonella* genotype are important variables that affect post-harvest susceptibility to *Salmonella*. For example, green tomatoes and tomatoes harvested in dry seasons are less susceptible to *Salmonella*. This suggests that quality and growing conditions have an impact on fruit susceptibility to *Salmonella* contamination.

SESSION III: *Pathogen Transference: Pre-Harvest, Harvest and Packing*



5.0 SESSION III- PATHOGEN TRANSFERENCE: PRE-HARVEST, HARVEST AND PACKING

PROJECT #9: *Escherichia coli* O157:H7 in bioaerosols from cattle production areas: evaluation of proximity and airborne transport on leafy green contamination (1/1/2011 - 12/31/2012)

Principal Investigator: Elaine Berry, Ph.D., Agricultural Research Service, USDA

Layman's Summary (Source: CPS Program Notebook – June 26, 2013)

A clear role for dust or wind in the transport of *Escherichia coli* O157:H7 from cattle to produce crops has not been determined. The research objectives are to: (1) Determine if *E. coli* O157:H7 is transported by dust or wind from cattle production to leafy green crops, and (2) Determine the impacts of environmental conditions and proximity on any dust/wind transmission of *E. coli* O157:H7. In each of two years, spinach will be planted in plots at distances from 60 to 180 meters from a cattle feedlot. Spinach plants will be collected every two weeks and examined for *E. coli* O157:H7 and nonpathogenic *E. coli*. Weather data, including rainfall volumes and intensity, air temperature, wind direction and speed, and relative humidity will be recorded at 15-min intervals by an on-site weather station. Thus, if *E. coli* O157:H7 is found to be transmitted to spinach by dust or wind, the effects of distance and other environmental factors on the transport process can be determined. This information is critical to the produce industry for understanding the risks associated with growing crops in close proximity to cattle production, and for determining safe distances between cattle feedlots and crop production.

Technical Findings:

The research objectives were to a) determine if *E. coli* O157:H7 can be transferred by dust, wind (bioaerosols) or flies to leafy greens and then b) determine the environmental conditions affecting transport. From nine plots of leafy greens planted at 200, 400 and 600 feet from a feedlot, every 2-3 weeks sampling was conducted throughout summer. Fly, feedlot surface manure, air and leafy green samples were collected. For weather data, an onsite weather station collected rainfall, wind and humidity data. Sampling occurred six times each year and enrichment cultures were processed using real-time polymerase chain reaction (RT-PCR).

The following are key findings (obtained from the June 2013 CPS Symposium presentation, the panel discussion and the final project report):

Finding 1: *E. coli* O157:H7 was found in samples at all three plot distances demonstrating that the pathogen can be transferred from cattle lots at distances of 200, 400 and 600 feet to leafy green plants. Levels of *E. coli* O157:H7 decreased with increased distance away from the feedlot.

What does this mean for you?

This finding supports the need for a pre-season environmental risk assessment that includes an evaluation of the surrounding land prior to planting. Also, it demonstrates the importance of risk and mitigation factors related to the recommendation of a 400-foot separation from feedlots in some guidelines. This validates the reasoning behind increasing or decreasing this distance depending on these factors. Factors increasing risk (e.g., feedlots upwind or uphill from

production land) and risk mitigators (e.g., wind breaks, ditches) should be evaluated and weighed in determining which crops to plant on production areas. This also suggests that a distance of 600 feet is safer, but even that distance, in some cases, may be insufficient to mitigate risk.

Finding 2: Dry and dusty feedlot conditions can increase *E. coli* O157:H7 transference from feedlots to leafy greens.

What does this mean for you?

When assessing adjacent land for potential food safety risks, in addition to requirements for physical distances separating feedlots from crops, consideration should also be given to weather conditions and how this affects animal movement, density and migration. Animal movement during dry and dusty conditions may increase the contamination risk from animals stirring up dust and potentially transmitting *E. coli* O157:H7 via wind. Growers can examine seasonal weather patterns and identify days that may be more risky than others and include this information in their risk management plans.

Finding 3: *E. coli* O157:H7 positive flies were found at distances of 200, 400 and 600 feet from the feedlot.

What does this mean for you?

While *E. coli* O157:H7 positive flies were recovered at all distances sampled from the feedlot, the study did not pursue *E. coli* transfer from the flies to leafy greens. However, previously CPS-funded research by Dr. Astri Wayadande's laboratory demonstrated that filth flies could transfer *E. coli* O157:H7 to spinach in the laboratory setting. Scanning electron microscopy images showed *E. coli* O157:H7 on fly body parts and in fly regurgitation spots on spinach leaves. *E. coli* was detected on flies up to 13 days after the initial exposure. Additional research is needed demonstrating *E. coli* O157:H7 transfer by particular fly species to leafy greens under field conditions.

