2012 HUMAN PATHOGENS ON PLANTS WORKSHOP
POSTER ABSTRACTS

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ABSTRACTS:

Detection and Diagnosis
Poster #1

Outbreaks of salmonellosis have been associated with fresh fruits and vegetables. Detection of Salmonella is based on conventional enrichment and isolation on selective media which is time consuming and labor intensive. The objective was to evaluate the utility of an accelerated plating procedure and rapid screening techniques for Salmonella detection. Food matrices tested included Romaine lettuce, cilantro, jalapeno peppers, tomatoes and cantaloupes. Produce was inoculated with Salmonella at ~ 2.5 cfu/sample, 7.5 cfu/sample and 25 cfu/sample. Six replicates at each inoculum level were tested per food matrix, as well as six uninoculated controls. After 24h preenrichment, subcultures were made into TT and RV broths. After 7h incubation at 42°C, plates were streaked for isolation of Salmonella and the selective enrichments were reincubated for a total of 24h and used for conventional BAM cultural isolation and the VIDAS-SLM assay. At both 7h and 24h incubation, portions of the TT broths were used for Neogen Reveal and RapidChek Salmonella tests. RV broths were also tested at both 7h and 24h but only on the Neogen devices. The seven hour accelerated plating procedure worked well for 4/6 to 6/6 of all produce samples inoculated at the lowest level. Both the RapidChek and Neogen Reveal tests worked as well as the VIDAS-SLM after 24h enrichment for most strains tested but failed to detect the pathogen after 7h selective enrichment in nearly all of the artificially contaminated samples. This seven hour selective enrichment procedure accelerated the isolation of Salmonella from contaminated produce.
Are adequate methods available to detect protist parasites on fresh produce? D. MACARISIN (2), M. Santin (2), G. Bauchan (1), R. Fayer (2). (1) USDA-ARS, Electron & Confocal Microscopy Unit, Beltsville, MD, U.S.A.; (2) USDA-ARS, Environmental Microbial & Food Safety Laboratory, Beltsville, MD, U.S.A.

Human parasitic protists such as Cryptosporidium, Giardia and microsporidia contaminate a variety of fresh produce worldwide. Existing detection methods lack sensitivity and specificity for most foodborne parasites. Furthermore, detection has been problematic because these parasites adhere tenaciously to plant surfaces and cannot be enriched in culture medium like bacteria to produce large numbers that facilitate detection. Cryptosporidium parvum oocysts and microsporidia spores were inoculated onto spinach leaves. Using laser scanning confocal microscopy it was shown that both parasites strongly adhered to plant surfaces and resisted vigorous washing. Low temperature electron microscopy analysis of the inoculated leaves revealed attachment of large numbers of parasites and frequent incidences of infiltration of oocysts into natural openings of the leaf such as stomata. Additional attempts to remove C. parvum oocysts from leaves with tetrasodium pyrophosphate detergent (Alconox), which was recently reported to be superior in Cryptosporidium recovery from fresh produce, were conducted. Alconox wash (P≤0.05) improved the percentage of oocyst recovery from 53.7 ± 7.2% in water to 73.6 ± 3.2% in 0.1% Alconox solution. However, after all washing treatments, samples were still positive for C. parvum DNA. This, suggests that in spite of the improved recovery, some oocysts were not removed from the contaminated leaves. Because some oocysts remained on all contaminated samples, the new Alconox elution technique must be further enhanced before it can be recommended as a standard method for removal of protozoan parasites from fresh produce.

Gradient Finder: A new ArcGIS tool to detect and display plant disease gradients from satellite images. F. W. NUTTER (1). (1) Iowa State University, Ames, IA, U.S.A.

The global monitoring of invasive biotic plant pathogens prior to their introduction into the U.S. remains a key challenge in the effort to safeguard our nation’s agricultural biosecurity. In order to minimize injury to susceptible crops, a precise and accurate early warning system is needed to detect, identify, and respond to the presence of new plant pathogen threats. The integration of Global Positioning System (GPS), Geographic Information System (GIS), and remote sensing technologies offers tremendous opportunities for meeting U.S. agricultural biosecurity needs. The overall objective of this study was to obtain temporal and spatial "signatures" unique to soybean rust epidemics, and to establish the discrimination needed to distinguish soybean rust from other plant diseases.

Bacterial pathogens in Norwegian onion production. J. PERMINOW (1), A. Sletten (1), I. Akselsen (1), E. Borowski (1), A. Hermansen (1). (1) Bioforsk Plantehelse, Aas, Norway

Norwegian onion growers have in recent years complained about bacteria like disease problems of bulb onion, Allium cepa L., both in the field and in storage. In order to find out which bacteria that are involved in theses rotting symptoms, a small survey was carried out. From Norway’s main onion production areas 16 samples of diseased onions were sent to our laboratory. Bacteria were isolated and dominating colony types were collected. Several biochemical, physiological and biological tests were carried out. Interesting isolates were identified by fatty acid profiling (MIDI System). Fatty acid profiling showed that there was a lot of different bacteria present in the diseased onions. Many of them were ordinary water- and soil residents, which are not known to cause diseases of onion, such as Enterococcus, Salmonella, Cedecea, Stenotrophomonas, Chryseobacteriuim and Curtobacterium. But there were also bacteria present which are known to cause diseases of onion in other countries, such as Burkholderia gladioli pv allicola, Pseudomonas viridiflava, Enterobacter chloae and Pantoea agglomerans. This limited survey has confirmed that different bacterial
Pathogens are present in rotting onions and causing problems in Norwegian onion production. Some of these are also opportunistic human pathogens, which may make this topic an issue of food safety as well.

**Poster #5**

**Primer with 5’ flaps improve the efficiency and sensitivity of multiplex PCR assays for the detection of Salmonella spp. and Escherichia coli O157:H7.** C. Timmons (1). (1) Oklahoma State University, Stillwater, OK, U.S.A.

Foodborne illnesses caused by Salmonella spp. and Escherichia coli O157:H7 are worldwide health concerns. Rapid, sensitive, and robust detection of the causal pathogens in foods and in clinical and environmental samples is essential for routine food quality testing, effective surveillance, and outbreak and traceback investigations. The aim of this study was to evaluate the effect, on PCR sensitivity, of adding a short, AT-rich, overhanging nucleotide sequence (flap) to the 5’ end of primers specific for the detection of Salmonella spp. and E. coli O157:H7. Primers targeting the invA gene of Salmonella and the rfbE gene of E. coli O157:H7 were synthesized with or without a 12-bp, AT-rich 5’ flap (5’-AATAAATCATAA-3’). PCR sensitivity assays were conducted using purified bacterial genomic DNA, crude cell lysates, and genomic DNA/ crude cell lysates spiked with DNA extracted from tomato and jalapeno pepper surface washes to examine background effects on detection. When targeting individual pathogens, PCR assays in which primers were flap-amended were more efficient than those in which they were not, with 20.4% and 23.5% increases in amplicon yield for Salmonella and E. coli O157:H7, respectively. In multiplex PCR assays, a 10- to 100-fold increase in detection sensitivity for these pathogens was measured when the primer flap sequence was incorporated. This improvement in both singleplex and multiplex PCR efficiency and sensitivity can lead to improved Salmonella and E. coli outbreak and traceback investigations and also has potential applications in biosecurity and microbial forensics.

**Poster #6**


Synthetic biology is an emerging field that engineers biological pathways toward novel applications. Previously the principles of synthetic biology have been used to construct transgenic biosensor plants that develop a measurable or visible response in the presence of specific small molecules. This strategy required the incorporation of three components into the plant: a receptor that relays a signal in the presence of a chemical input, a response-relay protein that carries the signal to the nucleus, and a measurable reporter activated in response to the signal. With further development, the technology has potential for use in inexpensive bioassays or continuous field-based monitoring systems. We have made significant progress toward engineering plants capable of detecting biotic agents; i.e., small molecules produced by plant or human pathogens. This poster summarizes our recent progress toward plant-based detection of a small diffusible signal factor produced by the bacterial plant pathogen Xylella fastidiosa, and highlights planned and potential applications of the technology toward in-plant detection of indicators of human pathogenic bacteria.

**Poster #7**

**Evaluation of Chromogenic Media for Isolation and Detection of Salmonella spp. from Produce.** G. Zhang (1), E. Brown (1), T. Hammack (1). (1) Food and Drug Administration, College Park, MD, U.S.A.

