Food Safety Research Advances and Priorities for Fresh Produce

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Final Report

Food Safety Research Advances and Priorities for Fresh Produce

HG 09068

Joseph Ekman

August 2010

Project HG09068

Food Safety Research Advances and Priorities for Fresh Produce

A travel / study tour

Project leader

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Purpose of this report

This report is on travel to an international research symposium and research priorities setting workshop held in the USA during June 2010. The report details information from of the symposium and workshop which are particularly relevant to the Australian fresh produce industry and their implications for Australia.

Acknowledgement

Funding for this project was provided by Frontline Services Australia Pty Ltd and Horticulture Australia Limited.

Date of Report

August, 2010

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Media Summary

The latest developments in fresh produce food safety research were presented and discussed at the Produce Research Symposium convened by the Center for Produce Safety (CPS) at the University of California, Davis in June 2010.

The CPS was established as a collaborative research centre in 2007 with funding from industry and government, following a significant national outbreak of *E.coli* O157:H7 contamination in 2006, attributed to bagged spinach. This inaugural symposium presented the results from the first eleven research projects funded by the Center, targeted at better understanding the risks for on-farm microbiological contamination of fresh produce.

The Produce Research Symposium was followed by the Produce Safety Research Priorities workshop, where industry and government met to identify and discuss the future directions, strategies and mechanisms for funding fresh produce food safety research.

Over 200 leading fresh produce food safety researchers, industry leaders and government agency representatives participated in the Symposium and workshop. The success of these events will shape future investment in USA food safety research and the science conducted will have global implications for improving fresh produce food safety.

This forum also provides opportunities for Australian researchers to engage in collaborative research projects and communication with the CPS through the strong Produce Marketing Association linkages and relationships that have been established in Australia. The next CPS Produce Research Symposium is scheduled for Florida in 2011 and this event series will become the premier international forum for advances in fresh produce food safety research.

Following the conclusion of events at UC Davis, a tour of Salinas Valley leafy greens production areas provided vital insights into the on-farm food safety practices implemented in recent years to minimize the risk of further outbreaks associated with fresh produce.

Background and Objectives of Travel

This project enabled Joseph Ekman to participate in important international symposia in fresh produce food safety:

1. the inaugural 'Produce Research Symposium' organized by the new Center for Produce Safety (CPS), University of California,(UC) Davis

2. the 'Produce Safety – Research Priorities' workshop presented by the Center for Produce Safety (UC Davis), Joint Institute for Food safety and Applied Nutrition (JIFSAN, University of Maryland) and the Western Center for Food Safety (UC Davis)

These two events were organized to present the latest information from CPS funded research projects and to identify the research priorities for future funding.

Objectives of the visit included:

- Meet with and establish linkages with the Researchers, Extension Specialists and Administrators at the Center for Produce Safety (CPS) at UC Davis

- Participate in the Produce Research Symposium at the CPS

- Participate in the Produce Safety – Research Priorities

- Communicate outcomes with the Australian fresh produce industry

Expected Outcomes

The expected outcome of attending the CPS Symposium and workshop was:

- to review latest research information for fresh produce food safety issues that may be relevant to management of fresh produce in Australia

- identify USA research priorities for future funding and potential collaboration opportunities

Produce Research Symposium

The following is a summary of notes from the oral presentations, CPS project reports and panel discussions at the Produce Research Symposium.

The Produce Research Symposium was divided presentations into three key session themes:

I. Survivability of *E. coli* in field conditions

II. Enhanced testing methods for pathogens in produce

III. Potential vectors for pathogen transfer during field production

Each session concluded with a panel discussion that included representatives from research, extension and industry sectors and managed by a session moderator.

SESSION I: Survivability of *E. coli* in field conditions

Survival of attenuated Escherichia coli 0157:H7 ATCC 700728 in field-innoculated lettuce

Linda J. Harris, Ph.D. University of California, Davis

From 1995 through 2006, 22 outbreaks of *Escherichia coli* O157:H7 in the USA were associated with consumption of leafy green vegetables. Most of the outbreaks have been associated with lettuce that was harvested in late summer or early fall (Autumn). A better understanding of how *E. coli* O157:H7 survives in the field is needed and this research project used a non-pathogenic strain of *E. coli* with characteristics similar to the pathogen to inoculate growing lettuce plants in the field. The long-term goal is to generate data that will help inform growers of strategies that could mitigate the risk of the organism surviving on lettuce after a contamination event.

Previous data suggested that after a contamination event *E. coli* does not survive very well. Large, rapid decreases in numbers of the organism are observed. However, a small number of *E. coli* do survive on the lettuce plants for much longer periods. Soil and lettuce plants at a field trial site in the Sailnas Valley were inoculated with *E. coli* 10^{-7} (mock contamination event) a single time (soil 5 days post-seeding, plants 33 days post-seeding).

Results

For both trials, a rapid population decline of 3 log was recorded during the first 2 hours. Enrichment techniques (addition of substrate and use of selective media and temperatures) were required to detect *E. coli* O157:H7 after only 2 days post-inoculation (dpi). 85% of the plants tested during spring and 74% tested during fall (Autumn) were below the limit of detection (10 CFU/plant). By 7 dpi, more than 90% of the lettuce plants for both trials were below the limit of detection for *E. coli* O157:H7.

However, *E. coli* O157:H7 could be recovered from lettuce plants by enrichment through 28 (spring) or 35 (fall) dpi. The percentage of *E. coli* O157:H7-positive plants decreased from 93% at day 2 to 7% at day 28 (spring) and from 88% at day 2 to 0.6% at day 35 (fall).

For both trials there were no significant differences in counts of *E. coli* O157:H7 on plants from day 0 to day 7. For enriched samples, significant differences in the number of positive plants were identified on only a few of the sample days. Greater numbers of positive plants were identified for overhead sprinkler irrigation for the first 2 weeks post-inoculation in spring while the opposite was true for the first week of the fall trial. However, during the first week after inoculation of the fall trial, 35 mm of rain fell which likely impacted these results.

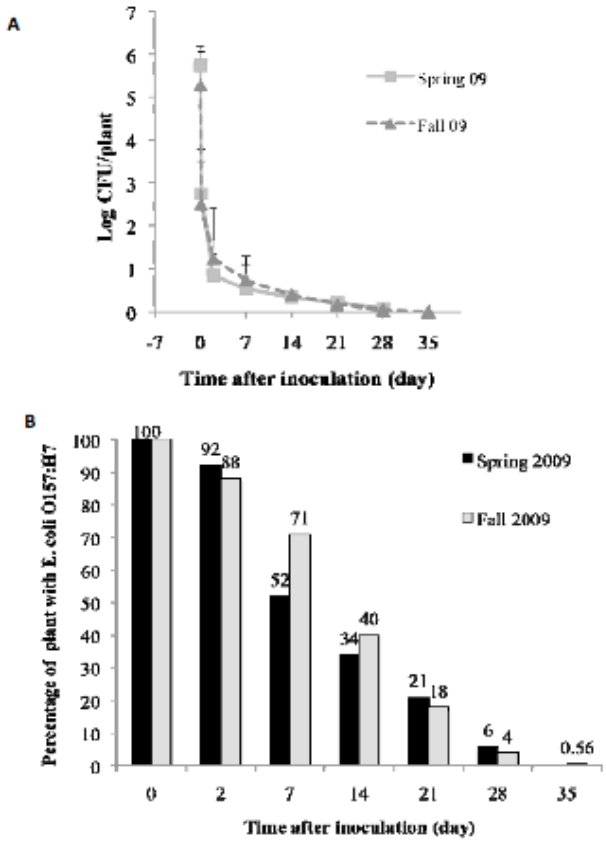

Figure 1: *E. coli* O157:H7 survival on lettuce plant. A) Population dynamic. B) Percentage of plants positive for *E. coli* O157:H7 after enrichment.
A) Romaine lettuce was inoculated 4 weeks after planting and was harvestable at day 28 during spring and at day 35 during fall. Each point represents the mean population size of *E. coli* O157:H7 ± SD. SD is shown only for sampling time (day 0, 2 and 7) when a plate count was possible. From 14 to 28 days after inoculation *E. coli* O157:H7 was detected only by enrichment. For both graph, data were combined for drip and overhead sprinkler irrigation treatment. n=120 at day 2, 7 and 14 post-inoculation for both trials. n=150 at day 21 and 28 post-inoculation for spring trial. n=120 at day 21, 156 at day 28 and 360 at day 35 post-inoculation during fall.

