A Practical Guide to the Scientific Research Presented at

The Center for Produce Safety's 2012 Research Symposium

Prepared By:

pma

Western Growers
Fresh produce from our families to yours

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1.0 Background

This report is intended as a summary and interpretation of the technical sessions presented at the third annual Center for Produce Safety Research Symposium held at the University of California, Davis, CA, on June 27, 2012. The Center for Produce Safety (CPS) was founded in 2007 and operates in collaboration with industry, government, scientific and academic partners. CPS is a leader in the delivery of science-based food safety research for the produce industry. Having funded 69 projects totaling $10.6 million, CPS is recognized for their partnering in research programs; linking scientists with stakeholders and for their scientific research funding process, research project management, translation of scientific reports into what the data mean for produce operations and industry outreach.

At the 2012 Symposium, results were presented for 18 CPS funded projects in four separate sessions: Good Agricultural Practices — Buffer Zones and Animal Vectors, Good Agricultural Practices — Irrigation Water Quality, Good Agricultural Practices — Inputs, Cultivation and Harvest and Wash Water and Process Control. In each session panels consisting of industry executives and scientists, regulatory leaders and academic scientists discussed the findings. The discussions highlighted how the research results are being used or could be used with a particular commodity, product or process. There were also poster presentations for an additional 33 projects. Poster presentations are available on the CPS website at https://cps.ucdavis.edu/poster_session.php. Final reports for fourteen of the projects, which have not been subject to peer review, are available on the CPS website at https://cps.ucdavis.edu/grant_opportunities_awards.php.

This report includes research from: a) the 18 projects covered during the presentations, b) information from the annual or final reports for each project, c) prior research funded through CPS relevant to the current findings, d) posters on display at the symposium and e) panel discussions following session presentations.

The authors' intent is to provide this report as a guide that the produce industry will find of use in understanding the latest research and in determining how to apply the research to their day-to-day operations. We have attempted, in addition to a review of each research project, to present key findings from each project along with an interpretation of what those findings mean for growing, harvesting and processing operations. The report also includes observations and recommendations regarding industry issues and opportunities and a review of several emerging food safety tools that demonstrate potential benefit for the industry.

2.0 Comments Regarding this Summary

This report has been prepared as guidance for the produce growing, harvesting, handling and processing communities and does not supersede any regulations nor does it constitute legal guidance. Recommendations and observations made along with statements of "What does this mean for you?" are solely the interpretation of the authors of this document.

3.0 Observations

In reviewing the CPS Symposium research presentations and subsequent panel discussions, the authors identified several issues that face the industry and emerging opportunities for addressing food safety concerns. These issues and opportunities require further research and industry attention.

- **Can we act to prevent animals from becoming pathogen vectors?** Over time as various animal species have been found to shed human pathogens the trend has been to create lists of "high risk animals" that need to be restricted from entry into production fields. However, research presented at 2012 CPS Symposium (Dr. Andrew Gordus and Dr. Michele Jay-Russell) and at the 2011 symposium (Wayadande 2011) indicates that numerous animal species are potential pathogen vectors. Even if pathogen prevalence levels among individual animals are low, numerous animals, including species of birds, reptiles, amphibians, deer, elk dogs and sheep can test positive for *Salmonella* and/or *E. coli* O157:H7. Further research is needed to understand how animals are infected by and transfer pathogens. Of particular interest would be the identification of reservoirs in the environment that serve as sources for animal infection. Concentrated animal feedlots (CAFO), for example, continue to elicit concern when located in close proximity to production fields (Dr. Elaine Berry). CAFOs, however, are only one potential pathogen reservoir of concern. The value of this research would be to move beyond current practices focused on avoiding animal intrusions to one of focusing efforts on preventing animal infection from occurring and thereby avoiding the risk of pathogen transfer to crops.

- **What is an optimal buffer zone?** Although buffer zones can be effective in mitigating contamination risks, further work is needed to determine how best to employ buffer zones, understand minimal effective distances from the contamination source and the value of physical barriers that might be employed in combination with buffering distances. While the CPS research findings have supported the concept of buffer zones to reduce cross contamination risks when evidence of animal intrusion is observed in production fields or adjacent land uses raise concerns for product contamination (Dr. Elaine Berry and Dr. Bruce Hoar), further work is needed to determine specific relationships between optimal distances and contamination sources, e.g. single location fecal contamination within a production site versus adjacent grazing or CAFO operations. Along with better defining buffer zone distances by specific risk, the industry would benefit from corresponding work examining the add-on effects of employing physical barriers like hedgerows or tarped fences, ditching, etc. to augment buffer zones.

- **How can we better manage irrigation water quality?** Current best practices for irrigation water testing can be costly for growers and the results have been shown to have limited use as predictors of potential pathogen risks. Still, food safety standards rely on them. Industry-derived irrigation water standards, e.g. the LGMA metrics, are based on the available science which is the U.S. EPA's risk assessment for recreational water use and associated action levels. Most metrics focus on generic *E. coli* as an indicator of possible fecal contamination. Dr. Anita Wright, however, demonstrated in her research that generic *E. coli* and fecal coliform testing has little or no predictive value for *Salmonella*. Dr. Edward Atwill's study indicates microbial water quality results are heavily dependent on seasonality, sample volume and the location where the sample was taken (*E. coli* detection probability in samples taken from a lateral canal are 10 times higher than those taken from a main canal.) With these results the question remains, what organisms are most appropriate for determining potential fecal contamination and when should those samples be taken?

Programmatic irrigation water testing, i.e. generic *E. coli* tests taken a defined time intervals may be less valuable than targeted, high volume, risk based testing of irrigation water sources. Dr. Atwill's collection of leafy greens industry irrigation water testing data (testing every 30 days throughout the growing season) in coastal Central California indicates very low frequencies of samples that exceed current EPA recreational quality water tolerances for generic *E. coli* (235 MPN/100 mL). In effect, growers, continue to test deep wells, on-farm reservoirs and other water sources month after month with most samples delivering "non-detect" results. However, after five years of collecting irrigation water data, might growers be better served using that data to identify specific water sources and/or specific times in the growing season when elevated generic *E. coli* levels have been observed and focusing more intense sampling efforts and assessments on those sources while reducing the frequency of testing on those sources that are observationally functioning properly and have no history of yielding detectable samples? The net effect might be that growers would perform the same number of total tests per year, but those numbers of tests would be focused on times when the risk for contamination are greatest based on historical trends and observations. This type of risk based testing requires active engagement by growers using their accumulated data to identify trends and on-farm observations.

- **Food-safety requires end-to-end systems solutions.** As scientific knowledge of the contamination risks associated with the production and delivery of safe fresh produce increases, it is becoming clear that these systems need to be viewed as complex biological systems that are highly interdependent. Teplitski, Schneider and Wayadande have reported that the genetic make-up of the commodity may play a role in its susceptibility for contamination by human pathogens and we have also come to understand that the genetic composition of the pathogen itself can determine its virulence, environmental survivability and reaction to common sanitizers. This interactive complexity requires a *total systems approach* to developing risk management practices and preventive controls. For example, it is important to understand the efficacy of composting (Jiang) and soil amendment preparation to be sure these processes are well controlled across several critical variables, pathogen elimination is verified through a valid testing strategy and finished compost or amendment storage is conducted properly to avoid re-contamination. Similarly, irrigation water quality, sanitation practices, wash water disinfection, temperature management and other critical components of production and distribution have to be carefully monitored and verified to insure the entire production system is effective in preventing contamination and restricting pathogen growth if pathogens are inadvertently present. The effective management of each of these steps in the production and distribution of produce is critically important to its safe consumption and failure to properly manage one component can have unfortunate consequences downstream. For example, if the wash water disinfection system does not prevent cross contamination effectively, then should pathogens be present they might be expected to survive and cross contaminate large amounts of product in a production run. This by itself is concerning, but if further down the supply chain, proper temperature control is not maintained, then conditions might exist that permit pathogen growth so that the dose level reaches the point where serious illnesses can result. Without an effective kill step, produce relies on a multi-hurdle approach to risk management and a mis-step anywhere in the supply chain can jeopardize food safety

- **Supply line management challenges**. Gone are the days when growers manage everything from pre-planting to consumer sales on their own. As is apparent from recent food safety failures, all supply chain members are responsible for food safety and are accountable for their practices to other members of the supply chain. However, the industry is fragmented as companies specialize, making accountability more difficult. Some growers only grow crops; while harvesting is performed by another company. Some handlers grow, harvest and ship themselves. Pesticides may be produced by one company, mixed by another and applied by a third company. Harvesting, transporting, cooling and packing may be performed by four different companies before it reaches a consumer outlet such as retail or food service. These are only a few examples of the complexity of the industry structure. As a result of the complexity, it is difficult for one company, whether a grower or a handler, to be able to effectively manage its suppliers and partners. Other industries (everything from consumer products to aircraft products) have spent years understanding and improving their ability to manage supply lines.

Supply chain management issues were identified in Ms. Wetherington's study "Using Leafy Green Marketing Agreement audit data to determine non-compliance areas and preparation of training and recommendations for improvements in future growing seasons." In the study, gaps were identified when leafy green handlers manage suppliers ranging from growers, water testing companies, cooling companies, sanitation unit suppliers, and composters to harvesters. Her research determined the leafy green industry could benefit from an increased focus on managing the supply line accompanied with associated education and training.

The significance of supply chain failures has become only too apparent in recent cantaloupe industry product failures and the result has been human illness and death. As demonstrated with cantaloupes, without strong supply chain management, failures whether associated with inadequate testing or growers not following best practices, from a strictly financial perspective, can mean the difference between economic viability and bankruptcy.

- **Compost challenges.** Unfortunately, the produce industry does not have an easy formula for growers to follow to guarantee the safe use of compost. Dr. Xiuping Jiang 's project, "Developing and validating practical strategies to improve microbial safety in composting process control and handling practices," demonstrated how compost creation, management, storage and application is a dynamic microbial system requiring effective process controls to eliminate pathogens during processing, and to prevent reintroduction during holding and storage. Her research shows the complexity of compost management and revealed gaps in current control processes. At a minimum, companies would benefit from a how-to guide for managing compost. As an industry, a compost company certification program could be introduced to minimize grower risk.

- **Wash water system management.** In several studies (Dr.Yaguang Luo, Dr. Elliot Ryser, and Dr. Keith Warriner), organic and microbial loads were determined to adversely impact wash water quality resulting in potential cross-contamination. Managing wash water is complicated as it involves not only balancing pH and maintaining disinfectant levels but also regularly assessing water cleanliness, turbidity and presence of other matter such as plant debris, latex, soil, etc. Understanding how to prioritize and address wash water parameters is critical for proper process control and hence avoiding the potential for cross-contamination.

4.0 New tools and technology

Several new tools, products and technologies were explored in CPS funded research projects. While for the most part the tools and technology are new or evolving, they have promise in detecting, minimizing or reducing pathogens on produce. Examples include:

- **Riboprinter® System.** One tool used to identify bacteria both in research and production environments is the Riboprinter® Characterization System from Dupont Qualicon. (The Riboprinter® System was used in Dr. Trevor Suslow's project "Microbial food safety on-farm risk assessment" in identification of coliform bacteria and presumption of *E. coli*.) The system relies on the highly conserved nature of ribosomal DNA (rDNA). Because the function of the ribosome is highly critical to protein production and therefore maintenance of life functions, mutations or random changes in rDNA are not tolerated and the rDNA "fingerprint" can be used to identify specific species of pathogens. Once a "fingerprint" is obtained, the equipment searches the Riboprinter® System database containing more than 1,200 genetic patterns for matches that identify the specific species and serotype of the pathogen. The advantage of the system is the ability to obtain results within hours and the ability to determine pathogen strains or serotypes quickly perhaps eliminating or reducing the dependence on time-consuming plating methods.

- **T-128.** T-128 is a food-grade GRAS chemical used in commercial wash systems as a chlorine stabilizer. This product improves chlorine's effectiveness under conditions when water sanitation may be compromised by high levels of organic materials or excessive product loading exceeds disinfectant capacities. Research from Dr. Luo indicates that when T-128 is used in produce wash systems, it helps maintain stable levels of free chlorine in water with high organic loads; reduces survivability of *E. coli* O157:H7 and *Salmonella enterica* in wash water with depleted chlorine levels. T-128 can also reduce *Salmonella enterica* on tomato stem scars, cantaloupe biofilms and stainless steel surfaces. Use of T-128 does not adversely affect product quality.

- **ZVI.** The zero-valent iron water filtration unit or ZVI is a relatively simple and inexpensive tool that can be added to existing sand filtration units to reduce the risks of pathogen contamination in water. The research demonstrates ZVI's potential to eliminate *E. coli* O157:H7 and *Salmonella* by binding the pathogens within the matrix of positively charged surfaces on the iron fragments. ZVI, as currently configured, may have more benefit to smaller production operations that require lower volumes of water for irrigation or cooling. While work still needs to be done to commercialize ZVI, it appears likely that ZVI will be a cost effective water filtration solution for certain size operations.

Even with the scientific advances, conducting hazard analyses and risk assessments are critical components of any food safety program.

5.0 Industry Recommendations

The four main sessions at the 2012 symposium focused on buffer zones and animal vectors, irrigational water quality, inputs, cultivation and harvest, and wash water and process control. However, several themes were common to all of the sessions and they are:

Hazard analysis and environmental risk assessments are critical.
Research presented at the 2012 symposium demonstrated clear advances in the science of food safety and at the same time highlighted the critical role growers, harvesters, handlers and others have in produce safety. Even with the scientific advances, conducting hazard analyses and risk assessments are critical components of any food safety program. Potential risks covered in the research presentations include: concentrated animal feedlots located in proximity to production areas, irrigation water quality degradation, animals as potential pathogen vectors, production and operational practices that may result in increased food safety risks, and compost production and management. Growers and handlers evaluate each of these risks regularly as part of their hazard analysis and risk assessments. Therefore, the recommendation is for companies to review the research findings and then revisit their hazard analysis and risk assessment practices and procedures. At a minimum the research could better inform the companies in conducting their assessments.

Industry partnerships with researchers are valuable.
Many of the sessions demonstrated the value of cooperative relationships among industry, government and academics to address food safety concerns and research. Working together ensures the research addresses issues that are priorities to the industry, taking advantage of the power of the lab and the reality of the field. Having industry involved with researchers in developing the project methodology and objectives ensures the project will more accurately reflect field level conditions and the results will be more applicable to actual operations. Partnering can also lead to process improvements as exemplified by Dr. Linda Harris' work on controlling pistachio temperatures during transportation and Dr. Edward Atwill's project evaluating increasing water sample volume to boost pathogen detection capabilities.