Conclusions/Recommendations:

This study can assist in the review of current risk and mitigation factors related to the transfer of *E. coli* O157:H7. As research findings such as these become available, industry would benefit from a well-defined process to review the findings and determine if any changes are needed in current industry practices or food safety guidelines. Without a formalized process for reviewing the latest science and consideration for its adoption, an opportunity is missed to better manage risks and potentially improve food safety. There are currently mechanisms in place to update industry guidance documents; this can actually be a means to ensure research findings are incorporated in these documents in a consistent manner.

PROJECTS #10: The likelihood of cross-contamination of head lettuce by *E. coli* O157:H7, *Salmonella* and norovirus during hand harvest and recommendations for glove sanitizing and use (1/1/2011 – 12/31/2012)

Principle Investigator: Jennifer Cannon, Ph.D., University of Georgia

Layman's Summary (Source: CPS Program Notebook – June 26, 2013)

Mandatory glove use when handling raw produce and the practice of sanitizing gloves with bleach is common despite uncertainty that these practices improve the safety of foods. Here, the impacts of glove use (frequency of changing and glove composition) and sanitation (using bleach and a novel sanitizer developed at UGA) on cross-contamination of raw head lettuce by bacterial (*E. coli* O157:H7, *Salmonella*) and viral (norovirus) pathogens during harvest will be investigated. Since build-up (soil and lettuce residue) on gloves likely impacts transfer of pathogens to and from gloves, accumulation of these materials on gloves over time (0.5, 1, 2 and 4 hr.) will be quantified for volunteer harvesters wearing either rubber or nitrile gloves. In the lab, natural accumulation will be mimicked on gloves and the likelihood of pathogen transfer to gloves and head lettuce will be investigated. Finally, a novel sanitizer will be evaluated for its efficacy in removing organic material accumulation and inactivating pathogens on gloves as compared to bleach. This study will provide a scientific basis for making recommendations on glove use and sanitation during harvesting head lettuce and will contribute to improving the safety of fresh produce.

Technical Findings:

This study examined the potential for gloves used in harvesting and sanitized under varying protocols to cross-contaminate raw head lettuce during harvest operations. Latex, latex canners and nitrile gloves were first tested to determine how to characterize and quantify the accumulation of organic material on gloves followed by testing under field conditions in the Salinas Valley. Field results were used to determine whether the transference of *E. coli* O157:H7, *Salmonella* and norovirus is possible and if so, to what extent. The study then examined the combination of organic load and sanitizers to determine the impact of sanitizers on pathogen inactivation. Gloves were inoculated with three pathogens — *Salmonella*, *E. coli* O157:H7 and norovirus and then placed in dunk buckets containing water and one of several sanitizers — Purell®, varying concentrations of free chlorine, and a unique sanitizer consisting of levulinic acid and sodium dodecyl sulfate (SDS), a surfactant commonly used in detergents.

The following are key findings (obtained from the June 2013 CPS Symposium presentation, the panel discussion and the final project report:

Finding 1: Glove use is no substitute for effective hand-washing.

What does this mean for you?

Ensuring workers wash their hands with soap for the Center of Disease Control and Prevention's recommended 20 seconds is a challenge for harvesting and packing companies. Study results demonstrate that norovirus is readily transferred from bare hands to gloves when gloves are applied supporting the spread of illness. Adequate hand washing is necessary to prevent the transfer of disease by hand contact (i.e., when putting on gloves).

Finding 2: Glove dunks are effective at reducing pathogen load, but need to be monitored and maintained throughout the work day.

What does this mean for you?

Use of disinfectant-containing glove dunks is an effective method for sanitizing gloves after workers apply them. However, glove dunks are less effective when lettuce and soil debris accumulate reducing the chlorine's effectiveness. Refreshing dunk buckets on a routine basis is critical to maintaining their effectiveness. Since chlorine is volatile and dissipates, covering buckets between uses is also critical to maintaining chlorine concentrations. This study demonstrated that disinfection of glove surfaces can reduce norovirus on gloves using a single glove-dunk bucket with both waterless hand sanitizers (reductions of approximately 1-2 log plaque-forming units (PFU)) and 50 ppm chlorine solutions at neutral pH (reductions of ~2 log PFU on latex gloves and ~3-4 log PFU on nitrile gloves). Bacterial contamination was completely inactivated by 50 ppm chlorine in glove dunk buckets when bacteria levels were low (<3 CFU/glove); and, when levels were >3 CFU/glove, it was reduced by 4-6 log CFU/glove.

Finding 3: In experiments investigating sanitizer effectiveness, there were greater reductions of viruses on nitrile gloves than latex ones.

What does this mean for you?

Log reductions of norovirus when gloves were soaked in water, water and 2% SDS, 200 ppm chlorine, 200 ppm chlorine and SDS, and 5% levulinic acid plus SDS were greater on nitrile than latex gloves. Studies like this one demonstrate how the material which gloves are made and work environment can affect pathogen survival and cross-contamination.