Salmonella is the leading cause of foodborne bacterial disease outbreaks. Isolation and detection of Salmonella from foods remains a big challenge. Lack of effective media is a major limiting factor. The objective of this project is to evaluate the effectiveness of chromogenic media for detection and isolation of Salmonella from foods. Xylose lysine deoxycholate agar, Hektoen enteric agar, and Bismuth sulfite agar

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were conventional selective media used as control. BIOLOG Rainbow agar *Salmonella*, BIO-RAD RAPID *Salmonella*, CHROMagar™ *Salmonella* Plus, HardyCHROM *Salmonella*, Brilliance *Salmonella* agar and R&F *Salmonella* chromogenic plating medium were selective chromogenic culture media selected for the study. Seventy eight *Salmonella* isolates, including all the subspecies, and 24 non-*Salmonella* isolates, including 15 species, were used for inclusivity and exclusivity tests of all the media. Tomato, pepper and lettuce were inoculated with 2 *Salmonella* isolates individually at 1 to 4 CFU/25 g and 5.5 log CFU/g food for enrichment trials. They were inoculated at 5.5 log CFU/g vegetable for enumeration trials. No typical *Salmonella* colonies were observed on any of the 9 conventional and chromogenic media when the 24 non-*Salmonella* isolates were streaked on these media. Preliminary results indicated that most chromogenic media studied were equivalent to or better than conventional media for detection of *Salmonella* spp., especially BIOLOG Rainbow agar *Salmonella*, HardyCHROM *Salmonella*, and BIO-RAD RAPID *Salmonella*, considering easiness to distinguish from background bacteria, enumeration result, inclusivity and exclusivity. Effective chromogenic media could improve the detection of *Salmonella* spp. in produce.

**Dissemination Mechanisms**

**Poster #8**

**Identification of microbiological hazards on the farm and during post-harvest processing of fresh fruits and vegetables: Epidemiological approaches to food safety research.** F. E. BARTZ (1), K. E. Mues (1), A. M. Fabiszewski De Aceituno (1), L. Jaykus (2), C. L. Moe (1), J. S. Leon (1). (1) Emory University, Atlanta, GA, U.S.A.; (2) North Carolina State University, Raleigh, NC, U.S.A.

Background: Several recent high-profile produce-associated disease outbreaks have highlighted the need to prospectively identify and quantify the relative importance of risk factors for produce contamination along the farm-to-fork chain. The goal of this work is to discuss field-based epidemiological approaches and results derived from these approaches that can address this need. Methods: We collected 923 samples of 14 produce types of domestic and imported origin from 15 farms and 8 packing sheds in the southern United States. Matched environmental samples (water, hand, and equipment surface) were also collected. Samples were enumerated for indicators of fecal contamination and for human pathogens. Simple and complex (multivariate hierarchical regression modeling) statistical approaches were used to identify relationships between the microbial quality of environmental samples and produce. Results: Most produce types had significantly higher microbial loads post-harvest than on the farm. Overall, the microbial quality of irrigation water and rinses taken from produce worker hand-rinses was poor. Produce type, season of collection, packing step, and microbial quality of equipment surfaces were significantly associated with the overall microbial load of produce samples. Conclusion: These field-based epidemiological studies can be used to identify specific mechanisms of produce contamination along the farm-to-fork continuum. These can then serve as targets for interventions to decrease the likelihood of contamination with food borne pathogens. The use of these and newer approaches will also be discussed in the context of a recently funded study to assess routes of produce contamination on farms on the Mexican side of the U.S.-Mexico border.

**Poster #9**

**Efficacy of pre-harvest chlorine-based sanitizers against Phytophthora capsici and human pathogen indicator microorganisms in irrigation water.** M. L. LEWIS IVEY (1), J. T. LeJeune (1), S. A. Miller (1). (1) The Ohio State University, Ohio Agricultural Research and Development Ctr., Wooster, OH U.S.A.

Contamination of surface water with plant pathogens and harmful human pathogens has the potential to significantly reduce crop yield and lower the microbial quality of produce, respectively. In cooperation with local growers, the efficacy of commercial chlorine gas (Cl₂) and chlorine dioxide (ClO₂) irrigation water injection systems to kill human pathogens and *Phytophthora capsici* was evaluated. Using baiting and *P. capsici*-specific PCR, *P. capsici* was detected in five different sources of surface water used for routine irrigation of pepper and cucurbit crops in Ohio. Although *P. capsici* was most frequently detected in source water adjacent to a field with a history of Phytophthora blight and crown and root rot it was also detected in
irrigation water sources near fields without a history of *P. capsici* infestation or a host crop. In small-scale studies, *P. capsici* was not detected in bait cucumbers placed in collected non-treated source water or pre- and post-rapid sand filtered (RSF) water treated with ClO$_2$ or Cl$_2$. However, *in vitro* studies using municipal water indicated that ClO$_2$ at the same concentration as that used in the field had no negative effect on direct germination of *P. capsici* and reduced zoospore populations by less than 50%. In Cl$_2$-treated irrigation water, residual chlorine was rarely detected and although coliform bacteria were reduced prior to RSF their levels increased significantly post-RSF. The efficacy of ClO$_2$ was variable throughout the distribution system and coliform bacteria never dropped below levels required by the OH-EPA for recreational waters. Many environmental factors contribute to the efficacy of chlorine-based chemicals to treat irrigation water and much more research is needed to better understand the characteristics of surface water before the costs of ClO$_2$ and Cl$_2$ injection systems can be justified. A model for a multiple barrier approach to treating surface water for irrigation is proposed.

Poster #10  
**Survival of Salmonella enterica in pesticide solutions and its persistence during pre- and post-harvest stages in fresh tomato production.** G. LOPEZ-VELASCO (1), A. Tomas-Callejas (1), D. Dawit (1), T. V. Suslow (1). (1) University of California, Davis, CA, U.S.A.

Foliar contact water refers to water used for overhead irrigation but includes micro-environment modification or treatment with diverse ag-chemicals, including pesticides. Foliar contact water contaminated with human pathogens has been implicated in produce outbreaks and previously shown to support bacterial growth when mixed with some ag-chemicals. In this study, we evaluated the *in vitro* survival of *Salmonella enterica* in 13 different pesticide solutions labeled for tomato under different conditions of temperature (10-37°C), time (0-96h) and water quality. Additionally, we studied post-application survival of an attenuated *Salmonella* Typhimurium on tomato plants under field conditions and following post-harvest washing with 50 mg/L of sodium hypochlorite, using selected pesticide solutions. Positive and significant correlation (p<0.05) was found between the growth of *Salmonella* and the temperature, time of incubation, pesticide and water source. Cabrio, Admire, Sulfur and Success allowed growth of Salmonella while Asana, Ridomil and Intrepid reduced survival. With other pesticides, the mixed *Salmonella* population (log 2 CFU/mL) was maintained during the incubation period. Analysis of the strain composition of the Salmonella cocktail after incubation in pesticide suspensions showed a greater recovery of *sv* Newport, followed by Michigan and Poona. Recovery of the bacterium from the field grown tomatoes declined from 80 to 15% after 3 and 10d of contaminated pesticide application, respectively but no differences among pesticides were observed. This study provides further evidence that pesticides can allow persistence or will support the growth of *Salmonella* and may elevate risk during foliar contact application beyond that of the water source alone.

Poster #11  
**Retention of Escherichia coli O157 by cut leaves, sprouts, and fruits.** S. L. Mathews (1), R. Smith (1), A. G. MATTHYSSE (1). (1) Dept. of Biology, University of North Carolina, Chapel Hill, NC, U.S.A.

In recent years there have been several outbreaks of food-borne illness caused by *Escherichia coli* O157:H7 carried on plant surfaces, particularly sprouts and lettuce and spinach leaves. Both *E. coli* O157 and K12 strains were retained rapidly by cut leaves. Significant numbers of bacteria were retained by the cut edge after 5 min. exposure. The number of bacteria retained increased slowly over the next 3 days. Bacteria were also retained by leaves in the region 4-5 cm above the cut edge. The increase in the number of bacteria retained by leaves after the first day appeared to be due to growth of bacteria already associated with the leaves rather than continued recruitment from the solution. *E. coli* was retained by cut leaves at a higher initial rate than *Agrobacterium tumefaciens* or *Sinorhizobium meliloti* suggesting that retention of *E. coli* is species-specific and not simply due to retention of small particles by cut leaves. Unlike O157 strains, K12 strains bound to cut leaves but not to sprouts. An examination of retention of *E. coli* by sprouts and cut leaves of several plant species suggested that the time course and characteristics of the interaction were largely determined by the
plant tissue rather than the plant species. Bacterial retention by cut cucumbers, green peppers and cherry tomatoes was similar in that cut surfaces retained more bacteria faster than intact epidermis. Mutations in genes required for the synthesis of polyβ-1, 6-N-acetylglucosamine, colanic acid, or cellulose affected bacterial retention by cut leaves and sprouts differently.