When immediate research needs are identified and time requirements fall outside the annual CPS RFP process, industry can also partner with the Center for Produce Safety researchers on rapid response projects. Rapid response projects are designed to bring resources to bear on immediate food safety issues and achieve results within a short period of time. An example of a rapid response project is one recently completed by Dr. Michele Jay-Russell entitled, "Investigation of potential reservoirs of shiga toxin producing *E. coli* and *Salmonella* in produce production areas of Arizona and New Mexico." Dr. Jay-Russell investigated the potential for wild and domestic animals in

Arizona and Mexico to be potential reservoirs of food-borne pathogens. Companies operating in these areas wanted to understand prevalence and total risk since Arizona and Mexico have high incidences of dog activity. Once the need was identified, CPS was contacted and a request was made for specific research to address the issue. Within a week, CPS responded and the project began at the start of the next growing season. Within a matter of a few months, and with the cooperation of leading companies in Arizona and animal welfare organizations, the prevalence of pathogen infection in wild and domestic dogs was determined and efforts can now be focused on mitigation practices.

Industry groups and associations should consider this approach when faced with a food safety issue that needs scientific study and results as quickly as possible.

Quantitative microbial risk assessments are powerful tools for advancing science and finding solutions to food safety issues.

The FDA is in the process of developing risk analysis tools linking the consumption of fresh produce to certain on farm production and processing practices. Included in the tools will be quantitative microbial risk assessment models (QMRA). A QMRA is a scientific process used to characterize hazards and then quantify the potential human health risk.

QMRAs provide the ability to determine whether exposure to a pathogen will have an adverse effect on human health. Since some pathogens are naturally occurring and can, therefore, be present in production environments, the question is whether the pathogen is present at a level capable of causing illness if the contaminated produce is consumed. The ability to answer this question provides valuable information for industry, regulators, and consumers. Using QMRAs researchers can estimate "positive" test results that may lead to financial loss or worse, consumer illness. With QMRA results, over time the industry may be able to move the FDA from a policy of zero tolerance to tolerance levels specific to individual pathogens. This is one example of how QMRA results can be used.

A more immediate benefit to growers, handlers, and processors is the ability to use QMRA results to evaluate the relative contribution of individual processes to overall risk levels and then to identify risk mitigation steps for reducing or removing those individual process risks (Dr. Linda Harris - temperature control processes during transportation, Diane Wetherington – need for validated environmental testing programs for packing houses. In both of these studies access to industry groups and individual companies resulted in findings beneficial to the industry that would not have been achievable otherwise.).

At the heart of QMRAs are data — specifically confidential grower data. Unfortunately, the industry has been slow to support researchers in their efforts to gain access to confidential data. The comment is often made that the data does not exist, or if data does exist, the concern is that somehow the data will be used against the industry as an "unintended consequence." While there are indeed data gaps and challenges associated with allowing researchers access to confidential data, if the industry embraces the challenges, tremendous opportunities exist for understanding actual risks. Initial assessments may have data gaps and may require the use of expert opinion or judgment. However, over time as additional data is collected, the QMRA will acquire greater specificity and ability to provide guidance on risk mitigation. Ultimately, risk assessments will be based on large pools of real data rather than on assumptions or estimates from laboratory studies or small research field experiments leading to tolerance level changes as the ability to control risks is enhanced. Commodities and grower groups should support the development of QMRAs for key commodities such as those more frequently associated with outbreaks and/or recalls.

PROJECT #1:

Escherichia coli O157:H7 in bioaerosols from cattle production areas: evaluation of proximity and airborne transport on leafy green crop contamination

Principal Investigator: Elaine D. Berry, Ph.D., USDA-ARS

6.0 Session I – Good Agricultural Practices – Buffer Zones and Animal Vectors

Layman's Summary appears as submitted by the Principal Investigator
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 the two years, spinach will be planted in plots at distances 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 and What They Mean for You:
The objective for this project was to assess whether of *E. coli* O157:H7 can be transferred by dust/wind from cattle production environments to leafy green crops, and if so, what is the impact of environmental conditions and proximity on the leafy green crops. The results presented represent the first year of a two year study. Included in the study was an analysis of spinach contamination and feedlot surface manure. Three plots of spinach were planted at 200, 400 and 600 feet from a feedlot. (An overhead irrigation system was used and it tested negative for *E. coli*.) Spinach samples were collected every 2-3 weeks between June and September. The prevalence of *E. coli* O157:H7 in feedlot surface manure was 50-90%, an average of 72%; in spinach 0-5.5%, at all distances. In the plot planted at 200 feet, *E. coli* O157:H7 occurred on average in 2% of spinach samples and at 400 and 600 feet 0.4% and 0.7%, respectively. Seventy-two percent of feedlot manure samples were positive, but only 0.98% of the spinach samples from all three plots were positive for *E. coli* O157. When fly traps from feedlots and spinach plots were analyzed, the number of *E. coli* O157:H7 positives were highest for house flies.

The following are key findings (obtained from the June 2012 CPS Symposium presentation, the panel discussion and the 2011 project report).

Finding 1: E. coli *O157:H7 can be transported by wind from cattle feedlots.*

What does this mean for you?
This study provides data to verify the need for and benefits of buffer zones or other mitigation measures/barriers to separate production areas from potential sources of contamination. This study demonstrates that *E. coli* O157:H7 can be transferred in the air from a contaminated concentrated feedlot to the surrounding areas. This work also supports the need to consider the risk of airborne contamination and utilize buffer zones and/or other mitigation strategies as demonstrated in the Leafy Green Products Handler Marketing Agreement (LGMA) guidelines for fields adjacent to concentrated animal feeding operations. The importance of understanding activities and operations on land adjacent to production areas and of conducting environmental assessments is highlighted by this study's findings. Further work still needs to be done on effective mitigation strategies.

Finding 2: *Even though nonpathogenic* E. coli *was detected on spinach at all lot distances, the percentage of samples testing positive and the concentrations detected decline as you move away from the feedlot.*

What does this mean for you?
Production fields located in proximity to feedlots can present a food safety risk, although the potential risk diminishes as the distance increases from the production field. Buffers may useful in helping managing the risk as may be windbreaks or other permanent or temporary barriers. When moving away from the feedlot, *E. coli* O157 survived although at diminished levels. Although *E. coli* does not survive well on spinach, when *E. coli* O157:H7 is present, even if the concentrations and detections are low, growers, processors and handlers cannot dismiss the risk because low concentrations have been shown to survive further processing and are known to cause disease. This finding highlights the need to determine effective and appropriate buffer zone distances and other strategies for managing this type of risk. More information on the appropriate size of the buffer zone should result from the second year of this research study. Additional work to evaluate the placement design and construction of barriers may also prove valuable.

Finding 3: *Seasonality is a factor in the transmittal of* E. coli *O157:H7 from feedlots.* E. coli *O157:H7 was not detected in any air sample at any distance in August and September. In 2012 additional air sampling will be added to enhance detection capabilities.*

What does this mean for you?
While field level research is optimal, environmental conditions are difficult to define and control. In this case, the feedlot research is dependent on wind and August and September are less windy than other times of the year in this experimental location in Nebraska. For produce companies, environmental assessments should include an evaluation of seasonality, topography and temporal factors along with adjacent land use, native wildlife, and other variables unique to specific regions of the country when identifying potential risks to food safety.

Finding 4: *Several species of cattle pest flies can carry* E. coli *O157:H7.*

What does this mean for you?
Insect vectors may be troublesome. While further research is needed to understand insect vectors, prior CPS-funded research by Dr. Astri Wayadande at the University of Oklahoma (Talley 2009) demonstrated that flies carry and are capable of transmitting *E. coli* O157:H7. When flies carrying *E. coli* regurgitated on leaves in a laboratory study, Dr. Wayadande found the regurgitated *E. coli* survived on the leaf surface.

Conclusions/Recommendations/Next Steps:
This project demonstrates the value of using opportunities for naturally-occurring pathogens to study food safety issues. It is clear that cattle feeding operations can serve as a reservoir for pathogen contamination of

adjacent lands and that, based on the data presented here, this risk diminishes the farther one gets from the source. Wind may play a role in dispersing pathogens from dust or dried feces though the very mild wind conditions experienced during this first year of experimentation limit our ability to draw more definitive conclusions. In 2012 Dr. Berry will complete further work looking for direct evidence that flies are a risk for pathogen transfer to leafy greens. Fly traps will be left unbaited to more closely replicate a real-life spinach production environment, and will include some live-netting.

The industry would benefit from research on effective methods to manage or prevent the potential transference by using natural buffers, beneficial insects and/or natural pyrethrins.

PROJECT #2:

Developing buffer zone distances between sheep grazing operations and vegetable crops to maximize food safety

Principal Investigator: Bruce R. Hoar, Ph.D., University of California, Davis

Layman's Summary appears as submitted by the Principal Investigator

Integrated livestock and crop operations are beneficial to producers of both products. Crop residues are an important source of food for livestock, however domestic and wild animals represent a potential source of food borne pathogens. Recent outbreaks of human infections with *E. coli* O157:H7 and other bacteria linked to consumption of California produce have raised concerns that sheep and other ruminants may elevate levels of pathogens within the soil, which have the potential to be transmitted to produce fields via aerosols. The California Leafy Green Product Handler Marketing Agreement (LGMA) of January 2010 lists sheep as one of the five mammalian "Animals of Significant Risk" species and any intrusion by such animals requires a detailed food safety assessment prior to harvest. "Buffer zones" between the crop production fields and livestock operations are important in order to prevent the potential transmission of pathogens from animals to crops. Currently, there is a paucity of information related to appropriate combinations of time and distance between the livestock operations and crop systems, particularly in terms of pathogen survival in animal feces, soil and aerosols, as well as the pathogen movements through wind, water or flies. The LGMA suggest that a distance of 400 ft. exist between a concentrated animal feeding operation and the edge of a crop and 30 ft. grazing lands/domestic animals, but recognize a lack of science on which to base this recommendation.[1]

This proposed research aims 1) to investigate factors associated with the survival of bacterial pathogens from initial deposition as feces, to presence in soil before and after irrigation events, to presence of pathogens in dust generated in fields with active livestock grazing, and 2) to investigate the distance over which pathogens can be transferred by aerosolized particles.

Technical Findings and What They Mean for You:

The overall objective of this research was to estimate survival of *E. coli* O157:H7 and *Salmonella* in feces and soil associated with sheep grazing, and to develop valid buffer zones for crop production fields. In the experimental plot, leafy greens were on one side of narrow road and an alfalfa field used to graze sheep was on the other side. Forty fresh fecal and 40 soil samples were collected at each sample collection. Air samples were collected up to 100 m from the field edge. In the study, 1.8% of 720 fecal samples tested positive

1 The "Commodity Specific Food Safety Guidelines for the Production and Harvest of Lettuce and Leafy Greens "or the LGMA metrics, provide a guidance distance for concentrated animal feeding operations along with the statement that "The proximate safe distance depends on the risk/mitigation factors …" Fencing and other physical barriers, typography, opportunity for water run-off, are some of the risk mitigation factors named along with corresponding recommendations to increase or decrease the guidance distance based on multiple mitigation factors. Furthermore, users are advised to, "Evaluate risk and document consideration of these factors." (Table 6. *Crop Land and Water Source Adjacent Land Use)*

for *E. coli* O157:H7; 0.8% tested positive for *Salmonella*. Of the 720 soil samples, 0.4% tested positive for *E. coli* O157:H7 (3 samples), and 0.4% were positive for *Salmonella* (3 samples).

The following are key findings (obtained from the June 2012 CPS Symposium presentation, the panel discussion and the final project report).

Finding 1: *Sheep can be reservoirs of* E. coli O157:H7 *and* Salmonella. *The research demonstrated a low prevalence of* E. coli O157:H7 *and* Salmonella *in feces (<2.5%) and in soil (<1%).*

What does this mean for you?

This finding validates the commodity specific guidelines, particularly, the need for environmental assessments to determine potentially risky activities and operations, and the use of buffers or other barriers to separate potential risk factors from product/production areas. Further work is needed to determine appropriate buffers and efficacy of alternative strategies.

Finding 2: *There was no association between duration of grazing and presence of bacteria or prevalence and management factors.*

What does this mean for you?

Sheep grazing and movement in fields where produce is grown should be prevented. If sheep are grazing near production fields and growers have a policy of preventing sheep grazing and movement, growers should ensure they maintain an appropriate barrier or buffer zone. The practice of sheep grazing in post-harvest fields or moving sheep between locations was demonstrated to result in positive *E. coli* O157:H7 and *Salmonella* in soil and feces samples associated with sheep grazing.

Finding 3: *While there was no vectoring by wind, seasonality appears to be a factor.*

What does this mean for you?

Very low levels of bacteria were detected in air—highest at 2 m, lower at 100 m. The researchers did not experience windy conditions during the sampling periods of these experiments, which likely help explain these results. There appear to be more positive samples later in the year; however, the number of samples is not large enough to determine significance. With all field studies, environmental factors are difficult to control and measure. Projects need to include environmental data during sampling. Likewise, growers need to consider environmental factors such as climatic conditions in their risk assessments and they would benefit from maintaining a record of those factors during the growing and harvesting cycles.

Conclusions/Recommendations/Next Steps:

Sheep represent a potential reservoir and vector for *E. coli* O157:H7 and *Salmonella* sp. Feces and soil from areas where sheep graze have been shown to contain low levels of both pathogens. It is important that growers that farm lands in areas where sheep may be grazed consider this risk when performing risk assessments for their operations. It is clear that more needs to be learned about setting appropriate buffer distances between animal feeding operations and vegetable production fields. As determined by these experiments, these studies are difficult to perform as field-level experimentation is subject to the vagaries of seasonal and climatic conditions. Requirements for buffer zones between livestock operations and production areas need to be confirmed with further research and other guidance specifying specific buffer distances or other preventive/protective approaches needs to be considered relative to the specific risks presented by adjacent land use and relevant seasonal and climatic conditions.

PROJECT #3:

Evaluation of amphibians and reptiles as potential reservoirs of foodborne pathogens and risk reduction to protect fresh produce and the environment

Principal Investigator: Michele Jay-Russell, Ph.D., University of California, Davis

Photo courtesy of Jessica Wheeler

Layman's Summary appears as submitted by the Principal Investigator

Our proposal will help the leafy greens produce industry determine if wild amphibians (frogs, toads) and reptiles (lizards, snakes) are potential carriers of *E. coli* O157:H7 and *Salmonella* in the central California coast produce production region. We will identify management practices and prevention strategies that reduce the risk of contamination of leafy greens and nearby waterways by these species. Statistical procedures and epidemiological methods will be used to complete three objectives: 1) determine if wild amphibians and reptiles are reservoirs of *E. coli* O157:H7 and *Salmonella* in the central California coast; identify farm production practices, environmental factors and control strategies that reduce the risk of contamination from amphibian and reptile species in the leafy greens produce growing environment, and 3) extend knowledge of preventing produce contamination by amphibians and reptiles to the produce community. The science-based data from this study will support co-management to promote food safety and environmental goals in the central California coast. Specifically, the results will improve pre-season and pre-harvest environmental assessments and interventions as required in the Leafy Green Marketing Agreement (LGMA) metrics, in particular those addressing animal intrusions.

Technical Findings and What They Mean for You:
This project evaluated whether wild amphibians/reptiles can be reservoirs of *E. coli* and *Salmonella* and studied the impact of farm practices and environmental factors on the risk of contamination from these species in the Central California coast and southeast Georgia.