The time from the "contamination" or inoculation event to the point of harvest significantly influences the probability of isolating *E. coli* O157:H7 from lettuce plants. Also:

- when the soil was inoculated prior to emergence, *E. coli* was isolated from the soil for up to 15 dpi but could not be recovered from the plants. After fifteen dpi, *E. coli* could not be retrieved from the soil;
- when plants were inoculated, the levels of *E. coli* rapidly declined and were only isolated after 7 days by enrichment; the percentage of positive plant samples steadily declined post-inoculation;
- a significantly higher number of positive plants was observed with overhead irrigation on some but not all sampling points, however no significant difference was observed at the end of the trials;
- pathogen transfer from the soil to the plant was not observed: *E. coli* was never retrieved from plants (10 to 50 per block) sampled at 7 or 15 days post- inoculation.

Contribution of phyllosphere microbiota to the persistence of Escherichia coli 0157:H7 700728 on field grown lettuce

Maria Marco, Ph.D. University of California, Davis

Linda J. Harris Ph.D. University of California, Davis

Plant surfaces (the 'phyllosphere') are colonized by indigenous communities of microorganisms (here termed microbiota). The composition of the phyllosphere microbiota can differ depending on environmental factors, including the time of year and moisture levels. Harris *et al* (unpublished) found that these environmental factors might also influence the persistence of *E. coli* O157:H7 on Romaine lettuce plants in the field. There may be approximately 100 million cultivatable bacteria per leaf.

This project addressed the current gap in knowledge of whether indigenous phyllosphere microbiota also contribute to *E. coli* O157:H7 persistence. Microbiota present in the phyllosphere of overhead and drip irrigated Romaine lettuce prior to and following inoculation with attenuated *E. coli* O157:H7 were identified. Bacterial diversity was assessed using culture-independent methods with high-density 16S rRNA phylogenetic DNA microarrays. Bacterial plant isolates with the ability to inhibit the growth of *E. col* O157:H7 were also identified.

Results

- inoculated plants had higher cultivatable bacterial populations.
- total bacterial phyllosphere population sizes on Romaine lettuce differed over time during the 4 week field-trials and season of planting (Spring and Fall 2009). During the Spring 2009, bacterial populations also differed significantly depending on method of irrigation and exposure to *E. coli* O157:H7. Additional field studies evaluating the effects of season, time, and irrigation on the bacteria associated with Romaine lettuce are required to establish the dominant microbial patterns on plants after an *E. coli* contamination event.
- population sizes of the phyllosphere bacteria on plants were negatively (Spring) and positively (Fall) correlated with the detection of viable *E. coli* O157:H7 on plants. Additional field studies needed to determine the effects of indigenous populations on *E. coli* O157:H7.
- a limited culture-independent analysis of the phyllosphere microbiota indicated that some plants contain only a few bacterial species while others harbor highly diverse microbial communities with members that are not easily cultivated on standard laboratory culture media. On-going analyses are aimed at identifying correlations between microbial diversity patterns or specific organisms associated with *E. coli* O157:H7 persistence.
- field-grown Romaine harbors indigenous bacteria that are antagonistic towards the growth of virulent *E. coli* O157:H7. A total of 28 inhibitory isolates were identified. The roles and applications of these stains to control *E. coli* O157:H7 remains to be established.

Comparison of surrogate *E. coli* survival and epidemiology in the phyllosphere of diverse leafy green crops

Trevor Suslow, Ph.D. University of California, Davis

S. Koike University of California Cooperative Extension, Monterey County
R. Smith University of California Cooperative Extension, Monterey County
M. Cahn University of California Cooperative Extension, Monterey County

Surrogate *E. coli* strains (safe substitutes for pathogens) were identified and used to study aspects of contamination, spread of contaminants in-field and survival during production of up to fourteen different types and varieties of leafy greens used in Spring Mix salads. A separate field trial investigated the effect of nitrogen application levels on surrogate *E. coli* survival and growth. Also, a team of Cooperative Extension Advisors was formed to conduct detailed microbiological grid-analysis (a 'Rapid Response' team) of grower/handler fields identified as

positive for foodborne pathogens, to help identify and report risk factors from localized contamination events.

Results

Studies found:

- generic *E.coli* was found to be more robust than attenuated *E. coli* O157:H7;
- survival of *E. coli* was very low in the first trial attempt within a commercial field. The field trial location was not ideal from crop management, especially weed management, and limited results were obtained in the 2009 trials;
- detectable survival was effectively zero 18 and 48h post-inoculation and a second identical inoculation was made on Day 5;
- survival was highly variable among the different Spring Mix salad varieties;
- low initial survival following both sprays was attributed to warm windy weather immediately following applications.

Although differences were observed in log CFU decline from the initial inoculum delivered per gram, it was regarded as premature to ascribe any significance to the reliability of these data due to problems in irrigation uniformity, weed density and other trial management concerns. The usefulness of the data resides in the observation that even under these stress-inducing conditions, survival of the att*E. coli* O157:H7 was confirmed at 8 and 14 dpi.

Other results from studies and 'Rapid Response' events include:

- from approximately log 4.3 CFU/ml inoculum applied to Romaine plants from one to three times - no att*E. coli* O157:H7 colonies were observed by direct plating at any date for any treatment level;
- evidence for viable residual contamination of applied strains was detected on plants within 14 days of the initial contamination date (Fig. 5) but rarely after that date and not associated with levels of N-fertilization;
- nitrogen dose had no demonstrable or practical effect on detectable survival of att*E. coli* O157:H7 applied as a foliar spray (Fig. 5). Survival detection by enrichment revealed positive plants in populations of 360 individuals at 14 dpi but never beyond;
- survival was detected in plants treated close to harvest for only one of 360 plants among the various treatment replications receiving all three applications. The one positive plant composite was associated with the plots not receiving additional N-applications above field residuals.

Examination of the survival and internalization of *E. coli* on spinach under field production environments

Steven Koike — University of California Cooperative Extension, Monterey County

M. Cahn — University of California Cooperative Extension, Monterey County
T. Suslow — University of California, Davis

This project monitored spinach plots inoculated with either a generic or a non-toxigenic *E. coli* O157:H7 strain for survival of this organism under coastal California field production conditions. The ecology and dynamics of *E. coli* were evaluated in an agricultural setting. A second aspect was to examine the phenomenon of 'internalization,' which holds that a plant can absorb a human pathogen (*via* root uptake) and transport the pathogen to leaves that will be later consumed. Internalization studies have mostly dealt with leafy vegetables grown in pots under artificial conditions whereas this study examined commercial, field grown spinach to see if internalization in the field can occur.