Poster #12
Survival of E.coli O157:H7, Salmonella, and Listeria in Manduca Hornworm Frass from Tomato Leaves. P. MILLNER (1), P. Martin (2), S. Reynolds (1), R. Reynnells(1). (1) USDA-ARS Environmental Microbiology and Food Safety Lab; (2) USDA-ARS-BARC-Invasive Insect Biocontrol and Behavior Laboratory, Beltsville, MD, U.S.A.

Larval pests, like Manduca sexta, can cause major chewing damage on tomato plants, and produce copious quantities of frass which have high contact potential as they fall onto leaves and fruits in the tomato canopy. Rainfall and condensation drops can facilitate spread and lodging of frass in cracks/crevices of tomato fruits. This study was conducted to determine growth and survival of E.coli O157:H7, Salmonella, and Listeria in frass produced by Manduca that have injected contaminated tomato leaves. Laboratory-reared Manduca larvae were fed once with tomato-leaf disks inoculated with a total of $2.9-6.8 \times 10^3$ cells of nonpathogenic E.coli O157, Salmonella, or Listeria. Frass was collected/assayed daily 4-da for survival of inoculated strains. E.coli increased 3-5 log$_{10}$, whereas Salmonella increased 1-3 log$_{10}$. Listeria was least prevalent and persistent in frass. Only E.coli was the only one of the three types of bacteria to show an increased population during all 4-da of frass assay. Salmonella cell densities did not increase after day 1. Infected hornworms can disseminate E.coli, Salmonella, and Listeria through their frass, excrement; E.coli and Salmonella can amplify cell densities 10-100,000 times from levels injested. E.coli is shed for at least 4-da after initial injection. Listeria survival is variable in hornworms. Manduca are capable of not only transmitting, but sustained shedding of E. coli and Salmonella, and for some larvae Listeria in their frass for at least 4-da post-injection, thereby making Manduca a potential vector for bacterial pathogens and a target to control.

Poster #13
Identifying and characterizing the associations between produce surface profiles, background microorganisms, and the attachment and colonization of foodborne pathogens. K. PEREZ (1), M. Akbulut (1), L. Cisneros-Zevallos (1), M. Taylor (1), A. Castillo (1). (1) Texas A&M University, College Station, TX, U.S.A.

Foodborne illness resulting from consumption of contaminated produce has become an increased concern in recent years. Although significant research on decontamination procedures for produce has been completed, there exists little data describing the interactions between native and epiphytic microbiota and human pathogens on produce surfaces. Such research may result in the development of interventions that prevent the attachment of pathogens to produce and assist in the decontamination of produce. The objective of this research will be to determine the role played by background microbiota in the attachment of enteric bacterial pathogens to produce surfaces with differing surface topographies and chemistries. Preliminary studies on green bell pepper have been performed to obtain an initial profile of key microbial groupings on a smooth surface produce. Aerobic microbes were enumerated on plate count agar (PCA), Lactic Acid Bacteria (LAB) on de Man, Rogosa, and Sharpe (MRS) agar, yeasts and molds on potato dextrose agar (PDA), and resulted in $3.1 \pm 1.0 \log_{10} \text{CFU/cm}^2$, $2.1 \pm 1.1 \log_{10} \text{CFU/cm}^2$, and $1.7 \pm 1.2 \log_{10} \text{CFU/cm}^2$, respectively. Further studies will be performed to identify predominant microbial groupings on surfaces of differing produce types (smooth, netted, leafy green) and the impact on the attachment and adherence of pathogens to various surfaces. The collective capacities of differing microbial groupings to inhibit foodborne pathogens from attaching will be quantified. These studies will generate data describing the interactions of background microbiota and human pathogens and the impacts of produce physiology, allowing the development of novel process interventions for inhibiting pathogen attachment and growth on produce.
Poster #14

**Pathogen Aerosolization and Deposition onto Nearby Leafy Greens through Various Farm Operations.** B. D. Smith (1), M. R. James (1), P. Milner (2), F. M. Hashem (1), C. P. COTTON (1), L. E. Marsh (1). (1) University of Maryland Eastern Shore, Princess Anne, MD, U.S.A.; (2) USDA-ARS, Beltsville, MD, U.S.A.

Transfer of pathogens from animal manure has been implicated in foodborne illness outbreaks of produce. Bacteria-laden manure dust/particulates from animal operations, including land application of manure and exhaust air from mechanically ventilated animal-housing units may be transported off-farm in air and deposited onto nearby crops. Our research has examined pathways by which airborne bacteria may be dispersed from typical poultry house (PH) and litter (PL) management operations across agricultural landscapes to contaminate fresh produce. One study examined airborne dispersal/deposition of *E. coli* (EC) from two different types of PH ventilation systems (high flow-rate tunnel vs. low-flow rate conventional) onto leafy greens (LGs) at several distances downwind. Analysis of lettuce and spinach leaves exposed to PH-ventilation exhaust showed that EC, which was prevalent in PL, was disseminated 11.1 and 7.5m downwind from tunnel and conventional fans, respectively. Another study examined airborne dispersal of bioaerosols (generated during mechanical cleanout and land application of PL) and deposition onto tomato plants stationed 7.5-120m downwind. Air and tomato-leaf samples exposed to airborne dust/particulates from PL-handling operations for 1-15 min were analyzed for EC and *Staphylococcus*. At 7.5-120m, *Staphylococcus*, present in high concentration in PL, predominated in air samples and on the tomato phylloplane; however, EC was not detected in PL or in air samples. Our initial results indicate that *Staphylococcus* in PL may be a good bioaerosol dispersion indicator for that matrix and that bacterial concentrations in spent PL will strongly influence concentrations resulting in air downwind of PL handling operations.

Poster #15

**Ingress and movement of Salmonella in tomato plants grown in different soils.** A. H. VAN BRUGGEN (1), G. Gu (1), J. M. Cevallos-Cevallos (1). (1) University of Florida, Gainesville, FL, U.S.A.

Several *Salmonella enterica* outbreaks have been traced back to contaminated tomatoes. A two-phase experiment was conducted twice to investigate the movement of *S. enterica* Typhimurium in tomato plants via leaf inoculation as affected by soil management and endophytic microbial diversity. In the first phase, 84 tomato plants grown in conventional or organic soils were inoculated 2-4 times before fruiting with two GFP-labeled *S. enterica* Typhimurium strains. Microbial communities in plants were investigated two weeks before and after inoculation. Inoculated and adjacent leaflets were tested for Salmonella survival from day 1 to day 30 after each inoculation. Fruits and seeds were also examined for Salmonella. In phase 2, extracted seeds including internally contaminated ones were planted in conventional soil, and infection of leaves and fruits of the second generation was checked. After inoculation, significantly more Salmonella CFU survived inside plants grown in conventional than in organic soil (P <0.05). All contaminated fruits were from tomato plants grown in conventional soil; the chance to detect contaminated fruits was about 3%. The contamination rate of the seeds from infected fruits was about 5%. There was a negative correlation between bacterial diversity in plants and the rate of internalization. In the second generation, no infection of leaves and fruits was detected. However, one plant from the 12 internally contaminated seeds died after germination in year 2; none of the control seedlings died. Thus, Salmonella can contaminate fruits and seeds through leaf inoculation, but the chance of seed transmission of Salmonella is very low.

Poster #16


Several *Salmonella enterica* outbreaks have been associated with tomatoes during the past six years.
However, the mechanisms of Salmonella dispersal in agricultural fields are poorly understood. The effect of Salmonella rdar morphotype and tomato trichome density on pathogen dispersal by rain splash or aerosol was investigated. Artificial rain matching intensity and drop-size distribution of Florida rain was used. Trichome density on tomato leaves was increased or decreased by applying jasmonic or salicylic acid, respectively. Rains of 50 mm/h or 100 mm/h were applied to 109 CFU/mL suspensions of GFP-labeled kanamycin-resistant Salmonella Typhimurium with and without the rdar morphotype. Cells dispersed by rain splash were recovered by making leaf imprints on LB agar with kanamycin. Aerosolized cells were recovered by sampling the air in lactose broth by using an impinger. Cells lacking rdar morphotype (MAE 119) showed a significantly higher dispersion than cells expressing rdar morphotype (MAE 110) when the trichome density was below 200 per cm². Conversely, MAE 110 cells showed significantly higher dispersal at trichome densities above 300 per cm² when compared to MAE 119. Salmonella aerosol formation after rain was only observed in MAE 110 cells in air samples and when a resuscitation step in lactose broth followed by enrichment in tetrazionate broth was used. Salmonella cells were detected on tomato fruits but not on leaves after aerosol formation. Results suggest that Salmonella rdar morphotype favors the pathogen dispersal at high trichome densities on tomato leaves and increases Salmonella’s ability to form and survive in post-rain aerosols.