For this one year rapid response study, live animals were captured and multiple samples (cloacal swabs, ventral swabs and fecal samples collected from a phosphate buffered saline bath) were collected from the animals. Water samples were also collected at the same location where the animals were captured. In samples collected in California, bull frogs (4.3%), newts (1 sample and it was positive) and western fence lizards (11.1%) tested positive for *Salmonella*. No *E. coli* O157:H7 was detected. Non-O157 STEC was detected in western toads, rough skinned newts and coast garter snakes. A greater percentage of *Salmonella* positives occurred in adult frogs as opposed to tadpoles. In the Georgia results, 15.6% of amphibians tested positive for Salmonella, higher than in California. For reptiles 18.4% tested positive for *Salmonella* with the highest percentage in the snapping turtle (80%). Of the paired water samples, 38.5% were positive for *Salmonella* in pre-irrigation ponds in Georgia. None of the pre-irrigation ponds in California tested positive; however, 16.1% of the non-irrigation water tested positive.

The following are key findings (obtained from the June 2012 CPS Symposium presentation, the panel discussion and the final project report).

Finding 1: *Confirmed that wild amphibians/reptiles can potentially shed* Salmonella *but* E. coli *O157:H7 and non-O157:H7 STEC detections are rare.*

What does this mean for you?
This validates other research (Austin and Wilkins, 1998; Parish, 1998; CDC 2003; Richards et al, 2004; Srikantiah et al, 2004; Mermin et al, 2004; Gray, 2007) identifying amphibians and reptiles as reservoirs and potential vectors in contaminations (iguana in cherry tomatoes). Growers should know amphibian and reptile populations native to their growing areas and understand the relative risk associated with these animals. Awareness of the seasonal, climatic and other environmental conditions that affect animal activities is important when developing hazard analyses and environmental risk assessments.

Finding 2: *In California,* Salmonella *was most prevalent in tailwater ponds but not detected in pre-irrigation reservoirs (which get water from stored well water), while in Georgia,* Salmonella *was isolated from irrigation ponds. California tailwater ponds had the highest concentrations of generic* E. coli*; irrigation reservoir samples had the lowest.*

What does this mean for you?
These findings demonstrate the differences between two regions – both in practices and in risk factors. It is important to understand that a risk factor in one growing region may not be a risk factor in another growing region. Unlike in Georgia where irrigation ponds are a primary irrigation water source, California growers rely on wells or surface delivery for irrigation water. Tailwater ponds are, however, a potential water source for dust abatement. If tailwater is used for activities such as dust abatement in and around production areas, growers should exercise caution and closely monitor water quality.

Finding 3: *In California* Salmonella *was not detected in pre-irrigation reservoirs where positive frogs and snakes were identified.*

What does this mean for you?
This result can be used in developing co-management practices. Frogs and snakes may not be risk factors for water in irrigation reservoirs. Fencing to keep out large animals may be sufficient protective measures. While the result needs to be confirmed through further data analysis, this finding can be used when evaluating environmental risks and developing risk mitigation plans.

Conclusions/Recommendations/Next Steps

This project points out the specific nature of risk assessment and the corresponding development of risk management practices. The two production areas studied here (Central California and Southeastern Georgia) demonstrate significant differences in irrigation pond prevalence of *Salmonella* and generic *E. coli* reflecting the very different sources of water used to replenish those ponds. These studies also highlight the importance for growers in understanding the amphibian and reptile populations present in their growing areas relative to the environment in which they reside. Lastly, the data presented here point out the importance of understanding common practices versus the risk of unintentional cross contamination potential. In an effort to make efficient use of tailwater, growers may be tempted to use this water in dust abatement around the ranch or for other purposes. The data derived from this project indicates that tailwater may contain significant levels of Salmonella and represents a cross contamination risk if it should contact fruit or vegetable crops. The Principal Investigator for this project indicated that next steps are to conduct statistical analysis to determine whether water quality data correlate with amphibian/ reptile data and to correlate serotypes/ genotypes with animal/ water data.

PROJECT #4:

Wildlife survey for *E. coli* O157:H7 in the central coastal counties of California

Principal Investigator: Andrew Gordus, California Department of Fish and Game

Layman's Summary appears as submitted by the Principal Investigator

We propose to continue testing wildlife for the human pathogenic strain of bacteria *Escherichia coli* O157:H7 in Monterey, San Benito and San Luis Obispo Counties. Since 1995, this pathogen has resulted in more than 25 outbreaks from eating leafy green vegetables (LGV); approximately half of these have been associated with LGV grown on the California central coast. Wildlife has been suggested to be a source of *E. coli* contamination of LGV. Because of this uncertainty, farmers are required to build deer- and wild pig-proof fences around their fields, and remove habitat and wildlife from their farms.[2] To date, however, there is minimal definitive data that wildlife is an important source of contamination. We propose to collect colon or fresh fecal samples from wildlife collected in relevant LGV production areas to determine if they are carrying *E. coli* O157:H7. This information will help us better manage and protect wildlife and provide food health safety information to farmers and to the food industry. The future of sustainable wildlife populations in the three central coastal counties is dependent on

having cumulative and accurate scientific data to properly manage wildlife and to protect human health.

Technical Findings and What They Mean for You:

To determine if wildlife is a potential vector *for E. coli* O157:H7 and

2 This is not a requirement of the Leafy Green Products Handler Marketing Agreement. Some buyers may require growers to implement measures such as fences and habitat and wildlife removal as stated here.

Salmonella in Monterey, San Benito and San Luis Obispo counties in California, small rodents, small birds, Canada geese, wild pigs, tule elk and coastal black-tailed deer were tested. As pathogens tend to wash downhill in a watershed and since animals tend to spend their entire lives in a watershed, nine central coast watersheds were evaluated. In the 2009-2011 study, feces from big game, birds and rodents were sampled. Rodents (7.4%), birds (2.7%), deer (2.3%), elk (3.9%) and wild pigs (5.9%) tested positively for *Salmonella*. Five percent of all animals tested positive for *E. coli* O157:H7 including one Dark-eyed Junco bird, elk (2.0%) and wild pigs (4.2%).

The following are key findings (obtained from the June 2012 CPS Symposium presentation, the panel discussion and the final project report).

Finding 1: *We cannot rule any animal out as a potential vector for* E. coli *O157:H7 and* Salmonella. *In this research all species tested positive for* E. coli *O157:H7 and/or* Salmonella.

What does this means for you?
A list of animals of significant risk, similar to the LGMA list, is no longer practical. This research demonstrates that by including *Salmonella* in the study, animals that would have been ruled out when only considering *E. coli* O157:H7, are now potential vectors (birds and rodents). Since other pathogens need to be considered (e.g. other pathogenic strains of *E. coli*) and other animals will need to be tested, the recommendation is that commodity groups and others developing food safety guidance remove the animals of significant risk focus and instead promote environmental risk assessments including animal densities and evidence of animal presence in a commercial crop as best practices in guidance and metrics.

Finding 2: *3,065 animals were sampled for* E. coli*; 1,082 animals were sampled for* Salmonella. *In this type of research it is unclear how many animals need to be tested to establish prevalence and quantitate risk for cross contamination.*

What does this mean for you?
Similar to final product testing, determining how many animals need to be sampled or how many final products need to be tested before declaring an animal or product free of contamination is difficult. Even if the density of animals in the field allows for this type of study (conducting these analyses is not practical for low density animals), the question remains as to why an animal tests positive. Is the root cause for the positive result a function of inherent characteristics of the animal, an environmental influence or both? Environmental risk factors could include the presence of a concentrated feed lot in proximity to the production area, irrigation water contaminated with pathogens that is also a watering location from animals, etc. From a risk management perspective, growers and handlers need to assume that most, if not all, animals are potential pathogen reservoirs and vectors and environmental risk factors should be considered when evaluating large animal populations in the production environment.

Finding 3: *The prevalence of* E. coli *O157:H7 and* Salmonella *is lower in native wildlife (tends to be less than 2%) and higher in non-native wild pigs (around 5%).*

What does this means for you?
This project validates research relating to the potential for wild pigs to act as vectors for both *E. coli* O157:H7 and *Salmonella (Atwill 1997, Jay 2007 and Feder 2003).* The presence of wild pig feces in the production environment can present a food safety risk. When conducting environmental assessments and daily harvest assessments, food safety personnel should search for feces or evidence that feces may be present in the production environment. To avoid the need to differentiate between deer and wild pig feces and other animal feces, the recommendation is to treat all feces present in the production environment as a potential risk and respond according to the best practices for the particular commodity (e.g. establishing a NO harvest buffer zone around the feces, etc.).

Finding 4: *While* **c***ertain bird species can act as pathogen vectors, high density flocks are of more concern than a single bird. It appears as if bird may obtain pathogens from local environments.*

What does this means for you?
This research confirms that certain bird species can act as vectors for *Salmonella* and *E. coli* O157:H7. The findings underscore the importance of environmental assessments and efforts to determine if local animal populations are potential risks. In evaluating birds as a potential risk, the food safety person needs to understand the natural history of the bird. With this information, growers can evaluate their own operations and bird populations and then derive different practices to control the environment and measures to discourage birds from locating in the production fields or in an area where direct contamination of the field might occur. During the panel discussion for this project, one grower mentioned his company's observing bird behavior on crops leading to a discovery that birds tend to land on his crops during sunset. With this observation, efforts were made to disrupt birds during sunset. After a period of time, the birds moved to other locations.

Growers should, however, concentrate on birds in high-density flocks. The research demonstrates that the presence of a single bird in a production environment may not present an immediate food safety concern, even with birds that are potential pathogen vectors. If there is a concentration of birds in the production environment, even if they have not been identified as potential pathogen vectors, they should still be monitored. As an example, though geese did not test positive for Salmonella in these studies, because they roost on sewage treatment ponds and are often found in large populations in production areas they should still be considered as potential pathogen vectors.

Conclusions/Recommendations/Next Steps:
Animals cannot be evaluated as potential vectors for pathogens in absence of a specific environmental knowledge of the farm in question. Since all animals tested thus far have demonstrated the ability to harbor human pathogens, they need to be assessed relative to the environments in which they exist, their feeding and migratory patterns and population densities. It is critically important for growers to consider these factors when performing risk assessments and to develop specific risk management practices based on animal behaviors.

7.0 Session II – Good Agricultural Practices: Irrigation Water Quality

PROJECT #5:

Epidemiologic analysis and risk management practices for reducing *E. coli* in irrigation source water supplies and distribution systems

Principal Investigator: Edward R. Atwill, Ph.D., University of California, Davis

Layman's Summary appears as submitted by the Principal Investigator

Our proposal will help the leafy greens (LG) produce industry identify risk management practices and remediation measures that reduce generic *E. coli* in irrigation water supplies. We will use statistical procedures and epidemiological methods to complete the objectives below. Objective 1: Working in close collaboration with the California and Arizona LG produce industry and allied organizations, finalize the master data file for statistical and epidemiological analyses of Objectives 2 through 5. Objective 2: Determine environmental, geographical, structural and operational risk factors for the occurrence of generic *E. coli* in irrigation water supplies. We will also determine the influence of different diagnostic methods on measured *E. coli* levels. Objective 3: Identify predisposing environmental, structural, and operational risk factors associated with generic *E. coli* exceedances in irrigation water supplies. Objective 4: Determine the ability if different mitigation measures to reduce the occurrence of an *E. coli* exceedances in irrigation water supplies. Objective 5: Develop more efficient irrigation water sampling plans for low- to high-*E. coli* risk source water

Photo courtesy of Mike Villaneva

supplies. Completing these objectives will assist the produce industry comply with microbiological standards for generic *E. coli* in irrigation water supplies, avoid future *E. coli* exceedances, and develop more efficient irrigation water sampling plans.

Technical Findings and What They Mean for You:

Data from growers, processors/shippers, and/or commercial labs was obtained and collated in order to characterize the occurrence of generic *E. coli* in four regions of California: north central coast, south central coast, central valley, and desert. Two different datasets were created (dataset 1 and dataset 2) with water samples from four sources (wells, canals, reservoirs, other). In dataset 1, there were more than 44,000 samples, of them 79% have no detectable generic *E. coli* and 0.86% of them have levels ≥ 235MPN/100 mL (the maximum allowable level for single sample pre-harvest foliar applications under the LGMA). In dataset 2, with more than 15,000 samples, 73% have no detectable generic *E. coli* and 0.71% had levels ≥ 235 MPN/100 mL. (The LGMA Metrics require monthly sampling, often more frequently, at points along irrigation distribution systems. Any single sample is required to have a rolling geometric mean of ≤ 126 MPN/100 mL, or ≤ 235 MPN/100 mL for any single sample.) In California the mean *E. coli* concentrations in the north central coast are 3.70 MPN/100 mL (wells) and 55.25 MPN/100 mL (reservoirs); in south central coast, the mean is 6.53 MPN/100 mL (wells) and 109.29 MPN/100 mL (reservoirs).Also in California, *E. coli* concentrations are lowest in the winter (ranging from 0.93 MPN/100 mL in the central valley to 20.66 MPN/100 mL in the desert), and peak in the fall in the central coast (25.81 MPN/100 mL in the south central coast) and in summer in the central valley (29.63 MPN/100 mL) and desert (36.82 MPN/100 mL).

The following are key findings (obtained from the June 2012 CPS Symposium presentation, the panel discussion and the final project report).

Finding 1: *On-farm reservoir samples have much higher concentrations of generic* E. coli *than well water samples in the central coast of California*

What does this mean for you?
Growers should consider their reasons for using on-farm reservoirs given the increased risk of *E. coli* exceedances associated with their use. These results from this study suggest that if you put "clean" well water in a reservoir, environmental influences can degrade the microbial quality of this "clean" water. Concentrations of generic *E. coli* can increase by approximately 300%. It is important to understand why values are higher in reservoirs at certain times. Further work should be done to understand the factors that influence or contribute to this degradation and methods to prevent the erosion of water quality.

Finding 2: *Seasonality is a factor in test results.*

What does this mean for you?
Further work needs to be done to understand the underlying causes for the various risk factors for *E. coli* occurrence, including seasonality, studied in this project. However, because seasonality is a factor, growers, harvesters and handlers should ensure their water sampling SOP's are reflective of the seasonality, regions and water sources trends in their production locations. The research indicates that for locations with relatively low variability in *E. coli* readings within a season, fewer samples are needed to determine water quality with a degree of certainty. On the other hand, locations with a high degree of variability within a season will need increased sampling to determine water quality with a degree of certainty. Companies can review prior year(s) test data for seasonality patterns and then modify SOP's to increase/decrease testing timeframes depending on historical test results as a risk management plan. (Note: changing company specific water testing SOP's would not necessarily change standards as outlined by customer specification or marketing orders or agreements, e.g. LGMA water testing commitments. The intent behind the recommendation is foster operation-specific risk assessment and the development of specific risk management practices.) The results of these individual company SOP seasonality studies, if made available as blinded data to the industry, could then be used to support risk-based modifications to food safety guidances like the LGMA and/or other food safety guidance water testing frequency requirements. Ultimately this type of investigation could stimulate a risk-based approach to water sampling and testing that might permit better targeting of when samples should be

taken and the frequency for that sampling as opposed to current programmatic samplings characteristic of several guidances, e.g. water testing every 30 days irrespective of seasonal or other risk factors characteristic of the growing region or operation.