Conclusions/Recommendations:

The inclusion of Norovirus in this study enhances its value. According to the Center for Disease Control and Prevention, norovirus is the leading cause of foodborne illness in the U.S. Since the virus can be transferred from person-to-person or from person-to-produce, the potential for contamination can be quite high. Harvest crews handle hundreds of products a day. Contamination can occur when an infected worker touches a product or when he or she touches a surface (e.g. glove) that then touches a product. Once contaminated, those same products are placed in cartons and bins with a potential for cross-contamination. The industry's ability to minimize the spread of norovirus is limited to identifying infected workers and removing them from the harvest crew while they are infectious, proper glove use if included in the company's Sanitary Operating Procedures, and training employees to understand the importance of effective hand washing and to notify their supervisor if they are feeling ill. Results from projects like this one are especially beneficial in providing support for the critical role of workers in fresh produce food safety that can be integrated into food safety training materials. This is important since employee training is considered one of the most effective methods for minimizing the spread of norovirus from one individual to another.

PROJECT #11: Pathogen transfer risks associated with specific tomato harvest and packing operations (1/1/2011 – 12/31/2012)

Principal Investigator: Michelle Danyluk, Ph.D., University of Florida

Layman's Summary (Source: CPS Program Notebook – June 26, 2013)

The establishment (of) standards related to removal of dirt and debris from tomato fruits during field pack operations and re-use of tomato cartons in re-pack operations within the Tomato Good Agricultural Practices and Best Management Practices (T-GAP) document is essential for the responsible harvesting, handling and packing of fresh tomatoes. Understanding risks potential transfer of pathogens onto tomatoes from used tomato cartons or cloths used to remove debris is a fundamental management prerequisite to providing customers with safe tomatoes. There is inadequate science-based data to base current standards and audit inspection criteria for re-use of tomato cartons and removal of dirt and debris from tomatoes. The purpose of this research project is to define risks associated with dirt and debris removal in the field and re-use of tomato cartons in re-pack operations. The research outcomes will allow for the assignment of research-based metrics for in field debris removal and re-use of tomato cartons for the fresh tomato industry.

Technical Findings and What They Mean for You:

This research addressed the potential of transferring *Salmonella* to/from cloths and tomatoes and to/from bins and tomatoes. Transfer coefficients between cloths and tomatoes and dirty used cartons and tomatoes were developed. The effect of using dirty and clean, wet and dry cloths was also examined. Cloth, tomato, and bin samples were inoculated with five *Salmonella* strains and dried for 0, 1 or 24 hours. After evaluating cloth and tomato contact times of 5, 10 and 20 seconds, 20 seconds was selected with three degrees of rubbing (none, mild, and vigorous). Cartons used in experiments were dirtied with a tomato debris and sandy soil mixture. Tomatoes and cartons were subject to three contact times — 0, 1, and 7 days and stored for either 12°C or 25°C (~53.6 to 77°F). The following are key findings (obtained from the June 2013 CPS Symposium presentation, the panel discussion and the final project report):

Finding 1: Low levels of *Salmonella* survived on and were transferred from clean and dirty cloths to at least 25 tomatoes touched with a contaminated clean or dirty cloth.

What does this mean for you?

Use of cloths in tomato harvesting and packing presents a food safety hazard. If cloths become contaminated with *Salmonella*, contamination can be spread to multiple tomatoes by re-use of a single cloth. When cloths are used preventive controls must be put in place to minimize the risk of contamination. Potential preventive controls include use of cloths made from antimicrobial materials, cleaning and sanitizing cloths at least daily, and replacing dirty cloths with clean ones routinely throughout the day.

Finding 2: The most important factor affecting *Salmonella* transfer between tomatoes and cloths/ cartons is moisture.

What does this mean for you?

Relatively low levels of *Salmonella* transferred to tomatoes under dry conditions. Preventive controls to minimize potential *Salmonella* contamination should include keeping cloths, cartons and other food contact surfaces used in harvesting and packing tomatoes as dry as possible.

Finding 3: At cool temperatures (12°C or 53.6 °F), *Salmonella* survived over 7 days on new and old, dirty and clean cartons.

What does this mean for you?

Many fresh produce commodities reuse cartons and/or bins in their harvesting, packing and storage operations. These experiments demonstrate increased contamination risk when tomatoes are stored in dirty cartons at storage temperatures recommended for slow ripening (12-13°C or 53.6 -55.4°F). Tomato handlers should implement preventive controls such as disposal or cleaning of dirty cartons to decrease the potential risk of *Salmonella* transfer from contaminated cartons to tomatoes.