Results

Various *E. coli* strains (mixtures of either rifampicin-resistant generic *E. coli* or rifampicin-resistant attenuated O157:H7 strains) applied as water-based sprays or mixed with sand and placed in mesh bags to simulate point sources of contamination did not survive in soil for long periods of time under commercial growing conditions in the Salinas Valley. Results included:

- spray or bag inoculum was not recovered, *via* direct plating, from the spinach plants growing through inoculated soil or next to bag inoculum;
- when mature spinach plants were spray inoculated and immediately disked into the soil, inoculated bacteria were recovered from field plots for over 85 days;
- when various *E. coli* strains were inoculated onto spinach roots by using a subsurface drip irrigation system, the above ground foliage did not test positive for the *E. coli* strains when using direct plating methods;
- surface sterilizing plants with mercuric chloride followed by enrichment culture resulted in only one of 80 whole plants being positive for the rifampicin-resistant generic *E. coli*.

The project also examined persistence in soil sprayed inoculum. Both the generic and attenuated O157:H7 *E. coli* rif strains, spray inoculated to soil at high rates (108 CFU/ml), post-seeding but pre-emergence, were recovered from the soil for relatively short periods of time (Fig.1).

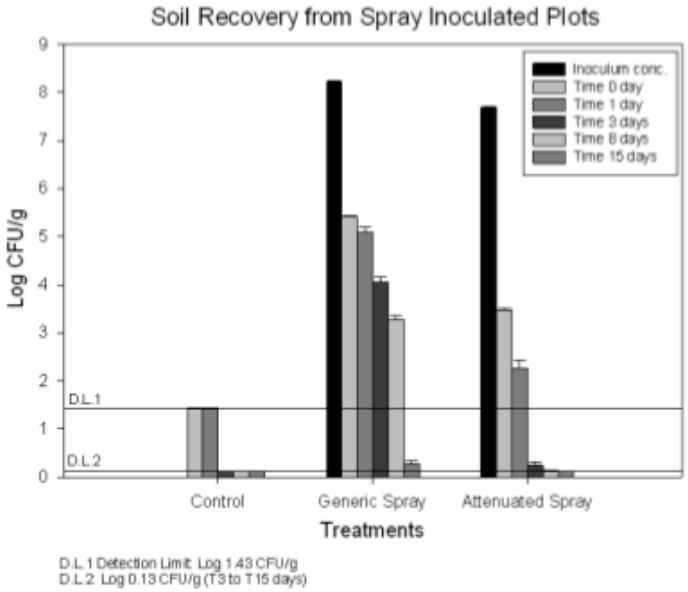

Fig. 1. Recovery of generic and attenuated O157:H7 *E. coli*^{rif} strains that were spray inoculated to soil after spinach was seeded but prior to seedling emergence. D. L. = detection limit.

Survival results show:

- the attenuated *E. coli* O157:H7 strain declined at a faster rate compared to the generic strain (Fig. 2).
- in general, a 100-fold (2 log) and 100,000-fold (5 log) reduction from 0 dpi to 8 dpi and 15 dpi, respectively, was observed for generic *E. coli* and a 5 log reduction from 0 dpi was encountered by 8 dpi for the attenuated *E. coli* O157:H7 strains.
- by one day after inoculation, all inoculated soil samples contained bacterial populations that were significantly lower than the original inoculum concentrations delivered to the soil surface.
- by 15 days after inoculation, recovery was below the detection limit by standard direct plating for both strains but the generic *E. coli* were still detectable following a centrifugation concentration enrichment.
- Generic or attenuated O157:H7 *E. coli* rif strains in uninoculated plots were not recovered, indicating that inter-plot contamination did not occur to a detectable level.

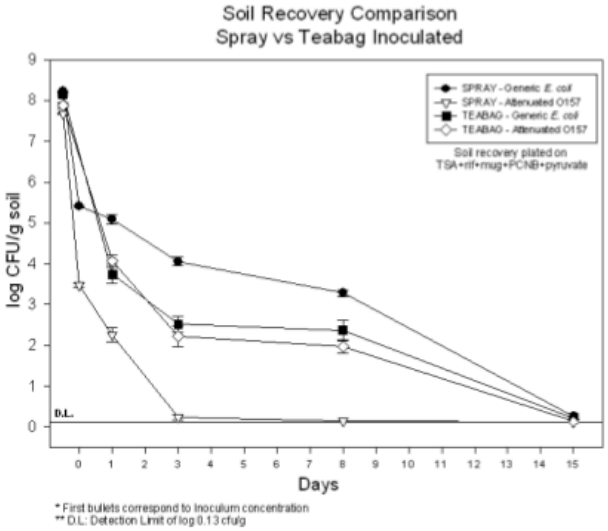

Fig. 2. Recovery over time of generic and attenuated O157:H7 *E. coli*^{rif} strains that were inoculated to soil as spray inoculum or inoculum in mesh bags. D. L. = detection limit.

Point source mesh bag inoculum

Both the generic and attenuated O157:H7 *E. coli* rif strains, inoculated to the soil by placing mesh bag inoculum on the tops of the beds post-seeding but pre-emergence, were recovered from soil adjacent to the bags (0 cm distance) for relatively short periods of time (Fig. 3).

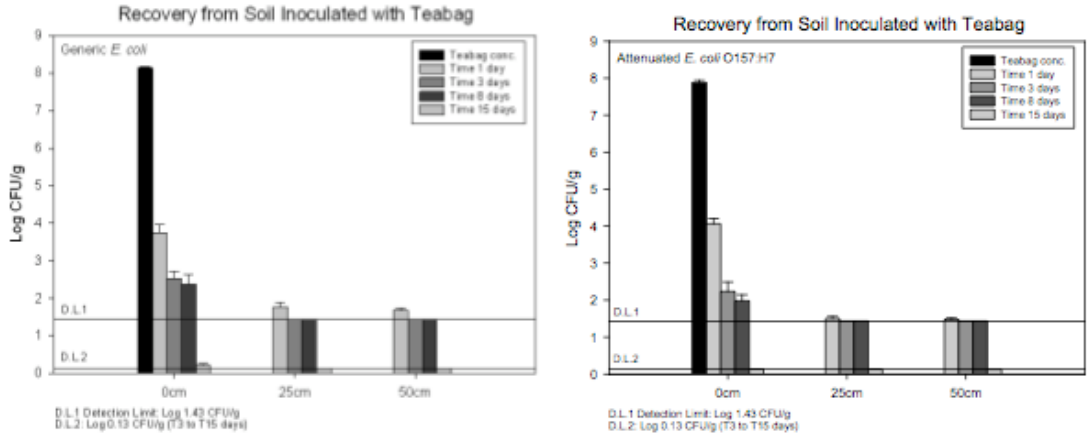

Fig. 3. Recovery of generic and attenuated O157:H7 *E. coli*^{rif} strains that were inoculated to soil by placing mesh bag inoculum onto bed tops after spinach was seeded but prior to emergence. Soil was taken adjacent to the bags (0 cm) and at 25 and 50 cm away. D. L. = detection limit.

Results show:

- by 3 dpi, all inoculated soil samples contained bacterial populations that were significantly lower than the 1 dpi recovered concentrations;
- by 15 dpi, recovery at 0 cm distance was below the standard direct plating detection limit for both strains but the generic *E. coli* were still detectable following a centrifugation concentration enrichment;
- the generic and attenuated O157:H7 strains declined at comparable rates (Fig. 2);
- there was no recovery of either generic or attenuated O157:H7 *E. coli* rif strains in uninoculated plots, indicating that inter-plot contamination did not occur to a detectable level.

For soil samples taken further away from the point source inoculum mesh bags:

- low populations of the generic strain were found on 1 dpi only, at both 25 and 50 cm distances (Fig. 3);
- after 1 dpi, no generic strains were recovered up to the end of experiment at 15 days;
- from the 25 and 50 cm distances, no attenuated strains were recovered at any time up to the end of experiment at 15 days.

Persistence on inoculated spinach plants

For all plant samples collected and tested by direct plating onto TSA amended medium, no generic or attenuated O157:H7 *E. coli* rif strains were recovered for any inoculum concentration or at any sample date.