Finding 3: *As the sample volume increases, the probability of detection of generic* E. coli *increases. Therefore, companies should consider amending current sampling volume practices by increasing the sampling volume to ensure more accurate assessment of generic* E. coli *levels in irrigation water sources.*

What does this mean for you?
Data presented in this project indicate that the current practice of single 100 mL grab sample has a lower probability of detecting generic E. coli than samples using higher volume, e.g. 1 liter (1,000 mL). As the goal is to generate water testing data that demonstrates the relative safety of specific irrigation water sources, it is important to use sample volumes and sampling procedures that give the grower the best chance of uncovering contaminated water sources and implementing management practices to curtail any potential for cross contamination.

Finding 4: *Industry collaboration is critical when developing risk-based monitoring programs.*

What does this mean for you?
Without access to industry water data, there is limited ability to determine *E. coli* occurrence levels in or across regions. The result is food safety guidance will adopt the most conservative monitoring programs deemed necessary to protect produce safety and hence human health, without supporting data. When companies collaborate on industry studies, the results can lead to risk identification and prioritization that is more reflective of actual conditions with the potential of more focused and possibly fewer test requirements. In this study, the availability of blinded test results and seasonality studies described above could lead to modifications to LGMA guidance and other produce guidance requirements for water testing that may result in industry cost savings.

Conclusions/Recommendations/Next Steps:
Sharing irrigation water testing data to facilitate evaluations of overarching risk factors and development of improved sampling/testing methods is critically important. Mechanisms can be developed to protect the proprietary nature of the data without limiting its value to the industry. This project clearly demonstrates the need to for growers to consider the source of irrigation water and how it is delivered to the plant when performing their risk assessments. This approach can lead to risk-based sampling programs that account for water source, method of conveyance, seasonality, environmental conditions and other variables as the industry replaces programmatic, generalized sampling schemes common in current guidances. The industry should also include consideration of increasing sample volumes to permit more probable detection of generic E. coli in water sources to facilitate improvement of risk models.

Commodity organizations and mechanisms such as the LGMA should consider how their programs can incentivize the collection, aggregation highest value use of industry data. Examples of these efforts might include revisions to guidance documents that define and recommend documentation of key data elements such as environmental conditions as contextual information for water samples, incorporating recommendations for larger samples and targeted (seasonal, risk based etc.) sampling. Governing bodies such as LGMA might request or even require data submission from members for use by academics and industry in research and guidance development.

PROJECT #6:

Science-based evaluation of regional risks for *Salmonella* contamination of irrigation water at mixed produce farms in the Suwannee River watershed

Principal Investigator: Anita Wright, Ph.D., University of Florida

Layman's Summary appears as submitted by the Principal Investigator

Outbreaks of human illness associated with produce have resulted in questions about the safety of the water used for irrigating these products. We have assembled an experienced team from the University of Georgia at Tifton and the University of Florida to address the water quality of vegetable irrigation ponds in the Suwannee River watershed. Irrigation water quality standards are not currently regulated or determined by scientifically based metrics. Coliform bacteria are widely used as indicators of fecal contamination, but their validity as indicators of bacterial pathogens is questionable. We propose to investigate the relationship between the occurrence and distribution of these indicator bacteria with that of a specific pathogen, namely *Salmonella enterica*. Growers in this principal produce production area of the United States have agreed to allow periodic collection of water samples from irrigation ponds to provide preliminary data and validate methodologies. Proposed research will systematically examine bacteria in and around 10 irrigation pond sites for a two-year period under a variety of environmental conditions. This research will identify management practices, environmental parameters, and locale characteristics associated with increased risk of pathogen contamination by irrigation water and will provide a research-based comparison of indicator organisms and *Salmonella* in a major fruit and vegetable growing area.

Technical Findings and What They Mean for You:

In this project the Suwannee watershed, including irrigation ponds, fruit and vegetable crops and variable buffers around ponds, was evaluated from Okefenokee to the Gulf of Mexico. The source of the water in the watershed is primarily rain, streams and groundwater though most water comes from rainfall. Pond physiochemical parameters, vegetation, microbial diversity, wildlife and domestic animals and crop rotation were all examined. Results from year one of the two year study included the finding of *Salmonella* in all ponds – in both water and sediment. Surface water samples were 45% positive; subsurface samples were 33% positive. There were no strong correlations seen with any of the 32 physiochemical parameter data collected from the ponds.

The following are key findings (obtained from the June 2012 CPS Symposium presentation, the panel discussion and the 2011 project report).

Finding 1: *Fecal indicator bacteria (*E. coli *and fecal coliforms) testing was of little or no predictive value for* Salmonella*. There was some correlation with fecal coliforms that may have been from a common source.*

What does this mean for you?
Growers need to know as much as possible about their water sources. Because a lack of correlation of indicators with pathogens has consistently been shown, analyzing trends in water test results and not just looking at individual test results continues to be critical for "knowing" your water source. If *Salmonella* is a known issue in the watershed, adjusting testing regimens accordingly is important for risk management.

Finding 2: *Seasonality is a factor in the* Salmonella *levels in the watershed. The highest levels were detected in July.*

What does this mean for you?
Growers need to ensure their water testing SOP's reflect the underlying risks associated with seasonality factors such as temperature, rainfall and recreational water use and that the risks are appropriately addressed in their environmental assessments.

Finding 3: *While multiple strains of* Salmonella *were present in high levels throughout the Suwannee River, there does not appear to be produce contamination when using the water.*

What does this mean for you?
This finding is informational only and is not actionable at this time. Further research is needed to understand if irrigation water contaminated with *Salmonella* in the Suwannee watershed has been associated with contaminated produce, given there are known cases in other areas where irrigation water has contaminated produce with *Salmonella*.

Conclusions/Recommendations/Next Steps
Sampling protocols need to be developed based on identified risks. Indicator organism testing, while informative, is not as effective as testing for the pathogen itself. Testing water for indicators and analyzing the data can give a grower a sense of the "base line" microbial variations for the water source and how it might fluctuate over the course of a year. Obviously, the more data points collected over time the more reliable this base line data is as a tool. However, it is increasingly clear that specific historical indicators like generic *E. coli* are not reflective of pathogen contaminations and we need to seek better indicator organisms or re-evaluate our testing programs to include pathogens or pathogen surrogates. Clearly, the decision to use pathogen-specific tests versus indicator tests is not to be taken lightly. It is important that the choice of test be matched against known potential contamination factors (e.g. known wild or domesticated animals, animal densities, environmental factors, delivery systems for the irrigation water, characteristics of the crop, etc.), likely pathogens that might be present, test costs and actions that could be taken to mitigate positive test results. As noted in the discussion of the project presented by Dr. Atwill and reinforced by Dr. Wright's data, our increased knowledge of water quality risk factors, prevalence of pathogens in specific water sources, sampling methods and testing limitations need to be weighed as the industry looks to develop more meaningful, risk-based irrigation water testing schemes.

The next research steps for Dr. Wright are to assess antibiotic resistance of the recovered Salmonella serovars, correlation of microbial data with pond characteristics and to look at wildlife as potential contributors to the observed *Salmonella* levels.

PROJECT #7:

Risk assessment of *Salmonella* pre-harvest internalization in relation to irrigation water quality standards for melons and other cucurbits

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

Layman's Summary appears as submitted by the Principal Investigator

The production of melons, including cantaloupe, honeydew, and watermelon, and other cucurbits (specialty melons, cucumber and squash) requires ample quantities of irrigation water of appropriate microbiological quality to ensure this essential input does not contribute to food safety risk to consumers. A great deal of uncertainty surrounds the setting of rationale and practical standards for growers to follow to meet these expectations. In open environments, it is unreasonable to expect that no pathogens of concern will ever be in surface water used for irrigation at some low level. Internalization of pathogens from soil and transfer to edible portions of fruits and vegetables has become a concern in recent years. The primary purpose of this research is to determine, by greenhouse and open field testing, the threshold level of *Salmonella* that would be required to represent a risk of fruit contamination by uptake of pathogen-contaminated irrigation water through the root system and subsequent transfer through the vine. We anticipate this threshold will be 10,000's times higher than levels of *Salmonella* reported in irrigation source water for domestic production of these crops. Food safety standards for melons and cucurbits will not need to remain preoccupied with the risk of internalization from roots.

Technical Findings and What They Mean for You:

To understand melon, predominantly cantaloupe, production risks relating to irrigation water quality, attenuated *Salmonella* was applied to soil in furrows and through subsurface injection in root zones. The presence of *Salmonella* in soil, root uptake, translocation through plant was then tested in more than 500 melons. *Salmonella* internalization in the vines and through the vascular system was determined to be based on the cultivar, *Salmonella* serovar and pathogen dose. Within two weeks; however, *Salmonella* became nondetectable. Contaminated water in furrows did not transfer laterally to the root zone of plants nor was it detected on top of the cantaloupe beds. In contrast, contaminated water applied via subsurface drip injected irrigation resulted in detectable pathogen in the soil and rhizosphere. Although Salmonella was detected in portions of the vine, in soil and rhizosphere, the results showed no evidence of fruit internalization.

The following are key findings (obtained from the June 2012 CPS Symposium presentation, the panel discussion and the final project report).

Finding 1: *The field studies demonstrate the lack of internalization of* Salmonella enterica *into fruit after various cucurbits (specialty melons, cucumber, zucchini, and squash) were drip-irrigated with* Salmonella *contaminated water that was shown to have also contaminated the surrounding soil.*

What does this mean for you?
For growers, it means that if *Salmonella* is present in the soil and in irrigation water, it is unlikely to migrate into the melon itself via uptake through the root system. However, *Salmonella* still has the potential to move from contaminated irrigation water and soil onto the cantaloupe rind surface and potentially internally if the rind is compromised. While this study demonstrates the lack of *Salmonella* uptake through roots for melons (cantaloupe, honeydew, and watermelon), and other cucurbits (specialty melons, cucumber and squash)), further work would need to be done to demonstrate whether the results are similar for other commodities (Note: this work has already been done for lettuce. As in this study, there was no root uptake following direct soil drench (Koike, 2009) In other experiments presented by Dr. Suslow, pathogens were shown to migrate a certain distance when experimentally introduced directly into injured surfaces, e.g. peduncle tissue and the degree of migration was based on tissue structure, plant maturity, melon variety, *Salmonella* serovar, and the dose. Since physical and biological factors seem to limit the ability to translocate pathogens, different commodities would have to be evaluated independently to identify specific risk factors. This finding stresses the importance of performing adequate quality examinations at harvest to be sure that cantaloupe tissues are not compromised (e.g. broken, cut or even bruised), which provides an opening for contamination should pathogens be present.

Conclusions/Recommendations/Next Steps
The research presented in this project is a good reminder that we need to apply proper perspective, good science and knowledge of industry practices when identifying and prioritizing contamination risk factors. We have seen a number of studies conducted in laboratory environments that show adherence of pathogens to root tissues. Often the inoculation doses used are quite high and the experimental conditions favor pathogen survival and growth; conditions that are not reflective of vegetable production environments. However, this is the second research report presented at CPS Symposia that demonstrate that in field conditions, root uptake into tissues does not occur. Taken together the studies should give growers and others confidence that appropriate quality irrigation water will not contaminate cantaloupes, melons, cucumbers or leafy greens through plant uptake.

These data also show that if *Salmonella* is artificially introduced into melon tissues, movement can occur but the characteristics of the tissues can limit this movement. As a grower or handler of produce, the best management tool for managing this risk is the very same management tool employed to control proper market quality — sorting product so that damaged fruit is culled out.

PROJECT #8:

Microbial Food Safety On-Farm Risk Assessment

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

Layman's Summary appears as submitted by the Principal Investigator

The purpose of this Rapid Response field study was to assess the potential public health hazard, within a unique on-farm data gathering opportunity, of a cantaloupe field adjacent to a small dairy operation. Our objective was to document the likelihood of presumed, localized dispersal of contaminants due to agriculture traffic, animal activity, and other direct and indirect transfer of fecal indicators and pathogens from the animal facility before the intended initiation of harvest at that location. In addition, the Rapid Response opportunity permitted the assessment of commercial kits and in-house UC Davis developed molecular methods (up to six different methods) to detect EHEC and Salmonella enterica in melons harboring low levels of stress-adapted pathogen populations against a high background of microbial populations and adhering soil.

Technical Findings and What They Mean for You:

The cantaloupe field under study received applications of raw manure solids from the dairy waste lagoon adjacent to the animal feeding operation (AFO). Prior to harvesting, a microbiological assessment of the cantaloupe field and surrounding area was conducted. The assessment included melon samples and environmental samples from irrigation water, run-off water, ditch sediments, dairy corral surface material, soil, manure, compost material, and air samples that were tested for total coliforms, fecal indicator bacteria (generic *E. coli* and *Enterococcus*), *Salmonella enterica* and pathogenic *E. coli.* Pathogenic *E. coli* was detected in melon, water, algae, lagoon, manure, and soil samples. In contrast, *Salmonella enterica* was detected in one composite melon sample, but was not detected in any of the environmental samples. Samples from the lagoon as well as algae, manure, compost, and water samples from the irrigation ditch were positive for generic *E. coli*. Generic *E. coli* levels in water samples taken from the irrigation ditch close to the cow corrals and lagoon were higher than from sources more distant from these two landmarks.

Using a Riboprinter®, a product that generates a DNA "fingerprint" of regions of the ribosomal RNA genes which are unique to a particular organism, total coliforms from the AFO were linked to those present in the melon field. Total coliform and *Enterococcus* spp. quantities were greater on melons adjacent to AFOs than on melons in other areas of the production parcel, and their concentration decreased the further from the AFO the measurements were taken. While melons throughout the rapid response field tested positive for generic *E. coli* and *Enterococcus*, these fecal indicator bacteria were not detected on melons from other regional fields.

Following the analysis, the crop was destroyed. A microbiological soil assessment conducted post-discing revealed a 3-log increase in total coliforms along the entire field parcel likely due to the incorporation of organic matter and the release of melon's nutrients and sugars. Detection of pathogenic *E. coli* markers also remained positive. After disking the crop, maize silage was planted in the affected field as a cover crop and grown for 60 days before harvesting the primary foliage for animal feed and discing the residual crop according to standard practice. Following harvest and discing, the soil was resampled with results showing that *E. coli* indicators had uniformly declined to below the detection limit in 92% of the samples. With the cover crop (maize), positive soil samples declined from 90% to < 8%. This has implications for using raw manure on production land as well as the efficacy of remedial programs for contaminated land that use a cover crop to reduce pathogen populations.

The following are key findings (obtained from the June 2012 CPS Symposium presentation, the panel discussion and the final project report).

Finding 1: *Sometimes the level of generic* E. coli *in a production environment can indicate a contamination event has occurred and that there is an issue that warrants further investigation. In this rapid response project case, generic* E. coli *levels in irrigation ditch samples were higher in areas that were closer to the AFO than areas farther away.*

What does this mean for you?
While generic *E. coli* tests are not optimal indicators of the presence of pathogenic *E. coli*, they are useful in establishing baseline water quality levels. Then, when levels deviate from the baseline, growers have some indication that an unusual occurrence might have taken place and further investigation is needed. For this reason, generic *E. coli* testing provides valuable information for assessing risk.