Conclusions/Recommendations:

Contaminated contact surfaces, such as cloths used to wipe tomatoes, are likely to present a risk of contamination to fresh produce. This study confirms that the use of clothes to clean dirt and debris from tomatoes during harvesting, whether these cloths are dry, wet or clean is not a best practice. This actually increases the risk that pathogens, if they are present, may contaminate the cloths and be transferred to tomatoes. While removing debris and dirt is a common practice, wiping multiple harvested tomatoes with a single cloth is not advisable for removing dirt and debris. Following alternative practices to washing and drying tomatoes should be considered instead of repeated use of a single cloth.

PROJECT #12: Survival, transfer and inactivation of *Salmonella* on plastic materials used in tomato harvest (1/1/2011 – 2/28/2013)

Principal Investigator: Lynne McLandsborough, Ph.D., University of Massachusetts

Layman's Summary (Source: CPS Program Notebook – June 26, 2013)

In an effort to improve sanitation, growers are increasingly using plastic materials to handle and pack fresh produce, replacing traditional wood crates and paperboard cartons. Further, many workers have begun wearing gloves in an effort to reduce pathogen contamination of hand harvested produce. However, there is a lack of practical, translatable research data that identifies what materials and cleaning/sanitization practices will most effectively manage food safety risks. Since bacterial transfer is a biophysical process that occurs between a surface and produce, we will evaluate the physical characteristics of glove and plastic bin materials, and their influence on bacterial transfer and cleaning/sanitization. We will then assess survival of *Salmonella* on plastic materials and the potential for cross contamination from bin and glove materials to tomatoes. Finally, we will quantitatively assess cleaning and sanitation efficiency of plastic materials at various stages in their lifecycle (new, repeatedly sanitized, abraded by cleaning brushes). Research results will be translated into recommendations of best practices for cleaning and sanitation to prevent contamination of produce. We will work closely with the Center for Produce Safety and our local, regional, and national industry partners to develop practical, science-based food safety training materials to support sanitary on-farm practices.

Technical Findings:

One project objective was to examine produce totes, bins, and gloves surface characteristics (e.g., hydrophilicity, surface energy and surface charge) and evaluate how aging and repeated cleaning and sanitization affect those characteristics. The second objective was to determine whether the totes, bins and gloves support bacterial survival and, if so, the potential for *Salmonella* transfer to produce. The third objective was to evaluate cleanability and sanitizer efficiency in reducing *Salmonella* spp. on bins/totes and gloves.

Glove and bin material hydrophilicity was determined by dropping water on gloves and bins and then measuring the water contact angle. Surface topography and roughness was determined using an optical profiler. To evaluate bacterial survival on bins, plastic coupons were inoculated with a 5-strain *Salmonella* cocktail in the presence or absence of organic matter or soil. Humidity levels (94%, 75%, 54% and 33%) were altered along with soil levels to understand how dryness impacts the results. *Salmonella* transfer from different glove materials to tomatoes was evaluated by rolling tomatoes over the inoculated gloves and determining levels of *Salmonella* transferred to tomatoes. The effectiveness of hypochlorite solutions on sanitation of sterilized new and worn harvest bucket materials inoculated with *Salmonella* was evaluated in the presence of various amounts of soil or organic material. Inoculated buckets were allowed to dry at 20°C (68°F) and 54% relative humidity for 24 hours prior to determining sanitizer effectiveness.

The following are key findings (obtained from the June 2013 CPS Symposium presentation, the panel discussion and the final project report).

Finding 1: When soil was added to the medium during plastic coupon inoculation, more *Salmonella* survived (decreased log reductions) on the coupons.

What does this mean for you?

It is important that laboratory experiments exposing materials used in fresh produce harvesting and packing attempt to simulate the actual environments as closely as possible. Bacteria are typically suspended in sterile nutrient-containing broths for inoculating the materials being studied. However, in actual harvesting and packing operations, materials being used are rarely without defects and free of dirt. Adding soil to the inoculation medium, as was done in these experiments, to more closely simulate real world conditions encountered by human pathogens during fresh produce handling, adds confidence regarding the results.

Finding 2: Low density poly ethylene and latex gloves had the greatest degree of transfer between gloves and tomatoes while vinyl and nitrile had lowest degree of transference.

What does this mean for you?

There has been a shift in the produce industry away from latex to nitrile gloves to avoid worker latex allergies. This research identifies a food safety rationale for switching glove material as well. Use of latex gloves is being phased out in a number of industries due to allergen health concerns, yet the broader health concern of transference and food safety has not been examined before this research project. When considering the purchase and use of any equipment (bins) or product (gloves) that comes in contact with produce, the buyer should ask whether the product has been tested for food safety performance. If so the buyer should review and understand the test findings and potential product limitations.

Finding 3: The presence of soil reduced sanitizer efficiency during sanitation of new and worn buckets.