Poster #17
Development of rapid alert systems for prevention of human enteropathogenic bacteria in vegetable production chains. L. VAN OVERBEEK (1). (1) WUR Plant Research International, Wageningen, The Netherlands

Outbreaks of *Escherichia coli* O104:H4 in Germany and France, last year, illustrate the seriousness of the threats of vegetable-borne food diseases for consumers. These outbreaks were associated with fenugreek seed lots that stayed “on the shelf” for almost three years. Based on these incidences and on our research on the transmissions of *E. coli* O157:H7 and *Salmonella enterica* via manure to freshly-consumed vegetables conducted in the past, we formulated new initiatives to study the fate and behaviour of human enteropathogens in plants and their surroundings. Key questions that will be addressed are: 1) what need to be improved in existing detection technologies for quick and reliable screens of human enteropathogens in vegetable-production chains?, 2) what are the possible transmission routes of human enteropathogens to seeds?, 3) where are these contaminations located in the seeds, with respect to eventual decontamination measures to be taken in the future?, 4) what is the interaction of human enteropathogens in the plant environment with respect to improved survival and acquisition of new traits from indigenous communities? A new project started up this year on the ecology and adaptation of human enteropathogens in plants. We state the hypothesis that these pathogens can be present inside plants, as endophytes, where they may come into close contact with typical commensal and pathogenic plant-associated bacteria belonging to the taxonomical family of *Enterobacteriaceae*. New projects on the transmission of human pathogens via vegetable seeds may be started up this year in close collaboration with academic and industrial partners.

Poster #18
*Filth Fly contamination of food plants.* A. WAYADANDE (1), J. Talley (1). (1) Oklahoma State University, Stillwater, OK, U.S.A.

Filth flies are higher order Dipterans that develop in necrotic tissue, rotting vegetation, fecal material, and other bacteria-laden habitats. This group is composed primarily of the muscid flies (house flies, Family Muscidae), the blow flies (Family Calliphoridae) and the flesh flies (Family Sarcophagidae). Filth flies have long been implicated as vectors of pathogens in hospital and restaurant settings, but little is known about their role, if any, in pathogen transmission to food plants. Recent work has shown that significant proportions of flies associated with animal production facilities are often contaminated with *E. coli* O157:H7, *Salmonella* spp., *Enterobacter* spp. and other human pathogens. When house flies are exposed to *E. coli* O157:H7-contaminated manure and they subsequently regurgitate onto plant surfaces, this pathogen replicates within the regurgitation spot to many times the original titer. Casual contact with food plants may
also result in contamination. *E. coli* O157:H7 and *Salmonella enterica* adhere to fly cuticular surfaces on the mouthparts and feet and detach during walking and surface sponging on spinach and other substrates. This suggests that flies may pose a risk for contamination of food plants if present in significant numbers.

**Poster #19**

**Comparison of Lettuce, Spinach, and Parsley Leaves for Internalization of *Escherichia coli* O157:H7 after Repeated Exposure to Low-dose Contaminated Irrigation Water.** C. C. WEBB (1), M. C. Erickson (1), L. E. Davey (1), A. S. Payton (1), I. D. Flitcroft (2), M. P. Doyle (1). (1) Ctr. for Food Safety, University of Georgia, Griffin, GA, U.S.A.; (2) Dept. of Crop and Soil Sciences, University of Georgia, Griffin, GA, U.S.A.

Irrigation water may contaminate edible leafy green tissue and a greater risk may occur if plants are repeatedly exposed to the pathogen and a moist environment. The fate of *Escherichia coli* O157:H7 (O157) on harvestable leafy greens when repeatedly exposed to contaminated irrigation water was determined in this study. A 3 log CFU/ml, GFP-labeled inoculum mixture, of virulent O157 strains was applied via overhead spray to mature plants (spinach, lettuce, parsley) with the application time (10-20 min) varying according to plant size and surface area. In the first trial, the spray procedure was repeated for seven consecutive days and leaves sampled on exposure day 0, 2, 5, and 7. In trial 2, spinach plants were sprayed for five consecutive days. Leaves were analyzed for internalized O157 on day five of exposure as well as two and seven days later. Leaves were either analyzed directly for total pathogen or dipped in 1% silver nitrate for surface sterilization and subsequent detection of internalized pathogen. Based on enrichment culture, in the first trial 19 of 160 parsley samples and 82 of 240 spinach samples were positive for internalized O157. In contrast, no internalized O157 was observed in lettuce plants. In the second trial, O157 was internalized within spinach leaves (25 of 40 samples) when analyzed on the fifth day of spraying but no internalized O157 could be detected two days later. Although internalization of O157 into leaves occurs with some types of leafy greens, the pathogen appears to be a transient resident.

**Emerging Issues**

**Poster #20**

**Listeria monocytogenes survival in the presence of malic acid, lactic acid or blueberry extract.** A. CONCHA-MEYER (1), J. Eifert (1). (1) Virginia Tech

The aim of this work was to study the growth and survival of *Listeria monocytogenes* strain Scott A in media acidified with malic acid, lactic acid or blueberry extract. Blueberry extract (pH 3.06) was obtained from previously blended and centrifuged fresh fruit. Bacterial growth was evaluated using tryptic soy broth with yeast extract (TSB+YE) adjusted to different pH (2.0, 3.0, 4.0 or 5.0) and incubated at 25°C for 48h. An optical density system was used to measure growth (turbidity) over time. Growth curves were produced by a BioScreen C Microbiology Reader equipped with an incubator and automated turbidimeter that determined optical density (OD) of the samples every 15 minutes. Complete inhibition of *L. monocytogenes* occurred in the presence of malic acid and with lactic acid at pH 4.0, 3.0 and 2.0, and with blueberry extract at pH 2.0. Peak growth reduction of *L. monocytogenes* was 39.7% with malic acid at pH 5.0, 39.4% with lactic acid at pH 5.0, and 55.1% with lactic acid at pH 5.0. Peak growth reduction with blueberry extract at pH 5.0, and 55.1% with lactic acid at pH 5.0. Peak growth reduction with blueberry extract at pH 4.0 and 3.0 was 37.8% and 41.2%, respectively. Our research indicates that *L. monocytogenes* can grow and survive in the presence of blueberry extract at pH 3.0. The acid tolerance response of this pathogen increases its potential threat if it becomes a contaminant in acidic foods. The optical density measurement technique consumes less media and is more rapid and precise than other methods to measure changes in growth over time.
**Poster #21**

*E. coli* O157:H7 in dual biofilms formed with resident bacteria isolated from fresh produce environment. T. LIU (1), X. Nou (2), A. M. Lefcourt (2), Y. M. Lo. (1) University of Maryland, College Park, MD, U.S.A.; (2) USDA-ARS, Beltsville, MD; U.S.A.

In produce processing plants, biofilms may potentially provide a supporting environment for pathogenic bacteria, which can result in enhanced resistance to cleaning and sanitizing efforts. The objective of this study was to examine the potential of bacteria strains isolated from a produce processing plant to co-exist with *E. coli* O157:H7 in dual species biofilms. Ninety-four isolates, including 15 species belongs to 9 genera, were recovered and identified. One isolate of each genus was selected for further study. Each isolate was allowed to form a monoculture biofilm in 96-wells microtitre plate. The total biomass of biofilms was measured to evaluate biofilm-forming capacity of each isolate. To investigate the interaction between isolates and *E. coli* O157:H7, *E. coli* was co-cultured with a 1 day old biofilm formed by each of the resident bacteria isolates. This 1 day old biofilm was used for simulating the naturally occurring biofilms in processing environment. *E. coli* O157:H7 was found be able to co-exist with resident bacteria. To verify the co-existence in co-cultured biofilm, fluorescence microscope was conducted. In two out of nine combinations, more *E. coli* O157:H7 cells were counted in co-culture compared to when *E. coli* O157:H7 was cultured alone. Based on our results, the biofilm formed by each of the tested bacteria recovered from a produce processing plant demonstrated the potential to provide a microenvironment for *E. coli* O157:H7. This microenvironment may protect *E. coli* O157:H7 cells from the sanitizing treatment, thus it becomes a potential source of cross-contamination in produce processing plant.