Finding 2: *When cantaloupes were planted in proximity to a small animal feeding operation, irrigation ditch indicator* E. coli *levels increased dramatically as the distance to the feeding operations decreased. This occurred even though the source water had very low levels of indicator* E. coli *levels.*

What does this mean for you?
This is an example of why environmental assessments are critical to a grower's ability to manage food safety risks. Irrigation water test data or any testing data has limited value if not analyzed within the context of the environment from which the samples were taken. In this case, the measurement of indicator generic *E. coli* may not have triggered further investigation without the accompanying risk assessment that identified the adjacent CAFO as a potential risk factor. It is important that any test data be viewed within the context of the production environment. It is important for growers or any operator that uses microbial testing as a tool to verify a food safety practice understands this principal so that informed decisions can be taken to insure the safety of the food.

Finding 3: The design and approach of a *microbial testing program should be risk-based. If sampling is simply a scheduled task, i.e. sample x, every y days, it is quite possible risks will remain undetected. While "cookbook" strategies are often attractive because they require less engagement, they may not be as effective as risk management tools.*

What does this means for you?
In this project sampling criteria was based on a hazard assessment of the production field, a subsequent review of the field, and then a determination of potential and presumed contamination risks. If sampling SOPs are only schedule-based and do not include sampling protocols when identified risks occur, companies may miss a contamination event since it was not detected during the normal sampling cycle. To increase the probability of detecting a food safety problem, companies should review their environmental risk assessments, prioritize

potential and identified risks and develop a sampling plan based on the risks and probable occurrences. For example, if rain events trigger higher generic *E. coli* levels in irrigation water, companies should revise their sampling plans to incorporate sampling following rain events. Reviewing sampling plans along with historical results is necessary to ensure sampling is effective.

Finding 4: *Field equipment was a mode of transfer when flail chopping and discing equipment were used to destroy the vine/fruit inoculated with* Salmonella.

What does this mean for you?
Nearly every food safety standard includes language that advises growers and harvesters to properly clean and sanitize field equipment to prevent crop contamination. The data presented here provide evidence that, indeed, contamination can occur when farm equipment has been in contact with contaminated soils or plant materials. Therefore, companies need to ensure field equipment is cleaned, sanitized and tested to verify the sanitization was conducted properly. It is prudent to consider risk factors and assume that pathogens might be present and make field equipment cleaning and sanitation a routine practice. In those limited incidences where pathogen testing is performed on water, soil or plants and they are found, then special care must be taken to clean and sanitize properly to avoid cross contamination risks.

Finding 5: *If pathogen contamination is discovered in a field, subsequent plantings of cover crops may be an effective practice to reduce contamination levels in the soil thereby reducing the risk of subsequent cross contamination on future vegetable crops.*

What does this means for you?
Data from this project indicate that the use of a cover crop might play a role in reducing potential crop contamination in subsequent plantings. In this study the use of a cover crop reduced the recovery of positive samples from 90% to <8% in a field where large amounts of raw manure solids from a dairy waste lagoon and cow corral were applied approximately 41 days prior to seeding and 135 days prior to intended harvest. There are likely several factors involved in the reduction of positive tests including cultivation practices, environmental factors and the survival capabilities of these organisms in harsh production environments, but these data offer evidence of mitigation practices that can be easily employed by growers when contamination is uncovered in fields. The question of what can be done with fields where product, soil or water testing has revealed indicator or pathogen presence may now begin to be addressed. Crop rotation practices where cover crops are periodically employed may be modified to address sporadic contamination events and restore grower confidence that a particular field is fit for planting vegetables or fruits. Clearly, more work needs to be done here to better understand cultural practices that can be used to manage these risks but the data presented here give growers and researchers a path to pursue to more fully address this issue.

Conclusions/Recommendations/Next Steps
This rapid response project demonstrates the value in partnerships between researchers and growers to address food safety issues. Microbial findings led to unfettered access for research teams to study an unusual and unique environment where a CAFO was suspected of contaminating a crop and provided a real time opportunity to advance our food safety knowledge base. This project helps to draw the linkage between the use of basic microbial testing and the necessity for coupling that testing to frequent environmental risk assessment. It also points out the importance of designing risk-based sampling plans to increase the likelihood of identifying a problem so that it can be addressed. It is important to note that performing active risk assessment and thoughtfully employing microbial testing programs requires operator engagement and proactive action and not a "follow the book" approach to food safety. We also saw the value of emerging technology in the use of riboprinting to validate that the microorganisms associated with the CAFO were indeed the same (i.e. genetically identical) to those subsequently identified

in the adjoining field of cantaloupes. This technology can be a useful tool in investigating contamination events and it is likely we will see its use increase. Lastly, this project provided some direction on what growers can do to ensure that land associated with a pathogen contamination can be restored (i.e., the risk of subsequent cross contaminations on following crops can be managed). The industry could benefit from further research in this area and that research may be best conducted through future rapid response projects like this one where thoughtful growers permit qualified researchers access to actual production environments where mitigation practices and their impacts can be measured. Lastly, it is logical to assume that if *Salmonella* (and perhaps other pathogenic organisms) are intermittently present at low levels in the production environment, then farm equipment needs to be routinely cleaned and sanitized to keep that equipment from becoming a source of cross contamination. Food safety standards and best practices recommend sanitation programs for farm equipment, and the data developed by Dr. Suslow support this recommendation.

PROJECT #9:

Mitigation of irrigation water using zero-valent iron treatment

Principal Investigator: Kalmia Kniel-Tolbert, Ph.D., University of Delaware

Layman's Summary appears as submitted by the Principal Investigator
Significant problems have occurred in the U.S. with regard to the contamination of produce by pathogenic bacteria. Minimally processed produce lacks the processing and preparation hurdles, such as cooking, to aid in reduction or elimination of the occasional and incidental contamination that can lead to widespread outbreaks and national product recalls. Greater emphasis has been placed on preharvest Good Agricultural Practices, and postharvest Good Manufacturing Practices, but the American food production and distribution system is vast, complex and global. Environmental fecal contamination is not uncommon in these foods, and transmission of human pathogens to plants through contaminated irrigation water has been documented under both laboratory and field conditions. This project proposes to develop and evaluate a high-volume treatment for irrigation water utilizing filtration through columns of zero-valent iron (ZVI) and sand. ZVI has been successfully used for over ten years in commercial water treatment operations to remove chemical contaminants. Evidence has described the adherence and inactivation of viruses and *Escherichia coli* by ZVI used in water treatment. The objective in this project is to optimize removal of E. *coli* O157:H7 and *Salmonella* Newport from water treated by passage through ZVI-sand columns under conditions modeling commercial use.

Technical Findings and What They Mean for You:
ZVI, essential ground up iron that can be sourced from recycling operations, has been used for some years in groundwater remediation. In the first year of this project, the research goal was to demonstrate ZVI's ability to remove/inactivate *E. coli* O157:H7. The phase 1 results demonstrating the efficacy of ZVI for removal/inactivation of *E. coli* 0157:H7 and Salmonella were reported out during the 2011 CPS Research Symposium. In the second phase of the project reported out at the 2012 Symposium, research was conducted to determine if ZVI is a potential high-volume solution for removing and inactivating bacterial pathogens from irrigation water.


Photo courtesy of Kalmia Kniel

The following are key findings (obtained from the June 2012 CPS Symposium presentation, the panel discussion and the final project report).

Finding 1: *ZVI is a useful addition to a sand filtration system to reduce* E. coli *O157:H7 and* Salmonella *contamination in irrigation water.*

What does this means for you?
In essence, this was a proof of concept project. When ZVI was used in conjunction with sand as a filter for water purposely contaminated with pathogens at various concentrations, the researchers demonstrated that the pathogens could be effectively removed over time. This data opens the opportunity for further research and reduction to practice studies to more fully determine where this type of filtration system might most effectively be employed, an analysis of achievable flow rates, and an evaluation of implementation costs and benefits. For growers with potential water quality issues, ZVI represents a good opportunity to undertake a collaborative development program to evaluate this technology in a commercial environment.

Finding 2: *ZVI looks to be a relatively simple and inexpensive tool that can be added to existing sand filtration units to reduce pathogen contamination risks in agricultural water.*

What does this mean for you?
ZVI may allow the use of water that otherwise would not meet irrigation water standards or the potential re-use of irrigation water. For growers with water issues, a ZVI sand water filter has the potential to be a relatively low cost tool as the iron used is commercially available, has a high surface area, and a long service life. Greenhouses, small production facilities and areas where acceptable water sources are scarce, in particular, may benefit from ZVI technology applications. Also, since a ZVI filter does not contain disinfectant chemicals, harmful chemical by-products are not released into the environment. Many people already use sand filters and ZVI could be added to current systems. That said companies will still need to conduct their own cost-benefit analysis prior to introducing the tool.

Finding 3: *The research results do not indicate what happens to the organism (is it being inactivated, retained on the ZVI matrix, killed off, destroyed or something else?).*

What does this mean for you?
There are numerous experimental variables relating to the performance of the ZVI filter (e.g. flow rates, dissolved solids in the water, pH effects, mineral content, etc.) and they require further research. It is important to know the fate of pathogens captured by the ZVI and the capacity of ZVI to capture microorganisms over long periods of time with varying volumes of water. Still left unanswered is the question of how long these filters might be effectively used although we know that ZVI offers high surface areas and "sites" to bind or capture bacteria. We also need to understand how these ZVI/sand filters might be "recharged" or reconditioned in commercial operations.

Conclusions/Recommendations/Next Steps
The concept of using ZVI and sand to remove pathogens from agricultural water shows promise. The task that remains is to move from the concept phase to reduction to commercial practice. It is unlikely that ZVI will be equally effective in all applications. Indeed, Dr. Kniel suggested that the technology might be better suited to applications where flow rates and delivered volumes are not high. Future research could also focus on what else can be removed from water besides pathogenic bacteria, e.g. viruses, organic contaminants like nitrates, etc.? Lastly, this research project is a stark example of how sometimes simple can work. A low cost, by-product of iron reclamation can be combined with sand to provide a tool to growers or others in the supply chain to treat higher risk water sources to manage cross contamination. The results of this study also present opportunity for close collaboration between the research community and growers in that next phases of study should include in situ testing in the field.

PROJECT #10:

Evaluation of the baseline levels of microbial pathogens on Washington state fresh market apples and mitigation measures used to eliminate contamination

Principal Investigator: Diane Wetherington, Intertox, Inc.

8.0 Session III-Good Agricultural Practices- Inputs, Cultivation, and Harvest

Layman's Summary appears as submitted by the Principal Investigator
The purpose of this proposed research project is to determine the occurrence of microbial contaminants on fresh market apples and identify practices industry participants are using to mitigate these contaminants. Intertox will work closely with the Washington Tree Fruit Research Commission and its partners to identify available datasets of microbial contaminants levels, ensuring the confidentiality of any private data, and to develop datasets if needed. Data will be evaluated for their quality and relevance, and compiled into a database. Once complete, this dataset will be used to derive statistics on baseline microbial populations. Finally, the effectiveness of mitigation practices on reducing microbial levels will be examined.

Technical Findings and What They Mean for You:
Surveys from 55% of the Washington state apple packinghouses provided details on their microbial food safety programs and practices. Survey results revealed the types of water, environmental and final product testing currently in place. All survey respondents follow at least one prescribed food safety program; SQF 2000 is the most commonly followed program. Seventy-eight percent use microbial swabs to check microbial levels at their packing facility — testing most frequently for generic *E. coli*, *Listeria*, and *Salmonella*.


Photo courtesy of Ines Hanrahan

Twenty-eight percent, at the time of the survey, conducted microbiological finished product testing.

Twenty-nine percent of Washington packinghouse companies provided access to their microbial test data from 2005-2010. The data was compiled into a database with more than 2,700 records.

The following are key findings (obtained from the June 2012 CPS Symposium presentation, the panel discussion and the final project report).

Finding 1: *Trust is critical for obtaining access to proprietary data. Industry groups and associations can play a pivotal role in enabling investigative relationships between data owner and researchers.*

What does this mean for you?
Cooperative management of data among industry associations, private companies and researchers is a model that can be used with other commodity groups in dealing with sensitive data. In this project confidential test data is protected through non-disclosure agreements and the removal of individual company identification before analysis. As with other commodity groups, there are challenges with obtaining industry data—competing priorities, concern about what is reportable to regulatory agencies as well as disincentive to share data and concern that data will be misinterpreted by the public. This level of data protection is often needed to convince companies to work together and provide data when there is no food safety crisis.

Finding 2: *Water used in packinghouses and finished products tested positive for pathogens; however, the prevalence of pathogen positives was <1%.*

What does this mean for you?
Commodity-specific test results, whether related to growing, shipping, packinghouse or processing operations are critical for establishing prevalence levels and ultimately for determining the risk level associated with specific production and handling practices for individual commodities. Known prevalence allows the industry to develop quantitative risk assessments based on actual versus estimated risk providing a solid foundation for science- and risk-based commodity-specific food safety guidelines.

Finding 3: *Microbial environmental monitoring programs vary widely. The industry would benefit from an environmental monitoring process validation study (to confirm location, frequency and types of testing).*

What does this mean for you?
While this study was specific to the apple industry, other commodities could benefit from similar validation studies to determine if environmental monitoring programs in place are necessary, effective and sufficient from both a cost and a food safety perspective. For example, sample location selection is critical for an effective environmental monitoring program. Sample collection needs to follow a protocol that was developed to address areas susceptible to contamination.

Finding 4: *Based on the data collected to date, there is no apparent correlation among individual positive microbial water, environmental and product test results. While additional data is being collected that may identify a correlation, without understanding the cause of the problem, the ability to determine what if any process(s) are out of control or if the event is random complicates efforts to address problems and avoid further positive results.*

What does this mean for you?
When faced with a positive microbial result without knowledge of the underlying cause or causes, companies need to re-evaluate and verify their processes and practices to understand what factors contribute to microbial and growth and then to determine if those factors are being adequately controlled. As previous

research has demonstrated for leafy greens, melons (Beuchat 2002, Leverentz 2001, Ukuku 2002) and now apples, differences in commodity characteristics (e.g., exterior surfaces), handling and sanitization practices, and worker practices may contribute to or inhibit microbial growth. This level of research is fundamental for determining commodity-specific risk factors. Without this information, it is difficult for growers, handlers, and processors to understand and to develop programs for managing risks.

Conclusions/Recommendations/Next Steps:
The next steps in this project are to use the data in the development of a quantitative microbial risk assessment model for fresh market apples. Using the model results, the industry will have an understanding of the relative risks associated with different processes and will be able to focus food safety efforts and resources toward finding effective management practices. This project highlights the conundrum faced by the produce industry as we collectively look to develop effective food safety programs. To date, the industry and researchers have focused considerable effort in identifying potential risks for pathogen contamination. However, we have not really been able to identify if all risks are actually equally significant or determine if some more concerning than others. We have not generally looked to quantify the impact of risk factors or developed management priorities. Therefore, we end up treating all risks as high priority and likely dilute the resources we can employ to effectively manage those activities that represent the greatest contamination risks. The produce industry routinely collects data (e.g. soil, water and product testing data, environmental testing data, process measures, audit data, etc.) that could be used to enable more quantitative risk assessments and identify key practices that can be prioritized to minimize the consequences of contamination events. This issue has emerged in earlier CPS-funded research programs and will be discussed further (see the discussion of the L. Harris project).