Inoculum transported in irrigation runoff

Runoff irrigation water was collected from furrows on 3, 17, and 29 dpi. Using the Colilert/QuantiTray 2000 system for analysis of *E. coli* in these water samples, only those plots treated with sprayed generic *E. coli* on the soil surface had substantially higher populations of total *E. coli* on Day 3 as compared to background *E. coli* positives in non-sprayed plots.

Analysis of positive QuantiTray wells revealed that *E. coli* in runoff water from the generic sprayed and mesh bag plots were rifampicin-resistant while those recovered from non-treated plots were rifampicin-sensitive and therefore not the applied strains.

No further detail on these results was given.

Inoculating plants at different development stages

Controlled dose contamination was applied to emerged and developing spinach leaves at First True Leaf (FTL), FTL + 7 days, and FTL + 14 days. Results included:

- for plants treated at FTL stage, recovery from collected leaves was possible only from 1 of 3 and 2 of 3 composite samples taken from the 576 and 57,600 MPN/100 ml doses in Trial 1 (September 2009) and none recovered in Trial 2 (October 2009);
- within 2 weeks all applied bacteria were not detectable;
- for plants treated at FTL +7, only 1 of 3 samples yielded detectable populations from the 57,600 MPN/100 ml dose of generic *E. coli* in Trail 1 and 2;
- for plants treated at FTL + 14 days, *E. coli* O157:H7 rif strains were detected in 1 of 3 or 2 of 3 samples at the 235 and 576 or 57,600 MPN/100 ml dose, respectively in Trial 1 but were not detectable in Trial 2;
- At FTL+14, generic *E. coli* were not detectable in Trial 1 but recoverable in 3 of 3 and 1 of 3 samples from 5,760 and 57,600 MPN/100 ml doses, respectively.

Survival in soil

When generic and attenuated O157:H7 *E. coli* rif strains were inoculated onto the mature spinach crop and the plants incorporated (by disc plough) into the soil, both strains were recoverable from field soil for an extended period of time (Fig. 4). From the day of crop

incorporation (day 0) through 85 dpi, periodic sampling continued to recover both generic and attenuated strains (Fig. 4). The grower irrigated all plots on 88 dpi and re-disced the plots on 101 dpi. The 106 dpi soil sample showed that neither generic nor attenuated O157:H7 strains were recovered by direct plating.

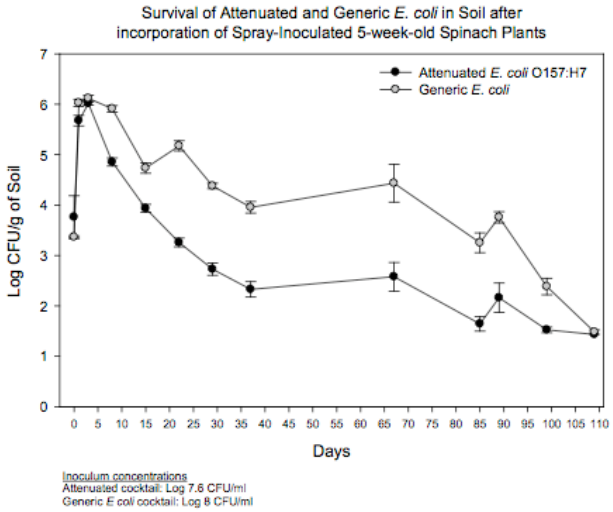

Fig. 4. Recovery over time of generic and attenuated O157:H7 *E. coli*^{rif} strains inoculated to spinach plants at crop maturity. Spinach was then disked and incorporated into the soil.

Inoculating roots and testing for internalization of *E. coli*

Bacterial inoculum was delivered to the spinach roots *via* a subsurface drip irrigation system. Inoculated water that was withdrawn directly from the subsurface drip tape tested positive for the respective strains. Results showed:

- irrigation water inoculated with generic *E. coli* resulted in a log 4.22/ ml contamination level and the attenuated *E. coli* O157:H7 inoculated water had a log 3.82/ ml level;
- when spinach foliage was tested by direct plating for either of the inoculated strains, no positive results were obtained;
- when plant tissues were processed in an enrichment step, only one sample tested positive for either strain (sample taken at 21 days from one of the generic *E. coli* replications);
- Soil collected adjacent to the subsurface drip tapes tested positive, by direct plating, for both generic and attenuated O157:H7 *E. coli* rif strains, confirming that viable inoculum was delivered to the subsurface soil area (Fig. 5).

Populations of both generic and attenuated O157:H7 *E. coli* rif strains declined rapidly over time. Generic or attenuated O157:H7 *E. coli* rif strains from water control plots (Fig. 5) were not recovered.

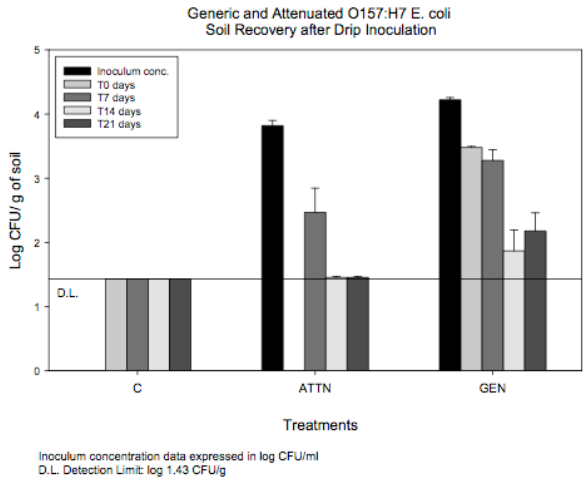

Fig. 5. Recovery of generic and attenuated O157:H7 *E. coli*^{nf} strains inoculated to subsurface soil and spinach roots by injecting inoculum into subsurface drip tape. D. L. = detection limit.

SESSION II: Enhanced testing methods for pathogens in produce

A high-throughput, culture-independent approach to identify an indicator species for *E. coli* 0157:H7 contamination

Gitta L. Coaker, Ph.D. University of California, Davis

J. Leveau University of California, Davis
T. Suslow University of California, Davis

The results from these molecular analyses to identify microbial indicator species include:

- the microbial population present on lettuce leaves is extremely diverse and 90-99.9% of the population is not culturable in the laboratory;
- a culture-independent approach is necessary to identify microbial populations associated with lettuce leaves;
- the pyrosequencing approach used was highly successful in identifying and quantifying bacteria associated with lettuce. The DNA sequence information obtained from pyrosequencing can be used to identify bacteria at the species level;
- there were lower levels of culturable bacterial populations present in Imperial and Yuma districts (inland, away from the coast) in the winter season than in Salinas during the spring and summer season;
- there were much higher levels of coliform bacteria present in Salinas than in Yuma and Imperial districts in the samples. Often, coliforms were present below the limit of detection in Yuma and Imperial samples;
- Coliform bacteria were more sensitive to environmental changes (higher humidity, higher temperatures) and their population size changed significantly across the summer season in Salinas. Therefore, select coliform species may hold significant promise as index/indicator organisms for *E. coli* O157:H7 contamination;
- The information obtained can be used to provide a benchmark for important bacteria associated with leafy greens.

Enhancing the effectiveness of human pathogen testing systems for the advancement of practical produce safety research and commercial management

Carol D'lima, Ph.D. University of California, Davis

T. Suslow University of California, Davis

This project's objectives were to:

1. refine and validate a novel, rapid method of screening diverse environmental and product samples for diverse pathogenic *E. coli*;
2. verify the function of a simple sample "banking" method to allow delayed pathogen testing;
3. apply the developed tools within a standardized scheme for investigation of natural contamination events of leafy greens.