**Poster #22**


*Aspergillus flavus* is a well-known pathogen of many important agricultural commodities and is a major producer of aflatoxins, which are carcinogenic polyketides that pose a serious health risk to humans and animals. Aflatoxin contamination in peanut exports worldwide accounts for as much as $450 million in losses. *A. flavus* is also an emerging human pathogen and is second only to *A. fumigatus* in reported aspergillosis outbreaks. Comparative genome analyses between *A. flavus* and *A. oryzae* have revealed large chromosomal inversions and duplications. Similar genome rearrangements and aneuploidies are observed in natural *A. flavus* strains and in progeny strains derived from intra-specific sexual matings. In the present study, we genetically examined the offspring from several crosses. We observed non-Mendelian inheritance of extra-genomic aflatoxin cluster alleles in crosses with partial aflatoxin cluster parents. The specific function of cryptic alleles is currently under investigation. Because *A. flavus* has multinucleate mycelium and conidia, we cannot rule out the possibility of cryptic heterokaryosis in which the parental strains harbor a small proportion of aneuploid nuclei that sort out in progeny in a non-Mendelian-like fashion. Preliminary evidence indicates that aneuploidy can be maintained to varying degrees in natural isolates but may be accelerated in strains that are serially transferred under adverse culture conditions. Further characterization of aneuploidies and their frequency in *A. flavus* populations will be important in understanding the role of genome plasticity in modulating toxicity and the adaptation of these fungi to changing environmental conditions.

**Poster #23**

The unique interaction of *Salmonella enterica* with *Arabidopsis* leaf. S. PANCHAL (2), C. Mecey (1), B. Rosa (1), S. He (1), M. Melotto (2). (1) Michigan State University, MI, U.S.A.; (2) The University of Texas at Arlington, Arlington, TX, U.S.A.

Recent studies have shown that stomata in the leaf epidermis are involved in the innate immunity of plants, as they actively close minimizing bacterial invasion of plant tissues. While the plant pathogen *Pseudomonas*
syringae pv. tomato strain DC3000 is able to re-open the closed stomata and become a fully virulent bacterium, the human pathogen Escherichia coli O157:H7 is unable to do so. This leads to an understanding that human pathogens are able to activate plant defenses through recognition of conserved pathogen associated molecular patterns (PAMPs), and may be largely restricted to the plant surface. However, a recent study demonstrated penetration of Salmonella enterica serovar Typhimurium inside the tissues of lettuce leaf via stomata. Here we report that this organism induces stomatal closure, but interestingly, the stomata re-open 2-3 hours after exposure to the bacterium. Our studies with Arabidopsis also indicate that Salmonella and E. coli have different behaviors and induce variable transcriptional responses in Arabidopsis leaf cells. Several genes involved in chromatin remodeling, heat stress, auxin metabolism among others are differentially regulated by Salmonella but not PstDC3000 or E. coli highlighting the unique response to this bacterium. As current decontamination efforts involve mostly sterilization of the leaf surface, there is an urgent need to implement additional control measures geared towards prevention of outbreaks associated with consumption of vegetables contaminated with pathogens such as Salmonella, which are able to penetrate and survive in the plant interior.

Poster #24
**Potential interactions between Ralstonia solanacearum and Salmonella enterica in Tomato Plants.** S. K. POLLARD (1), S. Rideout (1). (1) Virginia Tech, VA, U.S.A.

Approximately 80% of Virginia’s tomato production occurs on the Eastern Shore of Virginia (ESV). Since 2002, the ESV has been implicated in at least four outbreaks of Salmonellosis, and threatens the sustainability of this industry. Salmonella enterica Newport has been isolated from ESV ponds and poultry litter. The S. Newport isolated from ESV has been characterized by the FDA and identified to be strain pattern 0061. This S. Newport pattern 0061 is the same strain that was isolated from all of the Salmonellosis outbreak cases originated from Virginia. Not only does Salmonella contamination threaten the fresh market tomato industry, but so does the devastating plant disease, bacterial wilt, caused by Ralstonia solanacearum. Bacterial wilt is present in many ESV tomato fields and causes severe yield losses every year. Because of the prevalence of R. solanacearum and ESV’s association with Salmonellosis outbreaks, the relationship between the two pathogens is of interest. To investigate the relationship between these two bacteria, a series of greenhouse and fruit susceptibility studies is being conducted. Fruit samples are collected from plants infected with bacterial wilt and S. Newport, along with plants contaminated with S. Newport only. S. Newport internalization in fruit is then quantified and compared according to the different bacterial treatments. Preliminary results suggest that there is little difference in S. Newport internalization between the S. Newport contaminated plants and bacterial wilt-infected plants. However, this research has allowed for the development of a successful inoculation method that is both repeatable and requires few resources.

Poster #25
**Prevalence of Foodborne Pathogens in Fresh Produce in the US: Data from the USDA Microbiological Data Program (MDP).** S. REDDY (1), M. Tijerina(1). (1) M.S. US Dept. of Agriculture. Agriculture Marketing Service (AMS)

The MDP has provided data on microbial pathogens in select fresh produce since 2002. Commodities associated with high consumption and frequent illness/outbreaks are collected each week in 11 participating states. This statistically represents about 50 percent of the US population. Annually, more than 15,000 samples are analyzed in eight state laboratories for Salmonella, Escherichia coli O157:H7, non-O157 Shiga toxin-producing E. coli (STEC) and Listeria monocytogenes. MDP coordinates its activities with the Food & Drug Administration (FDA) and Centers for Disease Control and Prevention (CDC). In the last three years, sampling and analytical operations improved. Our data show from 2002 to 2008, there were 0.05% positives for Salmonella and 0% for E. coli O157: H7. From 2009 to 2011, the positives rates increased to 0.15% for Salmonella and 0.01% for E. coli O157: H7. During these two periods, MDP detected 0.11% versus 0.14% non-O157 STEC. L. monocytogenes testing that began October 2010, detected 0.4% positives. Isolates are characterized for serotypes, pulsed-field gel electrophoresis (PFGE), antimicrobial resistance and additional
virulence genes. Here we provide information on pathogen detection and data. This data is available to federal, state agencies and industry for food safety decision making purposes. The PFGE patterns of MDP isolates are uploaded to CDC’s PulseNet databases, facilitating early identification of common source outbreaks. FDA uses MDP data for investigations, initiating voluntary recalls, and developing guidance letters to growers. MDP is the only federal surveillance program for collecting microbiological data, including non-O157 STEC, on both imported and domestic fresh produce.

Environmental Issues

Poster #26


A study was established to investigate how long non-pathogenic *E. coli* and *E. coli* O157:H7 persist in an agricultural environment after application of raw manure to fields intended to grow produce. The study is currently being conducted at two geographically distinct sites: Princess Anne, MD (University of Maryland Eastern Shore, UMES), and Beltsville, MD (Beltsville Area Research Center (BARC), USDA-ARS. Uniform size field plots were laid out (2 m x 1 m) at both sites. At UMES, plots were treated with either UMES poultry litter or dairy manure solids (from the BARC dairy manure solid-liquids separation system). Non-pathogenic *E. coli* and *E. coli* O157:H7 were cultivated in UMES poultry manure extract and sprayed onto plots containing manure treatments. At BARC, manure treatments consisted of dairy manure solids applied to organic and conventionally-managed fields, as well as separate plots with UMES poultry litter, dairy manure solids plus liquids, and dairy manure liquid only. At both sites, plots received either a low or a high concentration of inocula containing several strains of *E. coli*. Data collection and analysis is ongoing at both sites and also will involve separate plots with spring application of manure and later leafy green cropping. Data collected here will provide valuable information for the Food and Drug Administration rule-making process for produce as part of the Food Safety Modernization Act. This study is being conducted with the Food and Drug Administration’s Office of Produce Safety and the University of Maryland Eastern Shore, Dept. of Agriculture, Food and Resource Sciences. This project is another collaboration between the USDA ARS and 1890 Land Grant Universities.

Poster #27

**Biofilms in irrigation pipes affect the microbial quality of irrigation water.** Y. PACHEPSKY (1), D. R. Shelton (1). (1) USDA-ARS, Environmental Microbial and Food Safety Laboratory, Beltsville, MD, U.S.A.