PROJECT #11:

Using Leafy Green Marketing Agreement audit data to determine non-compliance areas and preparation of training and recommendations for improvements in future growing seasons

Principal Investigator: Diane Wetherington, Intertox, Inc.

Photo courtesy of Mike Villaneva

Layman's Summary appears as submitted by the Principal Investigator
The purpose of the Intertox Leafy Greens Marketing Agreement (LGMA) Audit Data Evaluation Proposal was to use data collected by the California Department of Food and Agriculture (CFDA) during audits of leafy greens producers to determine if there are more efficient and effective methods for preventing the microbial contamination of these crops. This proposal consists of four elements: the collaboration with the CDFA and the LGMA Advisory Board to obtain confidential audit data for analysis; the preparation of training tools and training sessions for growers, and, recommendations to LGMA for any changes in best practices and/or the audit document.

Technical Findings and What They Mean for You:
For this project, audit data results from 2008-2011were obtained from the California Leafy Greens Marketing Agreement. Confidential client data was removed and a final dataset of 7,500+ records was created, consisting of 1,382 audits for 303 growers. While the LGMA audit database is organized by handler, the supply chain dynamics in the leafy green industry complicate the determination, from a quality control perspective, of whether the data reflects the handler, the grower, the harvester, the cooling company or some other third party company. This complication is problematic when an issue is identified and is not viewed as a shared issue/responsibility across companies in the supply chain.

For benchmarking purposes, in this research project each violation, deviation, infraction and observation in every audit was assigned a score. The scoring was coded as: potentially flagrant violation = 5 points; major deviation = 4 points; minor deviations = 3 points; minor infraction = 2 points; observation = 1 point; and no violation = 0 points. The scores were then summed by audit. Based on this scoring system, a “0” is an ideal score. Between August 2008 and March 2011, audit scores ranged from a high of 71 to a low of 0. The average audit score improved from 8.6 (2006) to 5.9 (2011). Then questions with greater than 5% non-compliance rates were examined. The highest non-compliance rates were improper work practices (26%), sanitation facility deviations (12%), improper water uses (11%) and evidence water sources and distribution sources may pose a contamination risk (10%). Using auditor comments to determine the root cause for the non-compliance, training recommendations were made to address the non-compliance at the supply chain level.

The following are key findings obtained from the June 2012 CPS Symposium presentation, the panel discussion and the final project report.

Finding 1: *Protecting proprietary data is essential for industry level research.*

What does this mean for you?
Cooperative efforts among researchers, the industry and in this case, the government, were needed to obtain access to confidential audit data. Unlike other projects where industry data is made available to researchers, this audit data is owned by the CDFA and not the grower or the handler. The CDFA data initially contained confidential grower, handler and third-party company details; therefore, a strong data management program was required along with accompanying non-disclosure agreements to gain access to the raw data. As discussed here, often research efforts that rely on access to industry data have failed as growers and handlers are reluctant to release proprietary data. Their principal concern is the “unintended consequences” of how the data or experimental results might be used or perceived by others in the industry, buying groups or regulators. This project demonstrates that it is possible to gain industry-wide support for providing data for industry level research if a strong data management program to protect the data owner’s identity is put in place. In this way, individual companies can use their data within the broader produce community to understand industry-wide risk factors, learn about effective risk management practices from each other and optimize their food safety efforts. In order to maximize the return on investment for data collection it must be put to its most beneficial use. While discrete company level data is important to individual companies for both improvement and compliance the aggregation and sharing of individual data is necessary for the advancement of food safety in fresh produce.

Finding 2: *Effective supply chain management is critical for produce safety.*

What does this mean for you?
Because of the complexity of the fresh produce supply chain, companies need to understand how to effectively manage a diverse group of relationships (e.g., harvesters, service vendors like portable toilet rental companies and packaging suppliers, soil amendment suppliers, testing laboratories, packinghouse operators, cooling facility operators, transportation companies, shippers, processors and ultimately retail or foodservice buyers). When one company in the supply chain has a food safety failure, it affects the entire supply chain and the industry. To reduce the risk that operational or handling failures compromise food safety, supply chain training is critical as are performance metrics accompanied by a system of checks and balances that are mutually supportive and therefore encourage trust. For example, every operation should ensure that all companies that provide services or materials to their operations are aware of and adhere to their food safety program and understand their role in producing a safe product.

Finding 3: *Benchmarking is useful for identifying and making improvements in industry wide and individual company food safety programs and performance.*

What does this mean for you?
Collecting data without using it to evaluate and, if

warranted, improve practices would be overlooking a valuable resource. Regular comparisons of process results with best practices help identify opportunities for improvement. Audit noncompliance areas are particularly useful for first determining how well the industry is performing and then evaluating individual company performance in relation to the industry (benchmarking). Risk areas that over time continue to be problematic for companies indicates processes may be out of control, that they need to be improved upon or further training is needed. Benchmarking can be used on a regular basis to identify performance gaps and to prioritize risk areas. These gaps can then be addressed through training, development of supportive collateral and resources and ancillary services.

Finding 4: *Quality circles are beneficial when addressing performance gaps.*

What does this mean for you?
As a result of this project, quality circles were incorporated into the LGMA handler training program focused on pH and chlorine testing. Quality circles are groups of workers, in this case field workers, managers and food safety personnel, who come together for the purpose of identifying and developing process improvements. The theory behind a quality circle is that workers are often aware of issues that need to be addressed and are in the best position to recommend solutions.) Having individuals work together to address a problem or to discuss how they have addressed a problem in a "quality circle" can be more valuable from a training perspective then classroom lectures and train-the-trainer efforts. With quality circles, employees become vested in identifying problems and finding solutions. Food safety becomes personal. Individuals learn, regardless of their responsibilities or organizational level, how important what they do is to product safety and ultimately to keeping consumers safe.

Conclusions/Recommendations/Next Steps:
Additional training sessions will be held to address other quality gaps identified in the research. This methodology would be useful to other commodity groups, particularly ones with audit programs. Formal and regular mechanisms to aggregate and review data should be established. Aggregated data should be put to its highest beneficial use allowing benchmarking of individual performance, continual food safety program calibration, development of training programs and collateral and continuous food safety improvements.

This program highlights an important aspect of food safety: the data generated by individual growers, handlers or operators can be used internally to improve the food safety performance of that operation and externally to benefit the food safety practices of the entire industry. In the produce industry, we often see audits as a mechanism to demonstrate food safety competence to a potential buyer or perhaps as a tool to help us improve our food safety programs for our ranch, harvest crew, packinghouse, cooler or processing operations. However, we often do not see audits as important data points where the answer to each question is a unique piece of data that taken with other data points from other operations can help us to identify industry training needs and knowledge gaps. In the produce industry, the key to accessing this data and creating a system where it might be shared with researchers to permit detailed analysis is protecting the identity of the data source. The program described here where the California LGMA and Intertox collaborated to share proprietary food safety audit data to identify handler training needs demonstrates that, with careful thought and experience in data management, it is possible to protect the proprietary nature of raw data so that it can be used to benefit industry food safety efforts. LGMA and Intertox are using the information generated from this joint program to develop targeted training sessions for leafy greens handlers. Several training sessions have already been held and additional training sessions will be scheduled to address other food safety knowledge gaps identified as a result of this research. This methodology could be useful to other commodity groups who have mandatory audit programs.

PROJECT #12:

Developing and validating practical strategies to improve microbial safety in composting process control and handling practices

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

Layman's Summary appears as submitted by the Principal Investigator

Compost as a soil amendment and organic fertilizer is a major source of nutrients for plant growth. Although the high temperatures generated by microbial activities during active composting can inactive pathogens, the survival or regrowth of foodborne pathogens during the composting process or in the finished compost can be problematic for vegetable production. This proposed study used a systems approach to address pathogen control during the composting process and subsequent storage and handling of finished products and to develop and validate some practical strategies, which can be readily adopted by composting operators or growers. In the proposed study, we'll validate the thermal inactivation data of *E. coli* O157:H7 and *Salmonella* in compost using naturally occurring isolates; optimize and validate the finished compost using a physical covering and/or straw as the base of passive static compost heaps and windrow compost piles; apply the pathogen growth model to determine the potential of finished composts to support the pathogen growth, and investigate the growth, survival, and control of foodborne pathogens in the finished compost. The results from this study will provide practical methods or practices on compost production and handling to eliminate or reduce pathogen contamination of compost, thereby helping produce industry to grow safe products for human consumption.

Photo courtesy of Xiuping Jiang

Technical Findings and What They Mean for You: The research program sought to validate thermal inactivation data in compost using naturally occurring strains *of E. coli* O157:H7 and *Salmonella.* A humidity controlled environmental chamber was used to simulate the early phase of the composting process: *E. coli* O157:H7 and *Salmonella* (outbreak and naturally occurring strains) were tested at various temperatures. The study found that inactivation rates of naturally present and outbreak strains are the same, although natural strains tended to survive longer than outbreak strains. Within the first day, pathogens increased in some compost. There was, however, a rapid reduction in populations within 48 hours, quicker in poultry compost vs. dairy compost, probably due to ammonia in poultry compost.

The following are key findings (obtained from the June 2012 CPS Symposium presentation, the panel discussion and the 2011 project report).

Finding 1: *Compost production, storage, handling and application are all components of a process needed to manage a dynamic microbial system.*

What does this mean for you?
Managing compost is a complicated process requiring process controls to reduce the potential for pathogen survival and/or re-contamination at every step from production through application. Prior research has demonstrated the technical requirements for the production process: accurate measurement of carbon/nitrogen ratios, achieving optimal temperatures, and rapid heat up times among others (see earlier Jiang project reports on the CPS website). This research demonstrates the importance of managing compost stored in the field. All of the research combined highlights the complexity of the composting process and the need for a systems management approach.

In reality, the burden of evaluating compost suppliers for adherence to best practices rests with growers purchasing compost. While there are some state-level compost regulations (California has requirements that composting meets certain criteria such as temperature, number of days, number of turns, and covering, they may not been updated to reflect more recent scientific findings. For companies that purchase and apply compost, at a minimum, the information from this research and from the prior research programs from the same scientist (Jiang, 2010) identifies critical process variables that need to be considered when evaluating compost companies and, if applicable, when growers monitor or audit compost operations for compliance with industry best practices and commodity-specific guidelines.

Finding2: *If not stored properly, finished compost can become re-contaminated.*

What does this mean for you?
Growers that purchase compost from a buyer or from a company that stores compost, need to understand how the seller manages compost to avoid re-contamination. That said, it is not clear at present, how to define proper compost storage. One can employ logic to storage of finished compost at a composting operation and suggest proper storage should include an assessment of: separation of finished composts from raw materials, use of different handling equipment and/or implementation of adequate equipment sanitation programs to ensure proper control against cross-contamination, storage in areas properly protected from likely pathogen sources (e.g. feedlots, runoff from treatment areas, etc.) and perhaps the use of covers. On farm storage precautions might include choosing locations protected from water runoff, not immediately adjacent to feedlots or dairy operations, providing protection against wind-blown contaminates and using handling equipment that has been sanitized prior to use. It may also be useful to keep storage times as short as possible prior to application to reduce cross contamination risks. It is also important to recognize that the environment can play an important role in compost recontamination and subsequent pathogen growth. For example, in a state like California where humidity can be relatively low and rainfall is not a factor during production seasons, the compost composition and subsequent storage risks will differ from compost produced in other areas of

the country where there is higher humidity and greater rainfall. In any event, growers need to understand risk factors associated with re-contamination for any soil amendments that they use.

Finding 3: *There is a difference in* E. coli *O157:H7 survivability depending on compost particle size and moisture levels.* E. coli *O157:H7 survives better in compost with large particles vs. small particles and in moderate moisture levels (30%) as opposed to compost with low or high moisture levels (20% or 40%).*

What does this mean for you?
This finding again points to the complexity of managing a dynamic microbial system. Using the results, companies that produce, store and handle compost need to revisit their processes to ensure humidity control points are monitored from production through application. Likewise, compost product testing should incorporate particle size as another variable when conducting and documenting pathogen testing.

Conclusions/Recommendations/Next Steps
This research project taken with the previous CPS-supported work in Dr. Jiang's lab and compost research performed elsewhere clearly points to composting as a manufacturing process with multiple variables that need to be recognized, managed and measured to insure pathogen levels are controlled. Indeed, the composting process starts with the raw materials and includes variables like pH, C/N ratios, temperature, heat-up times, product turns, microbial flora (antagonists) and as demonstrated in this report; humidity and particle size. This program also suggests that compost storage is a component of the process and needs to be executed so as to prevent recontamination of the finished product. This project also points out that, as with any process, the external environment needs to be considered. Humidity and moisture can be impacted by the production environment so that different production communities need to take their environment into account when developing process controls and assessing contamination and recontamination risks.

This research also opens up the potential to create an industry wide or regionally-focused best practice or checklist for purchasing and applying compost. One could even envision the development of a process certification for compost suppliers as part of commodity-specific audit programs. Interestingly, the Food Safety Modernization Act (FSMA) promises rules on preventive controls and it is clear from the language of the legislation (though the draft rules have not yet been released as this summary is being written) that FDA expects operators to demonstrate the preventive controls they use are effective. As applied to composting, that will likely mean that FDA will want compost producers and suppliers to fully define their production process and the preventive controls they employ to reduce the risk of pathogen survival in finished compost and/or recontamination of finished compost in storage. The research presented here and certainly the body of compost research that exists today will help inform the development of preventive controls and the methodologies used to validate they are effective and the verification measures that can be employed by composters to demonstrate the process was conducted in accord with their control limits.

PROJECT #13:

Assessing postharvest risks for *Salmonella* in pistachios

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

Layman's Summary appears as submitted by the Principal Investigator

In the past decade, outbreaks associated with consumption of raw almonds and peanut butter have been documented in the U.S. and in 2009 there was a large recall of pistachios when *Salmonella* was isolated from commercial products. However, with the exception of almonds, very little is known about the ecology of *Salmonella* in nut production and processing environment impeding the development of targeted commodity-specific intervention programs. Quantitative microbial risk assessment (QMRA) is an increasingly common tool that provides a framework for identifying critical data gaps and evaluating the overall effectiveness of risk-reduction strategies. This proposal will, through laboratory studies, identify points during post-harvest handling of pistachios where *Salmonella* may be reduced, controlled or amplified. Building upon a previous raw almond QMRA these laboratory data, industry data and expert opinion will be used to construct a pistachio QMRA. The overall goal of this research is to use the laboratory data and QMRA to develop scientifically-based food safety risk-reduction strategies for the pistachio industry.