To accomplish the first objective, diverse samples were inoculated with very low concentrations of human pathogenic *E. coli*. The highly specific DNA probe was verified to detect all *E. coli* O157:H7 and over 40 non-O157 types most associated with human illness. Sample DNA "banking" using a commercial system not requiring expensive freezing equipment, called FTA Cards, was effective with leafy greens for over two months with limited loss of detection efficiency. This technology will be used to more broadly secure grower permissions for farm access to conduct detailed grid-analysis of a crop, separated in time from marketing.

A large number of naturally contaminated samples, including irrigation water, animal fecal matter, manure-laden soil, compost and leafy greens field contamination events were tested during the project period. The probe for pathogenic *E. coli* proved useful for rapid molecular screening, confirmation tests, and mass screening of colonies for inclusion or exclusion to identify the source of the initial pathogen test reactions that suggested a commercial crop was contaminated.

A sensitive and specific molecular testing method for live Salmonella in produce

Beilei Ge, Ph.D Louisiana State University

John C. Beaulieu Ph.D. USDA – Agricultural Research Service

This project sought to:

1. design and optimize a Loop-Mediated Isothermal Amplification (LAMP) assay that targets Salmonella strains;
2. evaluate the sensitivity and specificity of the LAMP assay in detecting live Salmonella;
3. apply the assay in the detection of live Salmonella in experimentally contaminated produce items (shredded lettuce, baby spinach, sliced tomato, sprouts, and cantaloupe cubes) of various stages of maturity.

LAMP assays are less expensive than Polymerase Chain Reaction (PCR) assays – which can also be fast but require considerable skill and more expensive equipment. LAMP assays can target a specific sequence of a conserved gene from the target organism, and, coupled with use of propidium monoazide (PMA) that binds to dead and extracellular DNA can be used for accurate detection of live Salmonella on a produce sample.

This project successfully developed and tested a LAMP Assay with high specificity for Salmonella, evaluating a number of primer sets for speed, sensitivity, reproducibility, and quantitative potential.

SESSION III: Potential vectors for pathogen transfer during field production

Fly reservoirs of *E. coli* O157:H7 and their role in contamination of leafy greens

Astri Wayadande, Ph.D. Oklahoma State University

Justin Talley, Ph.D. Oklahoma State University

Filth flies can be mechanical vectors of several human pathogens. They are known to carry *E.*

coli, Salmonella spp, Shigella, and Campylobacter spp. to humans *via* prepared foods or by contamination of surfaces. It is not known, however, what role, if any, filth flies play in contamination of pre-harvest leafy greens.

In this study, flies were collected from seven areas in California in and near leafy greens production areas adjacent to animal production facilities and tested for the presence of *E. coli* O157:H7 by culturing on selective media. The results were:

- less than 1% of flies captured in the Salinas valley were positive when isolated on sorbitol MacConkey plates and serological confirmation;
- over 90% of the flies captured in the Imperial Valley were positive when tested by PCR.

The project also investigated *E. coli* O157:H7 colonization of the spinach phyllosphere after regurgitation of house fly vomitus. Results included:

- fly regurgitation spots were observed by scanning electron microscopy for evidence of bacterial attachment and growth over time;
- fly regurgitation spots from flies that acquired bacteria from bacterial lawns had numerous bacteria-like organisms attached to plant cell surfaces whereas very few organisms were observed for negative control fly spots;
- fly regurgitation spots from flies that acquired bacteria from manure-culture slurries had few bacteria-like organisms, but this was highly variable among spots;
- regurgitation spots examined one week after deposition had many more bacteria-like organisms and there was evidence of bacterial replication on the leaf surface.

These data, though not conclusive, suggest that *E. coli* O157:H7 survive in the gut of flies and can colonize the spinach phyllosphere after regurgitation under laboratory conditions.

Researchers also noted there was an association with aphid infestation in the crops - as flies are attracted to the volatiles and feed on honeydew.

Food safety risks associated with sheep grazing in vegetable stubble fields

Bruce R. Hoar, D.V.M. Ph.D University of California, Davis

Mark Trent University of California Cooperative Extension, Monterey County

The objectives of this project were to:

1. estimate the prevalence of fecal shedding of *E. coli* O157:H7 and Salmonella spp. by sheep grazing in different crop systems, plus measure the intensity of fecal shedding of commensal *E. coli* (*E. coli* spp not considered pathogenic) to support objective (3);
2. determine if rotational grazing between crop systems of differing forage quality and energy content alters the prevalence of fecal shedding of *E. coli* O157:H7 and Salmonella spp. by sheep;
3. measure the rate of inactivation of *E. coli* O157:H7 and Salmonella spp. as a function of parameters such as time, tillage practice, irrigation, ambient temperature, etc, and compare these estimates to the fate of commensal *E. coli*.

Initial research found that the prevalence of *E. coli* O157:H7 was relatively low amongst the sheep populations and locations in the Imperial Valley area surveyed. A total of 19 unique "bands" of sheep were sampled on 14 separate sampling dates - sheep generally not present in the Imperial Valley all year. 15 additional bands were identified and each sampled once, such that researchers estimate that approximately 28,000 sheep were in the population from which samples were obtained. Results were:

- samples were found positive for Salmonella on 6 of the14 sampling dates;
- no fecal samples were positive for *E. coli* O157:H7. Concern that this might be associated with pooling of samples resulting in lower detection sensitivity led to a trial where "spiked"

fecal samples with known numbers of *E. coli* O157:H7 were applied and then culture methods used to determine at what level the organism could be recovered. When 5 bacteria were placed into 10 g of sheep feces, the method would call the sample "positive" in greater than 90% of replicates;

- commensal *E. coli* was present at varying levels, ranging from 5×10^5 cfu/g feces to > 1×10^{10} cfu/g feces.

Objective 2 *was* not achieved for a number of reasons. The prevalence of shedding for *E. coli* O157:H7 and Salmonella spp. was too low to analyze any possible risk factors / associations that may have been present. Also, unlike previous winters, sheep were grazed entirely on alfalfa for the entire winter (with the exception of one week of sampling where Bermuda grass was grazed). Since there was no variability in the crop system used, it could not be determined whether forage quality or energy content would affect shedding of either pathogens or commensal *E. coli*.

Objective 3 results were restricted to summarizing for commensal *E. coli*, since pathogens were rare. Studies indicated that commensal *E. coli* could be recovered from soil for less than 5 days under field conditions. Since all the grazing was of alfalfa, no tilling was performed.

Low prevalence of pathogens meant that certain data analyses could not be performed, such as risks for shedding. Feed-lotted sheep have a very high prevalence of *E. coli* O157:H7 – up to 60%. In this study, expected variation in the crops utilized by the sheep for grazing did not occur and sheep were almost exclusively grazed on alfalfa. An unusually "wet" winter also affected the ability to collect samples.

Anecdotal reports from the industry are that sheep herd sizes have dropped by 2/3rds in the Imperial Valley due to food safety concerns with alfalfa.

Environmental effects on the growth or survival of stress-adapted Escherichia coli 0157:H7 and Salmonella spp. in compost

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Raw or inadequately composted animal manure is a potential source of pre-harvest contamination of fresh produce. Composting can inactivate human pathogens, but the outcome can be affected by many environmental factors. There is a need for developing composting guidelines and standards to apply to a wide range of conditions.

An extended mesophilic composting phase may induce a heat-shock response in human pathogens, which become resistant to the subsequent lethal temperatures during the thermophilic composting phase. Other sub-optimal conditions, such as slow heat-up of compost, low moisture contents and carbon-to-nitrogen ratio (C:N), may also enhance the survival of stress-adapted human pathogens during composting. Improvements are needed in the sensitivity of pathogen detection in compost. One potential method is to use bacteriophages to suppress indigenous microflora and the Pathatrix® system to concentrate the target pathogens from enrichment cultures.