What does this mean for you?

Sanitation with 50, 100, 150, 200 ppm total hypochlorite solution at a 2 minute contact time reduced *Salmonella* levels by only 3.5-3.8 log CFU/cm² instead of the required 99.999% or 5 log reduction. The study authors suggest this was due to the dying off of some organisms and the stress response of the survivors during the 24 hour desiccation period prior to experimental sanitation. If wet containers are allowed to dry before sanitation procedures the conditions in these experiments may represent real world conditions where surviving *Salmonella* may be difficult to kill when used containers are sanitized after drying.

Conclusions/Recommendations:

The findings support current guidance for examining and cleaning equipment daily. These findings could even enhance current guidance if they could be translated into recommendations for practical best practices for cleaning and sanitation. Future research could also include the effects of biofilm, which was not included in this study.

SESSION IV: *Hot Topics*



PROJECT #13: Sanitization of soft fruits with ultraviolet (UV-C) light (1/1/2012 – 3/31/2013)

Principal Investigator: Xuetong Fan, Ph.D., Agricultural Research Service, USDA

Layman's Summary (Source: CPS Program Notebook – June 26, 2013)

The U.S. Food and Drug Administration (FDA) Food Safety Modernization Act (FSMA) requires growers/packers of fresh fruits and vegetables to adapt preventive microbiological controls such as chemical sanitizers to minimize the risk of human pathogens. Tree-ripe fruits cannot withstand vigorous washing steps without impairing product quality, thus these fruits are manually packed without the use of water. To satisfy the FDA's requirement for preventive controls and to enhance microbial safety of the soft fruits, products must be sanitized by non-aqueous technologies. Our proposed project is designed to study the feasibility of applying ultraviolet (UV-C) light to tree-ripe fruits (viz., apricots and/or peaches). Specifically, we plan to assess the efficacy of UV-C in inactivating common enteric human foodborne bacterial pathogens and maintaining fruit quality during post-UV storage. Furthermore, to ensure and validate uniform UV-C exposure of all fruit surfaces, a rotating conveyor and the use of UV film dosimetry will be evaluated. In addition, the technology will be tested in commercial trials with our industry cooperator. Successful demonstration and implementation of UV-C technology will enable the fruit industry to meet the requirements of FSMA, while improving microbial safety and increasing the consumption of healthful fresh fruits.

Technical Findings:

Since soft fruit is prone to damage and cannot tolerate washes, non-aqueous interventions are needed. The objective of this project was to determine whether ultraviolet radiation in the form of short-wavelength ultraviolet light (UV-C) can be used to eradicate two human pathogens, *E. coli* O157:H7 and *Salmonella* spp. UV-C destroys microorganisms by damaging their DNA and disrupting their ability to perform essential cellular functions. UV-C ranges from 280 – 100 nm in wavelength, and the FDA has approved its use at a wavelength of 254 nm as a food disinfectant. The effectiveness of UV-C in reducing *E. coli* O157:H7 and *Salmonella* spp. was first tested in a laboratory and then in a commercial facility. Cocktails of five strains of pathogenic *E. coli* O157:H7 and four *Salmonella* strains were inoculated onto apricots and then dried. The inoculated fruit was treated with UV-C for periods of 0, 10 and 60 seconds followed by plating of the skins. Measurements were taken to determine changes in pathogen populations. To determine survival and growth of pathogens after UV-C treatment, inoculated apricots were also stored at either 2°C (35.6° F) for 21 days or 20°C (68°F) for 8 days and then tested.

In a commercial facility, fruit quality was examined following UV-C applications. A conveyor system with rotating devices was developed to ensure UV-C dosage was uniform. UV-C applications ranged from 10.3 to 16.8 mJ/cm² for periods of 0, 20 and 40 seconds.

The following are key findings (obtained from the June 2013 CPS Symposium presentation, the panel discussion and the final project report):

Finding 1: Treatment with UV-C reduced both *E. coli* O157:H7 and *Salmonella* spp. populations, but fruit surface structures may prevent UV-C exposure of all bacteria.

What does this mean for you?

UV-C treatment reduced *E. coli* O157:H7 and *Salmonella* spp. populations in both the laboratory and in commercial environments. There are, however, limitations to UV-C use. Electron microscopy images showed pathogens inside the stomata, adhered to crevices, and attached to the base of the surface hair-like structures. These results provide evidence that the shape of the produce item may influence UV-C effectiveness.

Finding 2: UV-C treatment requires fruit rotation for uniform treatment.

What does this mean for you?