Disease outbreaks caused by crops that are eaten raw have been linked to microorganisms in irrigation water. The purpose of this study was to examine the formation of biofilms in sprinkler irrigation systems and the effect of biofilms on bacteria concentrations in water passing through. An irrigation system was constructed from aluminum pipes and fed with water from the perennial creek in Beltsville, MD. Two-hour irrigation events were carried out weekly four times in 2010 and 2011. Water samples were taken from the creek and the sprinkler heads at hourly intervals during irrigation events and analyzed for *E. coli* concentration. Additionally, pipe sections were removed before each irrigation event, scraped, and analyzed for *E. coli*. Substantial differences were found between *E. coli* concentration in creek water at the pump uptake and in water from sprinklers, leading us to believe *E. coli* was released from the pipe’s inner surfaces to flowing water, or otherwise was captured from the flowing water. High *E. coli* concentrations were found in the water that resided in pipes between irrigation events, indicating the potential opportunity for *E. coli* growth between events even at extreme temperatures of 2011 summer. Because biofilms in irrigation systems can modify the microbial water quality, it is imperative to monitor the quality of water coming from sprinklers rather than at the pump intake. Currently, there is no peer-reviewed literature on pathogen and indicator
microorganism populations in biofilms in irrigation systems, and research is needed into occurrence and mitigation of this microbial reservoir.

**Poster # 28**

**Differential susceptibility of Spring- and Summer-grown Spinach to "Spinach Breakage".** A. C. WAYADANDE (1), S. Kamenidou (1), J. Hardin (1), R. Madden (1), J. Dillwith (1), J. Fletcher (1). (1) Oklahoma State University, Stillwater, OK, U.S.A.

Spinach, *Spinacea oleracea*, is grown during the spring, summer, and fall in the Salinas Valley of California. Spinach breakage, in which minimal processing of bagged spinach sometimes results in torn and physically damaged leaves, occurs primarily in the summer and fall months but not in the early spring. It has been suggested that faster growth during the warmer summer months contributes to weaker leaf structure. To test this, we grew “Silverwhale” semi-savoy spinach under conditions that mimicked spring (slow-growth) and summer (fast-growth) conditions in Salinas, CA. Leaves harvested at the same physiological and chronological ages were examined by scanning, light, and transmission electron microscopy. Cuticular lipid analysis and leaf mechanical properties were also investigated. Fast-growth (summer) leaves were 35% - 50% thinner than slow-growth (spring) leaves and had thinner cell walls, a higher proportion of intercellular space, and smaller cell sizes. Analysis of leaves at the same chronological age indicated that epicuticular wax composition differed between fast- and slow growth leaves. Mechanical tests indicated that fast- and slow-growth leaves had similar strength and strain properties immediately after being severed from the plant. However, one hour after harvest, slow-growth leaves failed at significantly larger strain levels than fast-growth leaves. These data support the conclusion that after harvest, spring-grown spinach is less susceptible to leaf breakage during processing and thus, less susceptible to contamination by food-borne pathogens than spinach grown under warmer climate conditions.

**Forensics and Traceback**

**Poster #29**

**Multiple Loci Variable-Number Tandem-Repeat Analysis for Strain Discrimination of Non-O157 Shiga Toxin-Producing *Escherichia coli*.** C. TIMMONS (1), L. M. Ma (1). (1) National Institute for Microbial Forensics and Food and Agricultural Biosecurity, Oklahoma State University, Stillwater, OK, U.S.A.

Human illness caused by non-O157 Shiga toxin-producing *Escherichia coli* (STEC) has become a growing concern worldwide. Currently, there is a need for a rapid discriminatory tool for surveillance and traceback investigations of foodborne illness outbreaks caused by non-O157 STECs. The purpose of this study was to develop a multiple loci variable-number tandem-repeat (VNTR) analysis (MLVA) assay for inter- and intra-serogroup strain discrimination of 6 major non-O157 STEC serogroups. The genomic sequences of STEC O103, O111, and O26 were analyzed for potential VNTRs and twelve loci were selected. Fluorescently labeled PCR primers for the 12 VNTR loci were designed and evaluated using a non-O157 STEC reference set, containing 4 strains (human isolates) of each of 6 major STEC serogroups (O103, O111, O26, O45, O121, and O145) and several strains of STEC O157. Preliminary results indicated that the selected loci allow high inter-serogroup discrimination for the “big 6” non-O157 STEC serogroups. Further testing is needed to determine their intra-serogroup discriminatory capability. The developed MLVA assay should provide a much needed tool for rapid DNA fingerprinting of non-O157 STECs in outbreak investigation.

**Human Pathogenic Viruses on Plants**

**Poster #30**

**Comparative Uptake of Enteric Viruses into Spinach and Green Onions.** K. HIRNEISEN (1), K. Kniel (1). (1) Dept. of Animal and Food Sciences, University of Delaware, Newark, DE, U.S.A.
Root uptake of enteric pathogens and subsequent internalization has been a large area of research with results varying due to differences in experimental design, systems tested, pathogen type and crops. While most of the research efforts have focused on the possibility of internalization of human pathogenic bacteria into crops, human enteric viruses also pose a threat to produce safety. The objective of this study was to determine the ability of two enteric viruses, MNV and HAV, to internalize into spinach and green onion tissue through root uptake in hydroponic growing systems. MNV and HAV (approximately 6 log genomic copies) were inoculated into two hydroponic systems, floating and nutrient film technique systems. On sampling days spinach and green onions were washed with Virkon® solution to remove any externally present viruses, homogenized in PBS and viruses were detected by qPCR. In a floating hydroponic system, MNV uptake in spinach was significantly lower than that of MNV in green onions with titers <1.6 log genomic copies on average, whereas no significant difference was observed for uptake of HAV in green onions and spinach (an average of 4.75 log genomic copies). Differences in root uptake were observed between the floating hydroponic systems and NFT, where greater levels of virus was taken up into a greater number of green onions and spinach plants in the floating hydroponic system as compared to NFT. In the NFT, oasis cubes holding the spinach and green onions served as a reservoir with up to approximately 5 log genomic copies of MNV and HAV. Understanding the interactions of human enteric viruses on produce can aid in the elucidation of the mechanisms of attachment and internalization and eventually in the better understanding of risks associated with contamination events.

Prevention and Control

Effectiveness of Lytic Bacteriophages in Reducing E. coli O157:H7 Populations Introduced Through Cross-contamination on Fresh Cut Lettuce. S. E. Ferguson (1), C. Roberts (2), M. SHARMA (2). (1) University of Maryland, Food Science and Human Nutrition Dept., College Park, MD, U.S.A.; (2) USDA, Agricultural Research Service, Environmental Microbial and Food Safety Laboratory, Beltsville MD, U.S.A.

Previous research has shown that lytic bacteriophages (phages) can kill E. coli O157:H7 on produce surfaces. The role of lytic bacteriophages in preventing cross contamination of produce has not been evaluated. A cocktail of three lytic phages specific for E. coli O157:H7 (EcoShield) at 10^8 PFU/ml or a control (phosphate buffered saline, PBS) was sprayed on to lettuce pieces (9 cm²). For suspension studies, lettuce pieces were immersed in either 500 ml of 10^8 PFU/ml Ecoshield or PBS for 30 s and 2 min. Phage-treated lettuce was...
spot-inoculated with 50 ul of $10^4$ CFU/ml E. coli O157:H7. Phage-treated, inoculated lettuce pieces were stored at 4°C for and analyzed for E. coli O157:H7 populations on days 0, 1, 2, 3, 6, and 7. Spraying phages on to cut lettuce reduced E. coli O157:H7 counts to 1.76 and 1.46 log CFU/cm², compared to spraying with PBS, which resulted 2.38 and 2.35 log CFU/cm², on days 0 and 1, respectively. Immersing lettuce pieces in a suspension of EcoShield for 2 min was more effective in reducing counts of E. coli O157:H7 than for 30 s. Lettuce pieces immersed in EcoShield for 2 min contained lower E. coli O157:H7 populations, 0.61 and 0.52 log CFU/cm², compared to pieces immersed in PBS, 2.27 and 2.04 log CFU/cm², on days 2 and 3. Phage titers from both spray and immersion treatments were ca. 4 log PFU/cm² on lettuce stored for 7 days. Spraying phages on to fresh cut lettuce resulted in a more immediate reduction of E. coli O157:H7 introduced to lettuce post-phage treatment than immersion in the phage suspension. Other data to be presented at this meeting suggest that increasing the concentration of bacteriophages suspension applied to lettuce can also increase the bacterial reduction. Our results show that phage treatments have the potential to reduce E. coli O157:H7 populations on leafy greens introduced through cross contamination.