The objectives of this project were to:

1. determine the thermal resistance of stress-adapted *E. coli* O157:H7 and Salmonella spp. in various types of compost at elevated composting temperatures in a humidity chamber by simulating early stages of on-farm composting;
2. apply competitive exclusion microorganisms as secondary treatment to eliminate the regrowth of stress-adapted pathogens in cured compost;
3. improve the sensitivity of pathogen detection from compost by combing bacteriophage enrichment and Pathatrix® detection system.

Results from these studies consistently demonstrated that:

- fresh compost with 40% moisture supported better survival of enteric pathogens than the compost with 50% moisture during composting;
- 'come-up' time (compost temperature increase time) was one of the most critical factors with longer survival being observed for the compost which simulated slow heating process (5 days come-up time) than the one with normal temperature rise (2 days come-up time), regardless of the moisture level and C:N ratio;
- thermal inactivation data for 16 out of 20 experimental series fit well into the mixed Weibull model, which in turn can predict the inactivation of *E. coli* O157:H7 during early stage of on-farm composting in previous trials.

Both plate count and modeling results suggested that microbial populations become adapted to the composting temperatures when the temperature rise during 'come-up' time is slow or the composting is conducted under suboptimal conditions. Composting processes need to be closely monitored to ensure a rapid temperature rise (~ 2 days) during the early phase of composting.

Results for studies to apply competitive exclusion (CE) cultures demonstrated that:

- finished compost can contain sufficient nutrients for a few pathogenic cells to grow under certain conditions such as warm temperatures (≥22^{o}C) and water activity maintained ≥$_{aw}$0.97;
- levels and types of indigenous microorganisms play an important role for controlling pathogen growth in the compost;
- for the dairy compost, the minimal level of 6.5 log CFU indigenous microflora/g was required to suppress the growth of *E. coli* O157 and Salmonella spp.;
- applying the competitive exclusion microorganisms into the compost reduced the growth potential of *E. coli* O157 and Salmonella spp., ranging from 10 to 2,000 fold compared with controls.

Results also revealed that the types of organic fertilizer inputs determine if the enteric pathogens can grow during storage. It is important for compost to be stored in a dry condition, maintain a sufficient level of natural microbial flora or add competitive exclusion (CE) cultures in the compost to keep the pathogens from growing, especially during warm seasons.

Bacteriophage cocktails specific for background bacteria in compost were isolated and characterized for the third project objective.

The two-step application of bacteriophages (phage) to the enrichment culture resulted in the increased detection of *E. coli* O157:H7 and the reduction of interfering background microorganisms on the selective agar. Although the phage cocktail does not greatly reduce background populations during enrichment, there seems to be some inhibitory effects that are allowing *E. coli* O157:H7 to grow better during 4-6 h enrichment. Considering that the Pathatrix® procedure is designed to be followed by PCR, a four-fold increase in cell numbers during enrichment can enhance the ability to detect pathogenic bacteria. Since the microbial isolates can be obtained easily, those new isolates can be used to propagate better phage cocktails to target those indigenous microorganisms unique to compost and manure. The use of phage cocktail enrichments to inhibit background interference, thus allowing the target pathogen to grow, is a method with potential.

Minimizing pathogen transference during lettuce harvesting by optimizing the design of the harvesting device and operation practices

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The objective of these studies were to:

1. determine pathogen levels required for pathogen transference to the edible portions of lettuce *via* contaminated coring knives;
2. reduce the risk of coring knife pathogen transference by developing improved coring knife design and sanitation procedures;
3. eliminate the potential for coring knife contamination via soil contact by separating the cutting and coring process;
4. identify post-harvest handling practices that can be used to effectively manage the potential food safety risks during coring-in-field (CIF).

The level of pathogens transferred from soil to harvested lettuce were found to be a function of:

- pathogen concentration in the soil;
- amount of soil present on the harvested lettuce, which are then impacted by a number of factors including lettuce growing and harvesting conditions;
- CIF lettuce harvesting methods;
- knife design and disinfection.

Optimizing harvesting practice and improving harvest knife design and disinfection can significantly minimize this potential risk.

The cutting blade and coring ring of a CIF harvest knife play significantly different roles in pathogen contamination and transference from soil to lettuce. CIF harvesting practice observed in California showed that the cutting blade has higher potential to be contaminated by the soil, but less opportunity to transfer pathogens onto harvested lettuce. However, while the coring ring has less potential to be contaminated by soil, if contaminated, it has a higher potential to transfer pathogens onto the harvested lettuce.

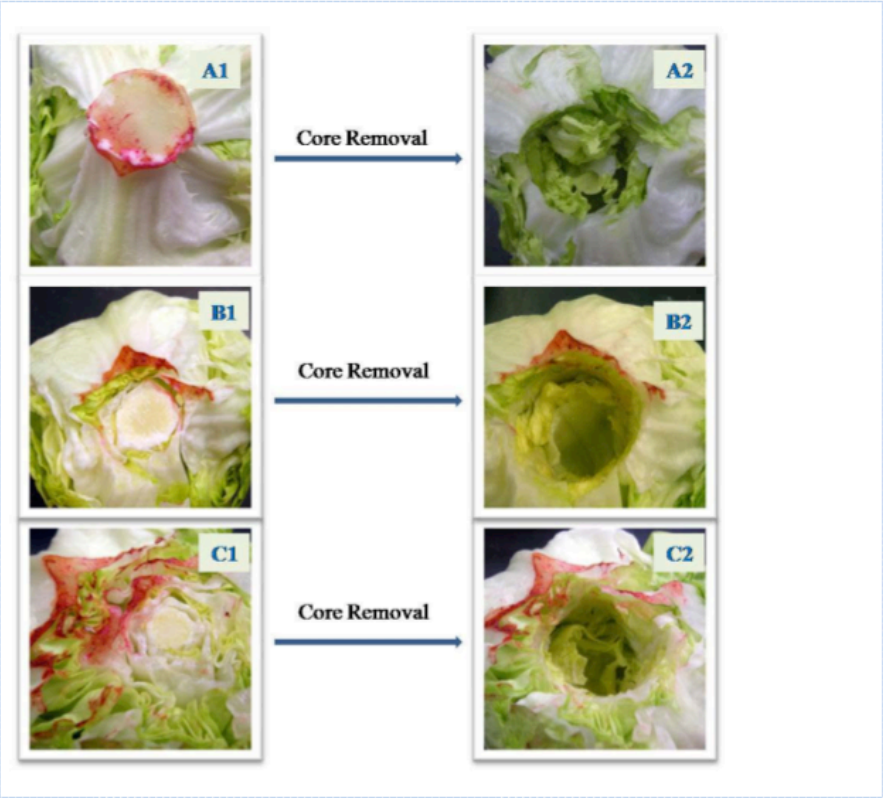

Figure 5. The impact of blade-to-lettuce contact on the transference of contaminants to the harvested lettuce. The blade was stained with red dye.

A. When the blade only contacts the stem (the waste), the contaminants will likely be removed during core removal.
B. When blade contacts both the stem and some amount of lettuce tissues located outside of the coring path, contaminants will remain on the harvested lettuce,
C. When blade contacts an extensive amount of lettuce tissue located outside the ring path, the majority of the contaminants will remain on the harvested lettuce.

The California Leafy Green Marketing Agreement calls for attention to CIF harvesting to minimize pathogen transfer; yet, detailed information is needed as to how to minimize pathogen contamination from soil during harvesting/coring operations. Since the cutting blade is used to cut lettuce off the stem that touches the ground, it is important to minimize the potential for the cutting blade to contact soil whenever possible. Since the blade-to-soil contact may be inevitable at some point during harvesting under the current CIF practice, avoiding contact between the blade and the edible portions of the lettuce plays a vital role in minimizing pathogen transfer.