For UV-C to be an effective pathogen control treatment, consistent exposure is needed on all areas of the fruit. In this study, experiments were conducted with and without a roller tray (i.e., random fruit rotation as it rolled down sloped belts). When fruit was rotated without use of a roller tray, nonpathogenic *E. coli* reduction was only 0.5-0.7 log CFU/fruit. With a roller tray, *E. coli* reduction ranged from 1.0 – 1.2 CFU/fruit. Consistent exposure will require equipment, currently not commercially available, that rotates the fruit without bruising or otherwise damaging the fruit. Equipment will also need to maintain throughput efficiencies while meeting UV-C exposure requirements of 20 seconds or longer.

Finding 3: Results from a consumer survey conducted as part of this research project suggest consumers may accept use of UV-C treatment for food safety purposes.

What does this mean for you?

When considering tools and technologies for improving food safety, not only should companies understand the impact the changes will have on produce quality but they should also evaluate the potential impact on consumer perceptions and ultimately purchase decisions. Given the current reaction to GMO products, the importance of consumer perspectives cannot be underestimated. Further studies are needed to understand consumer acceptance of UV-C treatment, particularly when used on fresh produce.

Conclusions/Recommendations:

The FDA Preventive Controls Rule will require implementation of a written food safety plan that focuses on preventing hazards in foods. Given there is no “kill step” for fresh produce eaten raw, alternatives such as the use of ultraviolet light to kill bacteria are needed. UV-C is promising given this study’s demonstration of its ability to reduce microbial populations in both laboratory and commercial processing/storage environments. However, as with all new technologies, a cost-effective, commercial system needs to be developed in order for UV-C to be useable by companies of all sizes. Compounding the issue with the use of UV-C is historic consumer resistance to the use of irradiation. As part of this study, a consumer survey was conducted. The results indicate an acceptance of the use of ultraviolet light (electromagnetic radiation) for improving produce safety as opposed to irradiation (ionizing radiation). In using UV-C light, the question remains will consumers be more concerned with produce safety or with the particular process used to improve produce safety?

PROJECT #14: Influence of the pre-harvest environment on the physiological state of *Salmonella* and its impact on increased survival capability (1/1/2011 – 12/31/2012)

Principal Investigator: Linda J. Harris, Ph.D., University of California, Davis

Layman's Summary (Source: CPS Program Notebook – June 26, 2013)

Salmonella spp. has been implicated in numerous outbreaks of foodborne illness tied to the consumption of fresh fruits, vegetables, and nuts, seeds and spices. Multistate outbreaks of salmonellosis due to consumption of tomatoes, mangos, melons and raw almonds have highlighted the ability of *Salmonella* to persist in a wide range of pre and post-harvest environments. Exposure to large swings in moisture, temperature, and nutrient levels are expected in these environments. The relative tolerance to these conditions is known to differ among strains of *Salmonella*. In addition, some of the environmental stressors may trigger a variety of survival response mechanisms in some strains providing further competitive advantage. While strain dependent survival phenomena have been documented, the mechanism of these differences is not clear. The proposed research seeks to increase our understanding of the environmental factors that trigger survival mechanisms in outbreak-related strains of *Salmonella* and to better elucidate those mechanisms related to desiccation tolerance and environmental persistence. The results will help the produce industry to better interpret *Salmonella*-positive test results and should assist in making informed decisions related to pre- and post-harvest risks of contamination.

Technical Findings and What They Mean for You:

Salmonella has a tolerance to drying/desiccation and is known to survive for long periods of time in dry environments and on dry surfaces (e.g., food and equipment). *Salmonella* strains that are able to produce cellulose and aggregative appendages called fimbriae (also called the rdar morphotype) survived better during desiccation than strains that did not. The research objectives were to characterize the role of *Salmonella* rdar cellulose production and its impact on desiccation tolerance and resistance to post-harvest treatments such as sanitizers. As a result of previous *Salmonella* strain research, this study evaluated the impact pre- and post-harvest environmental factors have on *Salmonella* rdar

For the research two *Salmonella* strains — rdar-positive (rdar+) and rdar-negative (rdar-) were grown on liquid broth and solid agar media. To determine desiccation tolerance, samples were dried using a desiccator to a relative humidity of 72% for 7 days and then tested. Next, the *adrA* gene (associated with cellulose and fimbriae) and a mutated *adrA* gene were studied to determine if gene expression is impacted by the substrate used. Cells were cultured in two broth and two agar combinations, plated and incubated at 28°C (82.4°F) for 2 days. Cells were examined at the end of the 2 days. Finally for determining resistance to post-harvest treatments, rdar+, rdar- and *Salmonella* Oranienberg were collected and cultured in agar and broth combinations with free chlorine and citric acid.

The following are key findings (obtained from the June 2013 CPS Symposium presentation, the panel discussion and the final project report):

Finding 1: *Salmonella* strains grown on solid agar medium survived significantly better during desiccation and persisted longer than when grown in liquid medium (broth).