Poster #33

Because fruits and vegetables are often consumed raw they are vulnerable to contamination by foodborne human pathogens. Continuing illness outbreaks and recalls erode consumer confidence in fresh produce. Why are these incidents increasing? Is the persistence of human pathogens on plants (HPOP) a new phenomenon, reflecting human pathogen adaptation to the plant environment? If so, when and why do bacteria internalize in plants, and what selective force(s) and genetic determinant(s) are responsible? More importantly, can colonization/growth of HPOPs be inhibited? A central dogma of plant pathology, the “disease triangle,” emphasizes that plant disease can result only in the convergence of three critical elements: a virulent pathogen, a susceptible host, and an environment conducive to their interaction. This concept, which serves as a framework within which plant disease management strategies are developed, can be applied with minor modifications to the problem of HPOPs. Research questions and approaches familiar to plant pathologists, in their work on a myriad of plant species and pathogen types, can be transferred smoothly to the study of human pathogens. Plant disease management strategies, including chemical and biological control, cultural practices, use of resistant plant cultivars, control of insect vectors, and removing sources of inoculum, can be examined for parallel applications in food safety. Thus, the plant pathologist’s disease triangle can inform our food safety efforts by providing structure and logic to the search for reasonable and effective approaches to outbreak prevention and mediation.

Poster #34

Tomatoes have been linked to multiple outbreaks of foodborne disease. In addition, tomato diseases are ranked the highest risk to greenhouse tomato productivity due to their destructiveness and the lack of effective management strategies. Systems approaches that integrate prevention and control of human and plant pathogens may provide a comprehensive, successful strategy to minimize food safety risks and achieve
high quality product. To address this, a multidisciplinary team of food safety experts and plant pathologists performed on-site surveys to identify greenhouse production methods and practices used by industry in US, Canada and Mexico. Standardized, pre-tested questionnaires were used to assess the practices. Points of pathogen entry, dissemination and proliferation were identified for human and plant pathogens throughout the seed-to-retail production cycle. Tomato greenhouse production process flow diagrams were constructed for large/medium/small growers that included a total of 293 practices performed during propagation, growing and post-harvest stages of production. Expert stakeholder groups performed impact analysis. Risks were ranked for *Salmonella* spp., *E. coli*, *Listeria monocytogenes*, *Clavibacter michiganensis* subsp. *michiganensis*, *B. cinerea*, Pepino mosaic virus and emerging tomato viroids. The results were merged into operational risk assessment profiles and high risk practices were identified. Identified points critical for simultaneous control of human and plant pathogens differed between large/medium/small operations. While interventions targeting quality of irrigation water and inter-planting would have the highest impact in large scale greenhouses, priorities for small greenhouses were identified in harvest and post-harvest stages of production. Development of a system-wide framework in which the introduction and spread of plant and human pathogens can be effectively managed will enhance public health and provide the fundamental basis for growth of the greenhouse industry.

Poster #35
**Pre-Harvest to Post-Harvest Strategies for the Reduction of Human Pathogens in Tomatoes and Leafy Greens: A Farm to Fork Systems Approach.** D. T. INGRAM (1), B. Zhou (2), C. Shen (2), Y. Yang (1), M. Sharma (1), X. Nou (1), P. Millner(1,4), Y. Luo (1,3); (1) Environmental Microbial and Food Safety Laboratory, USDA, Agricultural Research Service, Beltsville, MD, U.S.A.; (2) University of Maryland, Dept. of Food Science and Nutrition, College Park, MD 20740, U.S.A.; (3) Food Quality Laboratory, USDA, Agricultural Research Service, Beltsville MD, U.S.A.; (4) Sustainable Agricultural Systems Laboratory, USDA, Agricultural Research Service, Beltsville MD, U.S.A.

Tomatoes and leafy-greens are two produce commodities that are susceptible to foodborne pathogen contamination and subsequent association with foodborne illness. Outbreak investigations have associated water – through pre-harvest (irrigation) and post-harvest (cooling, wash-tank or rinse water) as potential vehicles for pathogen contamination of these commodities. In light of outbreaks frequently associated with water, our laboratories have successfully employed several approaches to mitigate foodborne outbreaks on two fronts: targeting the safety of both irrigation and processing water. We evaluated an inexpensive, rapid, in-line filtration system using a column of Zero Valent Iron (ZVI) to remove *E. coli* and *Listeria* spp. from pre-harvest irrigation water. Post-harvest investigations include the evaluation of new technologies and practices designed to enhance the efficacy of wash-tank and rinse-water typically used to remove potential pathogens and prevent cross-contamination. Investigations revealed operating parameters that affect the ability of foodborne pathogens to infiltrate tomatoes via stem scars, as well as a food processing aid (T-128) that improved the efficiency of chlorine as a disinfectant in post-harvest wash water. Investigations into current cold-storage retail display of fresh-cut produce reveal that this might also be critical food safety vulnerability. The 2009 FDA Food Code requires all packaged fresh-cut leafy-greens to be maintained under 41°F, but incidents of product temperature abuse occur frequently. Our lab has shown that new technologies and operational practices are available to ensure compliance with the Food Code, as well as maintain the quality, safety and prolong the shelf-life of fresh-cut produce from farm to fork.

Poster #36

Surface waters contain a vast community of living microorganisms, some of which are pathogenic to humans or plants. A surface water pathogen survey was conducted in over thirty actively used surface water irrigation reservoirs in New York throughout the growing seasons of 2010 and 2011. Survey results indicate that the exposure risk of crops through surface irrigation water to pathogens is high, making surface water a serious
threat to human and plant health. Generic *Escherichia coli* and *Salmonella* spp. were found frequently at varying levels from all water sources sampled while only one was found to be contaminated with the protozoan parasite *Cryptosporidium*. Over 25 species of plant pathogens have been identified so far including the oomycete *Phytophthora capsici* and the bacterium *Clavibacter michiganensis* subsp. *michiganensis*. Many potential pathogen isolates are awaiting identification. Currently, there is no ideal water treatment solution for growers using surface water for irrigation in the field due to the quality and volume of water used. An ultraviolet (UV) processing unit developed for food safety applications could be a solution for contaminated surface irrigation water; preliminary experiments involving pertinent human and plant pathogens have shown promising results. The UV system will continue to be evaluated in the laboratory and in the field as a potential solution for contaminated surface irrigation water as an effort to improve food safety and plant health by reducing the risk of pathogens spread through hydrologic vectors.

Poster #37
**Control of human pathogens on plant products.** L. M. MA (1), J. Fletcher (1), S. Dobhal (1), D. Gautam (1), C. Timmons (1). (1) National Institute for Microbial Forensics & Food and Agricultural Biosecurity, Dept. of Entomology & Plant Pathology, Oklahoma State University, Stillwater, OK, U.S.A.

Plant products, especially fresh produce, have been increasingly associated with outbreaks of foodborne illness and human pathogens. The food safety/biosecurity research group at National Institute for Microbial Forensics & Food and Agricultural Biosecurity, Oklahoma State University, has been addressing detection/discrimination, ecology, and control of human pathogens on plants. An improved PCR detection method for both *Salmonella* and *E. coli* O157:H7 was developed by incorporating a 5’ AT-rich flap into primer design. Colonization/internalization studies of *Salmonella* on cantaloupe have revealed that the plant pathogen, *Erwinia tracheiphila*, may facilitate the internalization of *Salmonella* into edible portions of cantaloupe fruits when the two pathogens are co-inoculated at the time of natural fruit cracking, whereas *Salmonella*, either alone or with *E. tracheiphila*, is capable of internalizing and persisting within the edible tissue of cantaloupe fruits developed from inoculated flowers. Through metagenomic analysis, the microbial community on fresh tomato surface is being assessed. Fungicides, such as Kocide, and insecticides, such as Assail, Mustang, and Ambush, were found significantly inhibitory to human pathogens. In the development of biological control agents against human pathogens on plants, a group of selected bacterial isolates from fresh produce have demonstrated strong inhibitory action against *Salmonella*, *E. coli* O157:H7, and *Shigella* in vitro. To control human pathogen contamination on plant products, it is essential to have reliable and sensitive detection methods, knowledge about ecological survival characteristics of human pathogen on plant hosts and agricultural production environments, and practical control measures.

Poster #38
**Fate and effect of sulfadiazine in bulk soil and in the rhizosphere of maize: a mesocosm study.** W. MAIER, Julius Kühn-Institut (JKI), Federal Research Ctr. for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany

*This came in late – no text. Will be hung up onsite but not in program book.*

Poster #39
**Cetylpyridinium Chloride Surface Treatment of Cantaloupe Reduces Salmonella Contamination.** R. SAUCEDO (1), J. Eifert (1), R. Boyer (1), R. Williams (1), G. Welbaum (1). (1) Virginia Tech, Blacksburg, VA, U.S.A.