Field observations of the current California CIF harvesting practices, and random samples received from California indicate that most harvesters in California (at least those harvesting for leading fresh-cut processors) are being careful to minimize contact between blade and soil, and between blade and harvested lettuce. Evaluation of random samples received from Florida and Mexico suggests that CIF harvesting in these regions needs improvement. A cutting blade that touches soils should NOT be used to touch the edible portions of the lettuce.

Harvesting CIF lettuce in a wet field (due to rain, irrigation etc.) represents a greater challenge to minimize transfer of soil to harvested lettuce. Extreme care is needed to avoid harvest knife contact between soil, and lettuce, if CIF lettuce has to be harvested under this condition.

Harvest knives widely used in the industry also need improvement. The rough weld between the cylindrical ring and its shaft harbors pathogens and is difficult to disinfect. The newly designed knife prototypes harbored fewer pathogens and were easier to disinfect.

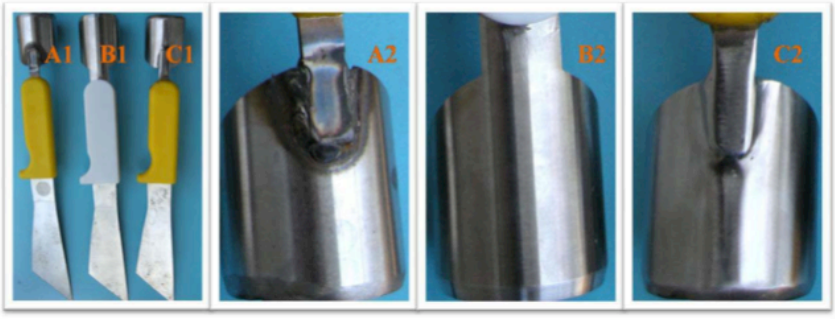

Figure 10. Development of prototype coring knife with improved food safety feature.
A1: Coring knife currently widely used in the industry. A2: Enlarged image showing the rough welding of the coring ring
B1: Prototype 1 – No-joint (one piece) design; B2: enlarged image showing no welding point
C1: Prototype 2. Current coring knife with rough welding point polished; C2: enlarged image showing the smooth welding pint.

The ability of ultrasound and surfactant to enhance chlorine disinfection efficacy was tested. While surfactant did not show any improvement on pathogen reduction, ultrasound improved chlorine efficacy at low concentration for pathogen reduction on the roughly welded surface.

Summary and Implications for Australian Food Safety

The decline in *E. coli* populations is rapid after soil and plants are inoculated. *E.coli* survival on Romaine lettuce and salad greens is low, with the majority of the population on the leaves or aerial plant parts (the phyllosphere) dying within days of inoculation and only small numbers surviving up to 35 days. Survival rates are variable across the crop with uniform contamination not equal to uniform survival.

Pre-emergence soil inoculation or inoculation of the growing crop *via* drip irrigation demonstrated that no soil-to-plant transfer of *E. coli* occurred. With only 1 in 80 samples of leaves showing any culturable *E.coli* (after enrichment), these studies suggest it is unlikely that field-grown spinach can take up *E.coli* via the roots and transport it within the plant vascular system.

Results also showed that although *E. coli* inoculum applied to the soil did not survive for 15 days, when the inoculum was applied to crop leaves and the crop ploughed into the soil, *E.coli* could be retrieved for 85 dpi until such soil was irrigated and re-ploughed. This suggests that *E.coli* may be able to survive in the soil for longer when available water and nutrient remain from crop trash.

Animal feces and simulated feces in experiments demonstrated that although these can cause local high levels of pathogens near the feces, pathogen populations decline rapidly in time and distance from the immediate impact site. Only the generic *E.coli* strain could be detected at 25cm and 50cm on 1 dpi and not thereafter. By 15 dpi only the use of concentration enrichment techniques could detect any strains of *E.coli* remaining at 0 distance. The current California Leafy Greens Marketing Agreement requires a 5 feet 'zone of no harvest' around evidence of animal intrusion or feces and this was deemed appropriate (if not a little excessive) in light of these results.

Initial work on the leaf microbiota has shown that up to 100 million microbes may inhabit a single leaf and the diversity and type of microbiota may siginificantly influence the survival of *E.coli* and potentially other food safety pathogens contaminating a crop. Although in their early stages, the molecular-based lines of inquiry promise many new insights into competition amongst microbiota within a crop. Inexpensive, rapid and accurate detection methods such as the LAMP assay for *E.coli* plus other research tools for identifying microbiota associated with *E.coli* that may used as indicators, will reveal new information and techniques for managing the risk of contamination and bring new perspectives in understanding microbial diversity and dynamics in crops.

Studies of nitrogen dose response showed no effect on *E.coli* survival, despite other researchers reporting such effects.

Studies on flies and sheep as vectors for *E.coli* contamination showed that it is possible but that the prevalence varied based on source, conditions and practices.

Compost standards may require review as the evidence suggests that the process control parameters may not be sufficient to ensure pathogens are destroyed. Results vary based on compost inputs, initial pathogen loads and process management. Pathogen heat-shock responses in compost with a slow mesophilic stage may enable survival and regrowth. New approaches using competitive exclusion organisms and bacteriophages promise improved food safety outcomes.

Re-design of tools such as the lettuce cutting and coring knife can significantly reduce the risk of pathogen transfer in-field.

Testing approaches and sample size can be misleading. Small pre-harvest leaf-mass samples of 25g can easily miss contamination in the field.

Produce Safety – Research Priorities

The Produce Safety - Research Priorities workshop was convened following the Produce Research Symposium to discuss the key issues and directions needed for further research effort and investment by the Center for Produce Safety.

The workshop was structured around 5 key theme areas:

- Composts and soil amendments
- Water (Field and processing)
- Co-management for food safety and the environment
- Worker health and hygiene
- Data mining

Selected 'Overview Speakers' provided an introduction for each theme, followed by a discussion amongst panelists and workshop participants. The discussion sessions were facilitated by a session moderator and an expert panel of industry, research and funding agency representatives.

The following summary notes the key issues raised and priorities identified from each of the themed sessions. Many of these recorded points are questions raised that need further research, improved communication or improved industry adoption to resolve.

Composts & Soil Amendments

Control parameters of mature compost process to ensure safety (process control)

The validity of process control parameters/critical limits specified for composts was extensively questioned regarding its adequacy for ensuring that human pathogens of concern for food safety were controlled. Discussions identified the need to:

- conduct further validation studies in terms of composting times and temperatures to ensure process control for target pathogens, emerging pathogens and the most heat resistant organisms is achieved;
- evaluate current control parameter effectiveness with respect to the types of raw material compost inputs and their pathogen loads – validate by feedstock/manure, raw materials, green waste, food waste, etc,.;
- establish process control requirements based on the most resistant pathogens at levels reasonably likely to occur in materials used for composts;
- evaluate if current control parameters monitored are adequate for food safety e.g. are C:N ratios, moisture levels and time/temperature enough? Need to validate other parameters that may impact on outcome, such as particle size, bulking agent addition, aeration, NH4, pH, raw materials, natural microflora (microbial community) of manure, age of manure, etc,. for their effects on kill times;
- identify the potential curing period requirements – e.g. times, temperatures, competitive exclusion (biocontrol);
- evaluate potential additional treatments for highly contaminated incoming raw materials;
- establish controls for covering of compost piles/windrows, the aeration influence of weather and agro-ecological regions on the safety of composts;
- manage the microbial community dynamics during composting processes;
- manage potential regrowth/recontamination/cross contamination of pathogens in composts;
- identify if biosolids can be safely used in some circumstances.