What does this mean for you? This finding suggests that *Salmonella* may attach to solid food and food contact surfaces and survive and grow if conditions are optimal. Some *Salmonella* strains survive better in environments that are dry and have varying temperatures and humidity levels. Dry facilities should consider reviewing their *Salmonella* testing programs based on this research.

Finding 2: *Salmonella* rdar strain types affect strain characteristics, which in turn affects long-term *Salmonella* survivability.

What does this mean for you?

When researching possible *Salmonella*, *E. coli* and *Listeria monocytogenes* issues in produce operations, strain selection is critical. In this study rdar+ strains are more tolerant to desiccation than rdar- strains. If rdar- strains had been used alone, the result might have been a conclusion that *Salmonella* would die off after some period of time (120 days). However, use of the rdar+ strain indicates *Salmonella* may survive for longer periods of time. This result highlights the importance of researchers using multiple strains or cocktails for their studies.

Finding 3: Strain type (rdar+ or rdar-) and the use of broth or agar for culturing do not appear to have an impact on *Salmonella* tolerance of 5% citric acid or 5 ppm free chlorine.

What does this mean for you?

Strain selection and characteristics along with the use of broth or agar for culturing do not appear to increase *Salmonella*'s ability to resist sanitizers applied post-harvest. This result implies strain selection may not be as critical as thought when testing sanitizer effectiveness.

Conclusions/Recommendations:

Strain selection, preparation, culture media, temperatures and humidity levels, are all factors influencing pathogen growth. When developing a research plan, scientists need to consider the pathogen being studied and then determine the appropriate strain selection, preparation, etc. to ensure the study is representative of actual produce environments. In this study, strain selection did impact long term survivability. If only one strain had been used, 7 day survivability would have been measured but longer term survival may not have been detected. (*Salmonella* survival has been detected in almond production environments for periods of several years.)

If desiccation is a study component, researchers need to determine how desiccation could be a factor and what optimal desiccation levels and exposure time should be used to replicate actual conditions. Research studies need to consider and mimic actual produce conditions as closely as possible. Similarly, companies relying on research findings to make decisions on sanitation and microbial testing programs need to analyze how the research results apply to their actual conditions before implementing changes. This study confirms the value of a validation study to consider specific environmental conditions.

PROJECT #15: Comparative assessment of field survival of *Salmonella enterica* and *E. coli* O157:H7 on cilantro (*Coriandrum sativum*) (1/1/2012 – 12/31/2012)

Principal Investigator: Trevor Suslow, Ph.D., University of California, Davis

Layman's Summary (Source: CPS Program Notebook – June 26, 2013)

The proposed research seeks to significantly narrow the knowledge gaps in food safety management of a very popular and widely used culinary herb, cilantro. The problem facing growers, foodservice and retail marketers, and public health officials is primarily the high detection prevalence of *Salmonella* on cilantro in produce surveys. Multistate outbreaks and multiple costly recalls have elevated these concerns over the past five years, in particular. Field-based research with non-pathogenic forms of *Salmonella* and *E. coli* O157:H7 will be conducted in California to determine the survival, growth potential, and postharvest removal efficiencies following simulated foliar contact contamination events during production. While the interactions between *Salmonella* and cilantro in laboratory studies have clearly shown that pathogen growth is likely at non-refrigerated temperatures, especially following dicing for foods such as salsas, our understanding of risk potential in more 'real-world' production conditions is largely absent. We anticipate that the outcomes of our research will foster the development and adoption of Best Practices in food safety management among cilantro growers and processors. This knowledge will be largely transferrable to other leafy culinary herbs including parsley and basil.

Technical Findings:

The project objective was to study the persistence and survival of *Salmonella enterica* and *E. coli* O157:H7 after cilantro contamination. For the study cilantro cultivars, Santo and Leisure, along with parsley and basil (controls) were grown in open fields. The cilantro was spray inoculated before maturity with a *Salmonella enterica* isolate and attenuated *E. coli* O157:H7. After plants matured, the cilantro was harvested, washed in a system containing sodium hypochlorite and then stored for 14 days at either 5 or 12.5°C. Pathogen populations were measured 12 hours post-inoculation and again on days 6 and 12. Post-harvest pathogen populations were monitored 7 and 14 days after washing.

The following are key findings (obtained from the June 2013 CPS Symposium presentation, the panel discussion and the final project report):

Finding 1: Pathogen populations as low as 1.7 log CFU/gram survived for up to 12 days — even after washing.

What does this mean for you?

If cilantro becomes contaminated just prior to harvest, pathogens will not only survive but will also be resistant to washing. *Salmonella* persistence is greater than that of *E. coli* O157:H7 even after washing and storage.

Finding 2: *E. coli* O157:H7 survival was dose-dependent at all three preharvest sampling time points (0.5, 6, 12 days) following inoculation. *Salmonella* survival was dose-dependent for 0.5 and 12 days post-inoculation, but not for 6 days.