Appropriate post-harvest washing and sanitizing procedures can help control *Salmonella* and other pathogens on cantaloupe or other melons. Since the surfaces of cantaloupes are highly rough or irregular, bacteria can easily attach and become difficult to remove. The application of cetylpyridinium chloride (CPC) to cantaloupe may be an alternative post-harvest technique to reduce the frequency and level of *Salmonella* contamination. CPC can also repel or reduce bacterial attachment, which can make surface bacteria more
susceptible to sanitizers or physical removal. We evaluated the efficiency of microbial log reductions of *Salmonella* on cantaloupe melons, utilizing CPC solution sprays. Additionally, we compared texture quality and color of treated and untreated melons. Cantaloupe (Athena cultivar) plugs (2.5 cm dia.) were inoculated with 100 μL of a broth culture of *Salmonella* Michigan. After 15 min, plugs were sprayed with 10 ml of a CPC solution (0%, 0.2%, 0.5% or 1.0%) and held at 37°C for 24 hrs. Melon plugs were diluted with Butterfield’s Phosphate Buffer, shaken, and the solutions were enumerated on tryptic soy agar. The texture quality and color of additional melon samples were evaluated after CPC spray treatments over 14 days storage at 4°C. A 0.5% or 1% (vol/vol) application of CPC reduced *Salmonella* levels by >1.1 log CFU/ml in comparison to the field control (p<0.01). No significant differences (p>0.05) were observed in the texture and color of CPC treated melons. A surface spray application of CPC can be an alternative antimicrobial post-harvest treatment to reduce pathogen contamination of cantaloupe.

Poster #40
**Fresh Produce Washing Aid, T-128, Enhances Inactivation of *Salmonella* and *Pseudomonas* Biofilms on Stainless Steel and Cantaloupe Rinds in Chlorinated Wash Solutions.** C. SHEN (1), Y. Luo (2), X. Nou (2), G. Bauchan (3), B. Zhou (1), Q. Wang (1), P. Millner (2). (1) Dept. of Nutrition and Food Science, University of Maryland, College Park, MD, U.S.A.; (2) USDA, Agricultural Research Service, Environmental Microbial and Food Safety Laboratory, Beltsville, MD, U.S.A.; (3) USDA-ARS, Electron & Confocal Microscopy Unit, Beltsville, MD, U.S.A.

Biofilm formation on plant surfaces and equipment used for food processing is concern for fresh-cut produce safety because biofilms protect against sanitizers. This study was to evaluate the efficacy of chlorine wash solutions, with or without the washing aid, T-128, on inactivation of *Salmonella* and *Pseudomonas* populations in biofilms on stainless steel coupons and cantaloupe rinds. Biofilms were formed statically on stainless steel coupons suspended in 2% lettuce extract or on cantaloupe rinds after inoculation with *Salmonella enterica* serovars Thompson or Newport, or *Pseudomonas fluorescens*. Coupons with biofilms were washed in chlorine solutions (0 to 20 mg/L, at pH 6.5, 5.0 and 2.9), with or without T-128. Cantaloupes with biofilms were manually washed for 5-min in chlorine solutions (200-1000 mg/L, at pH 5.0) with or without T-128. Cells on coupons or cantaloupes were enumerated by mini-MPN or spread plating. Biofilm cell responses to fluorescent viability stains after washing treatments were examined with confocal laser-scanning microscopy. For both *Salmonella* and *Pseudomonas*, the sanitizing effect of free chlorine (1.0-5.0 mg/L) was enhanced by ~1-3 log10 units when combined with T-128. Image analysis of surfaces stained with SYTO® 9/propidium iodide corroborate cultural assay results showing that T-128 aids in reducing pathogen viability in biofilms. For cantaloupes, free chlorine (500-1000 mg/L) sanitizing effects were enhanced by ~1-2 log10 CFU/cm² when combined with T-128. Conclusion: T-128 can aid in reducing pathogen viability in biofilms on stainless steel and cantaloupe rinds, and thus can aid in sanitizing food processing equipment or cantaloupes during fresh fruit processing.

Poster #41
**Enhancing Trade in Horticultural Crops through Food Safety and Phytosanitary Measures.** K. C. SHENGE (1), S. A. Miller (2), J. T. LeJeune (2), J. M. Erbaugh (2), R. A. Omolehin (1), C. M. Z. Whong (1), L. L. Yakubu (1). (1) Ahmadu Bello University, Zaria, Nigeria; (2) The Ohio State University, Columbus and Wooster, Ohio U.S.A.

Nigeria is Africa’s largest producers of fresh tomatoes, but growth of the industry is hampered by production problems and food safety issues. Surveys were conducted in Northwestern Nigeria to identify sources of microbial contamination of tomatoes pre- and postharvest, with the aim of developing a science-based Good Agricultural Practices (GAPs) curriculum and training programs. A rapid appraisal was performed with market vendors in the study area, and a socioeconomic survey was done with households to determine their knowledge, attitudes, perceptions and practices regarding produce food safety quality and plant health. Sources and magnitude of microbial contamination of tomatoes were identified during the farm and household surveys. Irrigation water samples were found to contain significantly high levels of contamination
with coliforms. Results also showed that tomato fruit samples from local markets had significantly higher populations of \textit{E. coli} than field samples, indicating that most of the fruit contamination happened postharvest. Information from surveys was used to develop GAPs guidelines to fit local market preferences, production systems and capabilities, and training modules for agricultural extension workers for scaling-out to producers and other segments of the tomato value chain.

Poster #42

**Microbial cross-contamination of tomatoes during washing with Tsunami 100 in a commercial packing line.** H. Wang (1), E. T. Ryser (1). (1) Michigan State University, East Lansing, MI, U.S.A.

Post-harvest packing of tomatoes typically involves the use of a sanitizer in dump tank water during washing. However, sanitizer efficacy is known to decrease with increasing organic load. The objective of this study was to assess the efficacy of Tsunami 100 (a peroxyacetic acid-based sanitizer) at 13, 52 and 72 ppm for reducing the microbial load on tomatoes and in the dump tank water during commercial packing. During three visits to one Michigan tomato packer, a series of tomato (~900 g), water (50 ml), equipment surface (100 cm²) and brush samples were collected during 4 hours of processing and quantitatively analyzed for mesophilic aerobic bacteria (MAB) and yeasts & molds using standard plating methods. Water samples from the dump tank were also assessed for sanitizer concentration, ORP, pH, temperature, COD, and total solids. Additional unwashed tomatoes obtained from the same packer were submerged for 2 min in 50 ppm Tsunami 100 and then brush-washed using a pilot plant-scale roller conveyor. Initially and after 4 h of commercial processing, MAB populations decreased 0.3 and 0.2, 1.5 and -0.2, and 1.1 and 0.5 logs on tomatoes using 13, 52 and 72 ppm Tsunami 100, respectively. Microbial counts in the dump tank water increased with organic load during processing. Both sets of brush rollers sampled before and after waxing were heavily contaminated. Based on the observed build-up of organic load in dump tank water and the heavily contaminated brush rollers, more effective microbial intervention strategies are needed to minimize cross-contamination during tomato packing.

**Sampling Challenges and Solutions**

Poster #43

**Effect of sample preparation in the detection and isolation of \textit{E. coli} O157:H7 from artificially contaminated produce.** K. J. Yoshitomi (1), K. C. Jinneman (1), W. M. Fedio (2). (1) FDA, Bothell, WA, U.S.A.; (2) New Mexico State University, Las Cruces, NM, U.S.A.

This study evaluated a composite rinse and a soak procedure to detect and recover \textit{E. coli} O157:H7 from produce. One of a five sample set was spiked with \textit{E. coli} O157:H7 at approximately 0.1 cfu/g or 1.0 cfu/g. All five produce samples in the set were individually rinsed with an equal volume Butterfield’s phosphate buffer, and 25mL from each were composited and added to 125ml 2X modified buffered peptone water with pyruvate. Composited samples were enriched for 5h at 37°C, antibiotics were added and incubation continued at 42°C for the remaining 24h. Enrichments were screened by real-time PCR and streaked onto selective agar plates (Rainbow and TC-SMAC) for cultural recovery. Napa cabbage, mung bean sprouts, jalapeño peppers, and cantaloupes were tested. Composite rinsate samples from Napa cabbage were positive for all inoculated samples. Jalapeño peppers resulted in positive PCR detection from 6/6 samples at the high level inoculum and 1/6 from the low. However, \textit{E. coli} O157:H7 was not recovered from any of the inoculated pepper samples. Bean sprouts and cantaloupes failed to yield positive results by PCR or be culturally confirmed. Individual Jalapeño peppers and cantaloupes were also analyzed by a soak procedure. This improved detection and recovery of \textit{E. coli} O157:H7 in cantaloupes, at both inoculation levels but was not successful for jalapeño peppers. This was attributed to reduced survival and growth of the inoculum with this food matrix. This study demonstrates the importance of validation for various sample processing techniques and food matrices.