Technical Findings and What They Mean for You:

Expert judgment, industry data and data obtained from a laboratory setup on-site at a pistachio processor facility were used in conducting the QMRA and in identifying post-harvest risk areas. Samples were obtained from multiple processor facilities during the 2010 and 2011 seasons for evaluating microbial populations in postharvest processes and for further analysis in the UC Davis laboratory.

Hulling, the first postharvest process studied, is a water-based process where machines are used to separate pistachio kernels from the shell, creating floaters (kernels) and sinkers (in-shell). After hulling, pistachios are separated using float tanks and are then dried prior to storage. Based on the samples taken, the prevalence of *Salmonella* in floaters (2.4%) was higher than in sinkers (0.71%). Maximum concentration in floaters was 8 MPN/100g vs. 1.8 MPN/100g in sinkers; sinkers made up the majority of the test samples (n = 984) vs. floaters (n = 168) in these studies. Temperature, humidity, time and moisture levels all impacted *Salmonella* levels. At higher temperatures, the growth of *Salmonella* was greater, but there was also a significant increase at room temperature over 24 hours. Overall, the results of the QMRA, based on industry data and current practices, show the potential risk for salmonellosis associated with the consumption of pistachios is low.

The following are key findings (obtained from the June 2012 CPS Symposium presentation, the panel discussion and the 2011 project report).

Finding 1: *Industry/researcher partnerships can be an effective mechanism to develop data that permits quantitative risk assessment.*

What does this mean for you?
Partnering is critical for the advancement of commodity-specific food safety knowledge and for addressing immediate issues facing grower, handlers and processors. In this project the ability to set up a laboratory on-site at a pistachio processor during harvest season provided access to both samples and experienced employees whose input benefited the research. Without this access, the research project would have been limited and meaningful data more difficult to develop. To insure specific types of applied research represents actual industry conditions, growers, harvesters, processors and others in the supply chain need to come forward and form collaborations to carry out critical studies.

Finding 2: *During this project it was noted that product temperatures increase when pistachios are left in trucks for several hours before processing (2-fold log increase after 6 hours) –providing an opportunity for Salmonella to grow.*

What does this mean for you?
Environmental conditions need to be considered when evaluating risk. While nuts are considered low risk foods since they are dry products, meaning the moisture levels do not support the growth of pathogens, altering environmental conditions can increase risk even in a low risk food. In this project, when temperature/humidity sensors were added to trucks, within two hours there was a rapid increase in humidity levels (almost 100%) and a slight increase in temperature. Likewise, *Salmonella* was shown to multiply at ambient temperature significantly after six hours for in-hull pistachios, pistachio hulls and pre-dryer pistachios. Process time between pistachio harvesting and pre-drying should be evaluated and minimized to prevent *Salmonella* growth. All commodities could benefit from similar types of analyses where process duration and holding times are considered especially as they relate to product temperatures.

Finding 3: *The results of this project demonstrate the value of a quantitative microbial risk assessment (QMRA).*

What does this means for you?
A QMRA is beneficial for identifying and prioritizing microbial risks. With this type of model, commodity groups can create "what if" scenarios and perform risk analyses and process evaluations. Often when industry groups discuss quantitative risk assessments, the limiting factor in actually pursuing QMRA is a lack of data. This project is an example of a quantitative risk assessment where the industry committed to its development and worked with the researcher to address data gaps. In this study, data gaps such as the percentage of floaters vs. sinkers, how long pistachios are stored in silos, temperatures in silos, post-process storage time and temperature were addressed using expert opinion. While there may be data gaps, expert opinion can be used in developing baseline risk assessments that as more data

becomes available over time, can be refined. In addition, QMRA may identify previously unidentified risk factors that need to be controlled for in a given process. During the on-site work for this project, researchers noticed that some trucks loaded with harvested product were sitting for longer periods of time than the usual 3-4 hours before unloading the pistachios at the processing facility. Researchers placed temperature and humidity sensors in the trucks and found that within two hours there was a rapid increase in humidity almost to 100%. Temperatures also increased slightly over a period of 12 hours. QMRA provides the opportunity to identify data gaps; an important process for the produce industry in focusing research endeavors and for companies to implement process controls. Over time, as more data becomes available, any QMRA model can be refined and further opportunities identified.

Conclusions/Recommendations/Next Steps:
This program demonstrates the value of QMRA. Identification of specific practices as contributing to Salmonella proliferation permits the development of mitigation steps to reduce this risk. This program also highlights the power of industry collaboration with skilled researchers to develop data that can be used to improve food safety performance. The produce industry has seen many efforts to develop commodity specific guidances and a number of potential risk factors for pathogen contamination have been identified, e.g. animals, water, compost, hygiene, etc. However, without a way to prioritize the impact of specific risk factors on the safety of the food or its threat to public health, all risks have to be treated as a top priority and resources are stretched and efforts to improve safety diluted. QMRA is a tool that may permit the produce industry to focus its risk management efforts and allow for the development of preventive practices and process controls.

PROJECT #14:

Pathogen transfer risks associated with specific tomato harvest and packing operations

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

Layman's Summary appears as submitted by the Principal Investigator
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 document is essential for the responsible harvesting, handling, and packing of fresh tomatoes. Understanding the risks for 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 project was developed in conjunction with the Florida tomato industry to measure the potential transfer of *Salmonella* between cloths (used to remove debris) and tomatoes and to determine whether the reuse of cartons increases the risk of transfer of *Salmonella*. The results show a difference in transference between new and used cartons under varying degrees of cleanliness. Used carton transfer was higher than with new cartons, especially with longer contact time. Dirty cartons facilitated the most transfer under short contact time. Generally, transfer of *Salmonella* was most efficient under wet conditions regardless of the age of the box. The data presented at the CPS Symposium is a mid-term report and should be considered preliminary as this project still has a year to run and additional research is ongoing.

The following are key findings (obtained from the June 2012 CPS Symposium presentation, the panel discussion and the 2011 project report).

Finding 1: *The FDA investigation of the 2008 outbreak of* Salmonella *originally attributed to tomatoes found used cartons being used for re-packing. This raised the question of whether used cartons could be a cross-contamination vehicle if the original tomatoes were contaminated with* Salmonella. *The early results from this partially completed study indicate that if contaminated tomatoes come in contact with cartons, whether new or reused, they could contaminate the carton. If the cartons are dirty and conditions are wet, transference is higher.*

What does this mean for you?
This preliminary data indicates that re-using tomato cartons in packing operations carries a risk for cross-contamination. This risk may be more acute if the cartons are dirty or show signs of fruit residues or moisture. This research program bears following for tomato packers and re-packers as it is completed in 2013. In the meantime, tomato handlers may consider carton inspection prior to re-use to mitigate any cross-contamination risks. More broadly, to reduce the potential risk of transferring pathogens to tomatoes from contaminated cartons, packers should insure proper carton storage. If containers are not properly stored and become moist and dirty, they can create an environment that supports pathogen growth presenting the risk of cross-contaminating tomatoes if pathogens are present.

Finding 2: *Early results from this partially completed study indicate that* Salmonella *does not survive for very long on dry, clean cartons.*

What does this mean for you?
This preliminary result is consistent with other previously funded research that indicates that *Salmonella* does not survive well on plant tissues in production environments. If these early results hold true, the finding that *Salmonella* does not survive for long on dry, clean cartons supports the general recommendations typically found in most food safety guidance and standards to store cartons in "dry, clean areas".

Finding 3: *While this research was specific to tomatoes, it may have applicability to commodities with similar surfaces that are washed and waxed (e.g., peppers).*

What does this mean for you?
Producers of commodities with similar surfaces (e.g., peppers) and similar environmental conditions (e.g. temperature and humidity) should consider these findings relative to their own operations. Any packing or repacking operation that reuses cartons, should review these study findings to understand the risk of pathogen transference. When deciding whether to purchase single use or reusable cartons, the potential for pathogen transfer should be a factor in the decision process.

Conclusions/Recommendations/Next Steps:
Clearly, the next steps here are to complete the research project and verify or reject the preliminary findings shared at the 2012 CPS Symposium. At this moment, the data support what expert assessment has always suggested; store packing cartons in clean, dry areas and protect them from dust and debris. While logic drove current best practices in this area, these data provide support that the basic premise was correct. These preliminary results also suggest that repacking tomatoes in damp or soiled cartons can result in transference of *Salmonella* (if present) to previously uncontaminated fruit. This result, if verified, could be significant in that many items are repacked according to size, maturity or ripeness postharvest but prior to shipment to retail and foodservice outlets. Cartons make up a significant proportion of overall product cost so that simply eliminating repacking in used cartons would likely have considerable impact of operational viability. Rather, risk mitigation measures will likely be a preferred path so that physical inspection of cartons might be implemented to prevent re-use of soiled, damp cartons. Other, disinfection treatments may also be applied to manage potential transference risks.

PROJECT #15:

Rapid testing of flume water organic load to better assess the efficacy of free chlorine against *Escherichia coli* O157:H7 during commercial lettuce processing

Principal Investigator: Elliot T. Ryser, Ph.D., Michigan State University

9.0 Session IV-Wash Water and Process Control

Layman's Summary appears as submitted by the Principal Investigator
In response to continued outbreaks involving *E. coli* O157:H7 and other bacterial pathogens, the safety of fresh produce has now become a top priority. Although bagged salad mixes and other such products available in supermarkets have been commercially washed multiple times in various chemical sanitizers to minimize the risks from hazardous microorganisms, such practices will not totally ensure end-product safety. As produce residues accumulate in the water during processing and reduce the effectiveness of commonly used commercial sanitizers, bacterial contaminants in this water are readily transferred to previously uncontaminated product. The study being proposed here will explore some of the water quality issues related to chlorine effectiveness with the goal being to identify several easily measurable water-related factors (example – the amount of lettuce debris in the water) that can be easily monitored by the industry to increase the effectiveness of chlorinated sanitizers.

Technical Findings and What They Mean for You:
This research evaluated the effect of organic load in wash water on the efficacy of free chlorine against *E. coli* in commercial lettuce processing, based on bench-top and pilot processing line analyses. The "organic load" - the amount of organic material resident in the wash water is considered important because the organic compounds can bind up the active chlorine moieties thus rendering them useless in water disinfection. The results indicate there was no recovery of *E. coli* in water that contained 0-1% organic load, but killing efficacy began to decrease at 2% and recovery was easily achieved with 5-10% organic load. The microbial population reduction was always less in water with higher organic loads even if the chlorine concentration was elevated upwards to 100 ppm. There was a greater reduction when chlorine-based sanitizer was acidified.

The following are key findings (obtained from the June 2012 CPS Symposium presentation, the panel discussion and the final project report).

Finding 1: *Proper pH control when using chlorine is extremely important for maintaining chlorine's disinfection efficacy.*

What does this mean for you?
It's not sufficient to add chlorine at a particular concentration and not monitor other parameters of the wash system that affect the efficacy of

chlorine as a water disinfection treatment. In order to properly function as an antimicrobial agent, wash water systems using chlorine need to be managed to achieve an acidic pH (approximately 6.5) to insure that the chlorine (sodium hypochlorite) in the water dissociates maximally to form hypochlorous acid, the chemical entity of chlorine that is most effective as a disinfectant. pH levels need to be monitored frequently to insure the acid environment is consistently maintained.

Finding 2: *Level of turbidity and solids also affect the efficacy of chlorine sanitizers. It is important for operations using water that contacts the surface of the fruit or vegetable to cool or wash the product to not limit management activities to just monitoring chlorine and pH, but to continually measure total organic load and change water when organic load reduces disinfectant efficacy.*

What does this mean for you?
Managing wash water requires a systems approach. The data suggest that it is important to manage organic build up in wash water in order to insure that sufficient chlorine sanitizer is available to control the microbial population that can build in wash or cooling water. Operators need to implement systems, e.g. turbidity measurement, to monitor organic load and be prepared to change out the water in the system to ensure proper disinfection occurs. Ideally, operators would conduct validation studies to measure the turbidity levels at which effective doses of active chlorine are no longer present in their system and use this information to set operation parameters, i.e. maximum limits that inform production personnel when water must be changed. Operators would then verify that these conditions are met during every production or cooling activity.

Conclusions/Recommendations/Next Steps:
Historically, industry guidances have used pH limits of 6.5 -7.5 for managing wash water. With our increasing knowledge of the dynamics of wash water systems, there is support for narrowing this pH range to more acidic conditions. An acidic pH closer to 6.5 is better. At a pH of 6.5, 95% of the available chlorine is in the hypochlorous acid state. As you move up in pH to 8.0, the hypochlorous acid decreases to 20% of available chlorine.

However, operating a wash water system that is effectively managing microbial populations requires more than just adding chlorine. Operators need to understand their equipment and how product moves through the system (i.e., product through-put, turbidity, quality of initial water, system capacity, water agitation, contact time, temperature, submersion, make-up water rates, areas where products may accumulate, etc.). Individual system validation is an essential part of risk management to ensure that a wash water system is effectively reducing microbial populations.

PROJECT #16:

Enhancing the efficacy of fresh produce washing operations through establishing monitoring methods and water disinfection technologies based on a combination of filtration and UV

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

Layman's Summary appears as submitted by the Principal Investigator
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 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 should 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 waste-water treatment.

Technical Findings and What They Mean for You:
One of the project objectives was to find a parameter that can be measured


Photo courtesy of Bob Whitaker

to accurately identify when the wash process is working well and when adjustments are required. To achieve this objective, three facilities took part in the study: two salad spinach and one shredded lettuce processor. In each of the facilities, approximately 300 samples of pre-, post-wash and source water were sampled. In the first year of this two year project, the results included a correlation of the log count reduction achieved with wash water parameters (temperature, turbidity, conductivity, oxidation reduction potential, oxygen consumption and impedance/capacity).

Log count reductions were the same regardless of methods used and whether the facility was state of art or not. Conductivity was positively correlated with log count reductions (LCR), and water temperature was negatively correlated with LCR. There was no correlation with: microbial loading of water, sanitizer concentration, turbidity, pH, and temperature differential. Analysis revealed a great deal of cross contamination (clustering of microbial population on spinach coming in, which is then distributed throughout after washing)

The following are key findings (obtained from the June 2012 CPS Symposium presentation, the panel discussion and the 2011 project report).

Finding 1: *Controlling wash water quality is critical to avoid cross contamination. A wash water system containing an antimicrobial agent is not a kill step.*

What does this mean for you?
Companies often believe they are effective in controlling wash water, without determining the underlying variables that need to be controlled. Regardless of the system used, companies need to know that they are actually controlling wash water quality and this requires validation studies. The research in this session points to a knowledge gap in the industry relating to variables affecting wash water quality and how to manage them. Managing wash water quality requires a systems approach. Water quality is dependent on temperature, turbidity, conductivity, oxidation reduction potential, oxygen consumption and impedance/capacity. Companies need to validate that their wash water control process is effective and then verify every day that is working properly.