What does this mean for you?

Contamination events closer in time to harvest will be impacted not just by the event but also the magnitude of contamination. Both pathogens survived better on preharvest cilantro when inoculated at 10^6 CFU/mL than when inoculated at 10^4 CFU/mL. The one exception was in samples taken at 6 days post-inoculation when *Salmonella* at both inoculation doses survived well.

Finding 3: Cooling delays impact growth potential. A delay of two hours had a negligible impact; however, 4, 6, and 8 hour delays resulted in *E. coli* O157:H7 growth.

What does this mean for you?

While this study focused on cilantro, other products would benefit from similar studies on how temperature and cooling delays impact pathogen growth. Companies should study their post-harvest storage and transportation operations to determine conditions for holding products before cooling. Also, plans need to be in place for handling situations when there are unexpected cooling delays.

Conclusions/Recommendations:

Pathogen persistence can extend beyond non-lethal postharvest processing including commercial washing with disinfectants and typical supply-chain conditions, particularly if temperature abuse occurs. The efficiency of different commercial washing and disinfection strategies is dependent on the contamination level and pathogen detachment. However there is always a risk of cross-contamination through the water or other contact-points. When simulating post-harvest contamination, the closer to harvest that contamination occurs, the more likely it will be present in the postharvest environment.

PROJECT #16: The role of riparian zones in bacteria dispersal to produce farms (1/1/2012 – 3/31/2014)

Principal Investigator: Martin Wiedmann, Ph.D., Cornell University

Layman's Summary (Source: CPS Program Notebook – June 26, 2013)

Riparian buffer zones have been implicated in the transmission of foodborne pathogens to produce fields and fresh fruits and vegetables, but no one currently understands how the growth of produce in close proximity to riparian zones influences the risk of contamination. The proposed work will measure the movement of fecal bacteria through riparian zones and onto produce fields by detecting the movement of genes from those bacteria. The measured movement of genes from field sampling will be compared to models that represent competing ideas about how the bacteria move. These models produce maps for lands. They tell us how bacteria move across the land in a manner similar to what roadmaps tell us about the movement of cars. The maps that agree best with the movement patterns of fecal bacteria will be used to advise growers about when, where and how riparian zones increase risk of foodborne pathogen dispersal onto produce. Ultimately, a web-based tool can be developed to apply the best model to new lands and help the produce industry evaluate crop planting decisions, pre-harvest surveillance practices and harvest practices to prevent product contamination.

Technical Findings:

Recognizing that pathogenic *E. coli* is found in more than 15% of wild ruminants, this research focused on improving the prediction of produce contamination by modeling the rules of pathogenic *E. coli* dispersal through riparian zones to produce fields. The research assumed if wildlife are a significant vehicle for pathogen movement then bacteria will follow the preferred movement pathways for wildlife. Movement pathways consist of corridors as well as the surrounding land. For the research, GIS models were developed for animal specific movement patterns (e.g. air or ground) and a cost model determined how much energy it takes for an animal to get from place to place. As an example, birds have lower movement costs since it is easier for them to move from one location to another. Forests and fields were examined and sampled for generic *E. coli* in two locations in New York. A total of 571 soil, drag, fecal, and water samples were collected from 23 produce field and 17 forest sites. Genetic markers identified eight *E. coli* subtypes all of which are found in wild and domestic animals and humans. Subtyping was used to estimate *E. coli* rate and distance of movement among produce field and forest sites.

The following are key findings (obtained from the June 2013 CPS Symposium presentation, the panel discussion and the final project report):

Finding 1: *E. coli* transmission was not uniform across the agricultural landscape. Local landscapes affected *E. coli* prevalence and dispersal dynamics across a particular area.

What does this mean for you?

E. coli prevalence and dispersal patterns in one area may not apply to another area with a different landscape. However, this study demonstrates that *E. coli* is terrestrially transported most likely by wild animals with open waterbodies, residential areas, and major highways serving as barriers to that transport.

Finding 2: Soil samples obtained from forested riparian areas were more likely to contain *E. coli* (70%) than soil samples from produce fields (48%).

What does this mean for you?

Forested areas may act as a reservoir for *E. coli*. Understanding contamination sources for a particular production area is key to monitoring and minimizing potential food safety risks.

Conclusions/Recommendations:

Animal intrusion is a known risk factor for pathogen contamination of fresh produce. Gaining a better understanding of the distribution of fecal indicator organisms such as *E. coli* in the production area and surrounding environment provides information that is valuable to decision-making during planting, growing and harvesting activities. Increased awareness of the potential pathogenic hazards. This study suggests the importance of studies to address co-management issues and questions. This research confirms that contamination does not necessarily occur when there is animal habitat in close proximity to production areas. The best strategies to actually mitigate any risk should consider fecal contamination and co-management issues.