Finding 2: *The log count reduction achieved by a wash process is essentially a function of the initial loading on the incoming material.*

What does this mean for you?
In the research findings, three different facilities processed spinach or lettuce using either PAA or an ORP controlled hypochlorite wash. In all cases there was a strong correlation between initial loading and log count reductions achieved. Facilities with better wash water quality did not demonstrate log count reductions significantly different from other facilities. Therefore, it appears initial loading is significant and wash water quality control is necessary to avoid cross-contamination. There is no kill step. This finding stresses the need for grower and handler food safety programs to minimize potential microbial contamination from pre-harvest through product delivery. Once a product becomes contaminated, the risk of contaminating other products and materials increases. Wash water processes can, at best, minimize the potential for cross contamination.

Conclusions/Recommendations/Next Steps:
Laboratory research is purposely conducted in a controlled environment in order to isolate and evaluate cause and effect interactions. As with any research conducted in a laboratory, its application to commercial production operations needs to be verified in an operational environment. Laboratory findings may not be directly reproducible in a commercial operational environment and often require modifications before they can be incorporated into actual working environments due to other factors not accounted for in the laboratory. An exhaustive review of wash water systems is in order to examine whether they are working to their optimal potential. No two wash water systems are the same – incoming products, flow rates, etc. need to be evaluated independently.

PROJECT #17:

Evaluation and optimization of postharvest intervention strategies for the reduction of bacterial contamination on tomatoes

Principal Investigator: Keith Schneider, Ph.D., University of Florida

Layman's Summary appears as submitted by the Principal Investigator
Tomatoes are a nutritious food and an important crop for the state of Florida and the US economy. Yet, foodborne illness outbreaks associated with tomatoes have negatively impacted public health, consumer confidence in tomatoes, and the industry's economic well-being. The Florida tomato industry has taken an active role in establishing tomato-handling standards to prevent pathogen contamination and infiltration. However, due to lack of scientific data applicable to commercial handling conditions, some of the standards rely upon recommendations previously developed for tomato quality maintenance. Therefore, scientific studies to evaluate pathogen contamination and infiltration under realistic commercial handling conditions are critical for developing handling practices to effectively reduce pathogen contamination. Proposed research will focus on evaluating current tomato post-harvest handling practices on *Salmonella* contamination and infiltration, and providing answers to the queries raised by the industry. Goals include defining operational limits for dump tank water management, identifying cost-effective dump tank water quality monitoring parameters, and developing/optimizing an overhead spray sanitation system to minimize tomato surface contamination while reducing water and chemical use. Project outcome will provide data for developing science-based guidelines to reduce food safety risks, and avoiding setting up costly regulations that may not necessarily advance food safety.

Technical Findings and What They Mean for You:
The first goal of this project was to evaluate the effect of dump tank handling conditions have on *Salmonella* infiltration into fruit. For this research tomatoes were submerged in water containing *Salmonella enterica* to determine the impact of tomato variety, temperature differential, immersion time, and the post-stem removal time on the incidence and severity of *Salmonella* internalization. Another objective was to evaluate the effectiveness of sanitizers applied by overhead spray rinse with a brush washer to remove pathogens from tomato surfaces versus submerging and washing tomatoes in a flume system. When evaluating changes in dump tank water quality and sanitizer levels in three different processors/ packers in Florida, dump tank sanitizer levels varied significantly among the three facilities during the day although each facility had similar ORP values.

The following are key findings (obtained from the June 2012 CPS Symposium presentation, the panel discussion and the 2011 project report).

Finding 1: *PAA achieved a higher log reduction on tomatoes after a 5, 15 and 30 second overhead spray treatment (2.8-, 4.7- and 5.5- log_{10}, respectively) than NaOCl (1.9-, 3.5- and 4.1- log_{10}, respectively) or ClO_2 (3.5- and 3.9- log_{10}, respectively). Neither NaOCl nor ClO_2 log reduction results were significantly different from treatment with water alone at any of the exposure times. In addition, at 60 seconds, there was no significant difference among the three sanitizers with all three reaching a 5-log reduction.*

What does this mean for you?
It is important for operations to evaluate their water disinfection programs no matter which sanitation system (PAA, NaOCl, ClO_2, and others) is employed. It is expected that choice of sanitizer is only one variable in the performance of the overall disinfection system along with equipment design, pH, initial water quality (minerals), turbidity, aeration, contact time with the product, etc. These data indicate that perhaps longer exposure times are required for NaOCl as opposed to PAA in the systems tested. Again, exposure time to the sanitizer would be expected to vary depending on the organisms, surface of the material where they reside, presence of biofilms and the mechanism of action of the sanitizer. It is important for each operator to understand the impact of these variables such as exposure time to the sanitizer and to develop process control measures to define exposure times, identify minimums/maximums and insure the process is conducted within these set points each day.

Finding 2: *Organic material in water greatly impacts the availability of disinfectants.*

What does this mean for you?
Tomato dump tank water quality deteriorates rapidly as organic material increases. Each time organic material is added; compositional variability is introduced in the water system and this variability is not necessarily captured and offset by the control systems. Operators need to understand this variability (organic load, pH, sanitizer, etc.) and have a mechanism in place to determine the corresponding sanitizer level adjustment required to offset the compositional changes. Each of the four research projects (Ryser, Schneider, Warriner and Luo) dealing with wash water disinfection presented at the 2012 CPS Symposium identified the importance of monitoring organic load and/or turbidity to better understand the actual availability of sanitizer in the wash or cooling system. One output of this research project was a reminder that oxidative reduction potential or ORP may not be a reliable measure of actual sanitizer level under certain conditions. While convenient to use as it offers a continual and reportable estimate of oxidative potential, under conditions where organic load is high, measurements no longer are linear and sanitizer levels may be severely underestimated. Therefore, even with automated ORP systems, operators would be wise to do routine measures of disinfectant levels to ensure they fall within the range the operation has set to deliver proper water disinfection.

Addition of sanitizers to tomato dump tank water reduces pathogen survivability and minimizes the risk of cross-contamination. Just controlling wash water quality; however, is not enough. Organisms may not only exist on the surface of fruits and vegetables, they may also be present in biofilms on the equipment (Luo, 2011). This highlights the importance of the condition of the incoming product and the cleaning and sanitizing programs in the packinghouse.

Finding 3: *Physical disruption, e.g. the use of brushes, can improve pathogen removal from fruit surfaces. Further, this project highlighted the need to assess any changes made in the wash process to determine if unintended cross contamination opportunities are created.*

What does this mean for you?
Physical agitation of the surface can help remove microbes. This project explored the concept of using brushes to dislodge surface microbes in conjunction with a water spray containing a disinfectant. The work was performed in a laboratory environment. Greater than a 3 log reduction was accomplished using this dual system. The concept of disrupting microbial adhesion to the surface of

the fruit or vegetable has been explored previously in other systems using both chemical (detergents and emulsifiers) and physical methods. Brushes are already used in many tomato process operations and may be an important tool in reducing overall microbial surface populations. However, operations incorporating brushes in their washing operations need to evaluate their sanitation SOP's, environmental testing programs, and testing requirements to understand how they measure and handle risks associated with the use of brushes. Even with high levels of sanitizer, there is still a potential for cross-contamination if equipment, including brushes, are not cleaned frequently.

Finding 4: *Tomato varieties differ in their susceptibility to pathogen internalization. Several tomato varieties were studied and all of them internalized* Salmonella enterica *in tissue below the stem scar. Further internalization, however, was dependent on the variety, time elapsed since stem removal and pathogen exposure time.*

What does this mean for you?
Growers need to follow research relating to pathogen susceptibility of the commodities and cultivars they produce. By being aware of and understanding variety-specific susceptibility to pathogens, growers can then evaluate the potential risks associated with pathogens along with the benefits of planting a particular cultivar.

Internalization of pathogens into fruit or vegetable tissues is an emerging area of potential concern. As we learn more about this potential risk factor, it is important that growers, packers and handlers understand that the potential exists for internalization of pathogens from wash systems; especially if the fruit or vegetable tissues are damaged so that an open wound can provide a point of entry for the organism. Stem scars, fruit cracking and other common commodity characteristics or quality defects need to be monitored carefully in regards to potential sites of entry for pathogens.

This research program also follow up on a concept first raised by Dr. Teplitski from the University of Florida at the 2011 CPS Research Symposium regarding the importance of the genetic and physiological status of the fruit or vegetable in harboring human pathogens. Teplitski showed that commercial and heirloom tomato varieties can differ in their susceptibility to *Salmonella*, and he recommended further research to explore the potential to develop more resistant varieties to minimize the risk of contamination. Wayadande (2012) further demonstrated that spinach leaf breakage is higher in summer and fall varieties and anecdotally, there seems to be a higher occurrence of pathogen contamination on fresh spinach at those times, indicating a possible correlation. While the reasons for breakage are not known, several have been proposed including a varietal or cultural influence or the result of rapid growth perhaps as a result of application of nitrogen fertilizer just prior to harvest. Further research is needed to understand why commodities vary in their susceptibility to pathogen internalization.

Finding 5: *In a laboratory experiment, overhead spray treatments of NaOCl were more effective at reducing* Salmonella *on tomato surfaces than on tomatoes washed in a flume system.*

What does this mean for you?
When tomatoes were subject to overhead spray treatments containing sodium hypochlorite (NaOCl), surface disinfection of pathogens was found to be more effective than when tomatoes were treated in a NaOCl-treated dump tank. Dump tanks are beneficial for loading tomatoes into the packing line without bruising, but this practice may increase the risk of pathogen internalization. Packing line systems that reduce the time tomatoes are fully or partially submerged in water (e.g., dry dump systems or systems with mechanical conveyors) may reduce the likelihood of pathogen internalization. Operators need to weigh the benefits and risks of dump tanks and/or flume systems versus dry dump systems equipped with spray bars. If using dump tanks to load tomatoes in the packing line, water retention times, temperatures, turbidity, sanitizer levels, pH and other water quality measures should be closely managed. Alternatively those employing dry dump systems and spray bars augmented with brush systems

also need to carefully evaluate their systems to insure proper wash water management and including designing systems that minimize fruit damage and ensure efficient equipment sanitation. In either case, it is important for operators to gain a complete understanding of their wash/conveyance systems and to validate that their system is successful in controlling Salmonella if present in the water. Operators must keep in mind that surface treatments will not compensate for any pathogen internalization that may occur if tomatoes are submerged in contaminated water upstream in the packing line.

Conclusions/Recommendations/Next Steps:
This research program examined a number a critical components in tomato washing operations. The utility of ORP as a measure of sanitizer levels, variable sanitation effects of PAA and NaOCl versus exposure time, the effects of physical treatments in removing pathogens from tomato surfaces, the potential interaction between pathogen internalization and tomato variety and the importance of monitoring organic loads/turbidity to optimize wash water disinfection. In sum, these studies draw us to the conclusion that it is critical for operators to have a complete understanding of their wash systems. There are clearly opportunities and challenges with any wash system design, choice of sanitizer, variety selection or measurement tool. The key is for any operator is to recognize potential cross contamination risks and to develop preventive controls to manage those risks and to further validate that those preventive controls are effective and can be verified throughout production. Interestingly, the research here indicates the possibility of breeding cultivars less susceptible to pathogen infection or supporting pathogen growth. This again points to the importance of considering the entire biological spectrum in contamination events that include not only the genetics of the pathogen but also the genetics of the fruit or vegetable and even those of consumers.

PROJECT #18:

Improving produce safety by stabilizing chlorine in washing solutions with high organic loads

Principal Investigator: Yaguang Luo, Ph.D., USDA-ARS

Layman's Summary appears as submitted by the Principal Investigator

Chlorine is widely used by the fresh and fresh-cut produce industry to reduce microbial populations and prevent the potential spread of human pathogens during produce washing. However, the organic materials released from cut produce quickly react with chlorine and degrade its efficacy for pathogen inactivation. A novel food-grade chemical mixture, F86-128, formulated by industry scientists purportedly stabilizes chlorine in fresh-cut leafy green wash systems with high organic materials and thus prevents reduced chlorine efficacy. Prior to commercial adoption, a highly experienced USDA-ARS project team, consisting of microbiologists, food technologists, and chemists, will conduct in-depth studies and expand the research scope to include herbs, tomatoes and cantaloupes. Laboratory-based studies will be augmented with semi-commercial pilot-plant trials to test real-world conditions, including realistic pathogen contamination patterns of fresh produce and diverse tests to determine appropriate operational conditions for use of F86-128. We will confer with CPS and industry to ensure that the findings will be not only scientifically rigorous but also commercially applicable. Successful completion of this project will provide objective and thorough scientific evaluation of the effectiveness of F86-128 for improving produce safety by stabilizing chlorine, thus facilitating the industry in making informed decisions on F86-128 development and commercialization.

Technical Findings and What They Mean for You:

Chlorine is depleted in wash solutions by organic materials present in the water, leading to potential pathogen survival and increasing the risk of cross-contamination. To address this issue, industry scientists formulated T-128 (previously called F86-128), to effectively stabilize or protect active chlorine from degradation due to chemical interaction with organic constituents. This research program was designed to evaluate the efficacy of T-128 in wash systems under various conditions and to establish appropriate operational parameters for its use in laboratory and process plant trials.

The following are key findings (obtained from the June 2012 CPS Symposium presentation, the panel discussion and the final project report).

Finding 1: *T-128 is a chlorine stabilizer that keeps wash water from becoming a source of contamination; especially in the presence of high organic load.*

What does this mean for you?

While the mechanism of action of T-128 against bacterial survival in high organic load wash water is not known, there appears to be a synergistic reaction with chlorine. T-128 does not appear to be a sanitizer on its own; it is effective only when used in combination with chlorine. When T-128 is used with chlorine in leafy green wash water systems, it appears to reduce the potential for chlorine being bound up by organic materials present in the wash water environment resulting in more available chlorine to act on microorganisms. Therefore, in a sense, T-128 seems to serve as a "safety net" of sorts for chlorine since it stabilizes chlorine as wash water organic load levels increase. This ability to preserve chlorine as organic load increases may permit operators to use wash water for a longer period of time between change-overs. In many vegetable processing plant locations, conservation of water not only affects operational costs, but also benefits the environment.

Finding 2: *T-128 can stay active even after long periods of time.*

What does this mean for you?
Because T-128 stays active for long periods of time, it resulted in some slowdown in chlorine degradation. Operationally this could result in the need to adjust chlorine levels less frequently. Slower chlorine degradation can lead to a reduction in chemical use and is potentially a cost savings.

Finding 3: *The use of T-128 significantly reduced* Salmonella *survival in biofilms on netted cantaloupe rinds and on stainless steel surfaces.*

What does this mean for you?
Biofilms are extremely difficult to get rid of once they are formed. Pathogens are protected and difficult to kill when they are associated with biofilms. The efficacy of T-128 against *Salmonella* in biofilms is an added bonus to its ability to stabilize chlorine in wash water. Although use of T-128 in wash water is no replacement for thorough cleaning and sanitizing practices, because of their intractability, operators are wise to utilize multiple methods to compact biofilm formation.

Conclusions/Recommendations/Next Steps:
Although the mechanism of action for T-128 is unknown, this study demonstrates the ability of T-128 to stabilize chlorine even under high organic load. This study also showed that T-128 also prevented the buildup of biofilms. Additional studies on wash water systems for other commodities would be beneficial to the industry as well as the effectiveness with other sanitizers.

10.0 References

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