The validity of end product microbial criteria/testing standards was also discussed with respect to the adequacy of current specifications/standards. Issues identified included:

- whether the use of *E. coli*/fecal coliforms were the appropriate indicators (e.g. does an *E. coli* kill = a Salmonella kill?) plus other detection methods and microbial ecology as indicators for process verification;
- can stress-adapted pathogens recover (heat-shock responses of pathogens exposed to sub-lethal temperatures may survive the 55^{o}C control temperature parameter);
- whether soluble carbon levels (available carbon needed for pathogen regrowth) also could be used as an indicator of safety for composts;
- investigating the validity and use of alternative indicators to determine if composting has been successful, (e.g. bio-assays such as grow-out of weed seeds from composted material to determine if temperature kill has been successful).

Other issues identified for further investigation to do with composts and amendments in relation to their use in proximity to harvest date of fresh produce included:

- determining the fate of pathogens in compost when applied in the field, such as identifying the influence of soil types (including soil microflora/communities), soluble carbon and use of abiotic/biotic metrics to determine fate of pathogens;
- identifying the potential pathogen transfer pathways from compost to crops in the field – what potential mechanisms exist other than *via* root or surface contact?;
- need for national commercial standards based on risk ranking by crop and use. Potential to use a risk rank by raw materials used?;
- identifying best practices by agro-economical region – survival of pathogens may be significantly different depending on the parameters of the environment in which they are used?;
- need for a HACCP-based approach.

Water (Field and Processing)

Workshop discussions focused on issues concerning the risk potential *versus* risk exposure and if current commercial tests based on generic *E. coli* are adequate. The risk potential for water is considered to be 'very high' and the issues identified included:

- how consistent is the methodology for establishing compliance/non-compliance (e.g. test kits, assay temperatures plus other assay conditions);
- what is the optimal sample volume and sampling protocol? How does water movement over landscapes affect sampling efficacy?;
- should there be separate approaches and standards to testing drip *versus* overhead irrigation water?; Based on CPS research that indicates the lack of pathogen transfer from water to edible portion without direct contact - is there sufficient evidence yet to eliminate metrics requirements for drip irrigators?;
- how adequate and affordable is the methodology for detecting and enumerating live, dead, viable-but-non-culturable pathogens and other members of the microbial communities?;
- are current predictive indicators for microbiological produce safety reliable - water standards are based on EPA studies related to safe recreational water uses;
- Salmonella and viruses are often under-represented in assays – do we need better indicators and risk factor assessments for these pathogens?;
- how valid are the methodologies for water testing in different regions? How do climate factors and environmental events interfere with testing outcomes?;
- does biodiversity around water sources cause contamination? (Trevor Suslow (unpublished data) stated his monitoring of 19 on-farm reservoirs found biodiverse water sources were always better);
- need industry data to be made available for inclusion into a meta-analysis.

Intervention and Mitigation Technologies

- how reliable are various copper, UV-filtration, chlorine dioxide, $CaOCl_2$, etc., on-farm tests and validations - especially for disinfection of the high-burden waters. What issues need to be addressed with dose monitoring?;
- is there the potential to bridge the scale issues - link commodity-specific technologies with watershed ecology of pathogens;
- potential for biological interventions;
- need to define approaches for disrupting attachment of pathogens to fresh produce. Postharvest treatment tanks need agitation to disrupt microbes attached to produce surfaces so that sanitisers can kill microbes in suspension;
- accessibility of the '–cides' to pathogens within biofilms in water delivery systems, in protected niches on produce, within biofilms and effectiveness of controlling biofilms (potential reservoirs for pathogens);
- what are the limitations to the efficacy of wash-water treatment for produce? Is there the possibility to develop an alternative/ultimate 'kill step' as wash water is never likely to provide this;
- social issues remain a challenge with effecting change and acceptance of new practices;
- audit risk criteria needed: e.g. interactions between source of water, mode of irrigation, crop traits and crop development stage – define the linkages between the presence of pathogens in water and presence/level in crops;
- meaningful science-based water standards are needed plus cost-effective sampling methods.

Post-harvest treatment of water

- need better information so that industry can make informed choices of chemicals, implementation technologies and their adequacy for target pathogens;
- 'hurdle technologies' needed (combining chemical and physical methods for produce sanitation).

Co-Management of Safety and Environment

Following the *E. coli* in spinach outbreak in 2006 some farmers in the industry were alleged to have illegally cleared remnant vegetation in proximity to production sites in order to minimize the incursion of wildlife, had culled wildlife or had installed barriers that cut through wildlife corridors. The environmental impact of food safety management practices has therefore brought a new focus onto the co-management of these issues in the USA.

The following identifies a significant number of unresolved questions and issues for co-management that the workshop participants regarded as needing further research or discussion at industry and regulatory agency level. Issues included:

- are there different food safety risk profiles associated with different wildlife?;
- what are the most important contributing factors in pathogen occurrence in wildlife?;
- what are the transport mechanisms for food safety pathogens from wildlife to produce?;
- what are the relative importance of prevalence and level of pathogens amongst wildlife?;
- why are animals coming into fields? What is attracting and what might deter them? What can industry do better for sustainable wildlife management around production sites?;
- what non-crop vegetation management should be expected?;
- water body management, especially tail water management, is needed to prevent/minimize pathogen contamination of irrigation waters and how significant is the risk from animals going to tail water reservoirs leading to pathogen transfer onto crops?;
- how much data is necessary to convince us that any particular animal species does not represent a risk?;

- are there thresholds for contamination that cause concern and how do we determine such thresholds? i.e. how do we determine the probability of a problem?;
- Is it possible to manage birds to reduce the risks associated with birds as vectors of pathogens?;
- Can we use existing data to feed into risk models to better manage wildlife?;
- Are some sites more attractive than others? Why? What are the factors that attract the birds and what are the factors that contribute to the fecal contamination among populations of birds?;
- can appropriate buffer distances regarding wildlife and domestic animals be defined?;
- do we need additional survey data to determine the impact of food safety interventions on environmental goals and can we quantify ecological impacts of individual interventions on farms?;
- what are the cumulative environmental effects of many small impacts on wildlife habitats?
- What are the acute and chronic environmental effects of habitat removal, use of poison bait and other food safety interventions?;
- do we need further research into the tools that we use to evaluate the impact of food safety interventions on habitat?;
- research has shown that pathogens can survive up to 9 months in sediments - how long does sediment have to be held before spreading it on fields? The sediment is subject to water quality laws;
- studies on a landscape scale rather than individual farm scale would be useful;
- what specialties should be involved on research teams to evaluate co-management issues?;
- can Qualitative Risk Analysis (QRA) be used to evaluate effect of wildlife on water quality?;
- what is happening in terms of habitat damage and why is it happening? Is it due to misunderstanding or misinterpretation?;
- should audits include 'not-to-exceed' statements?;
- are there other major reservoirs of EHEC other than cattle?;
- what is the impact of pathogen load in wildlife/environment compared to pathogen incidence on produce?;
- do pathogens move among herds of wild and feral animals and if so, how?;
- what is the role of strict liability in farmer co-management practices?;
- is there a form of consumer education that can mitigate this issue?;
- there is a need to establish achievable standards that recognize that zero tolerance of wildlife is difficult/impossible to meet;
- can postharvest tools be better used to reduce risks?;
- are we taking enough samples to effectively detect incidence of pathogens in the environment?;
- what is the level beyond which contamination is unacceptable from a public health point of view?;
- how do sampling schemes relate to the prevalence of pathogens in a field? How can we relate prevalence to food borne illness outbreaks?;
- does research in the central coast (California) represent a unique situation or can it be extrapolated to other agro-ecological regions?;
- what research should be done in regions outside the central coast and how does it differ from central coast research?;
- what other pathogens should we be considering that we are not currently tracking?;

- can vegetated strips and canals be constructed and managed to better sequester pathogens in the environment?

Worker Health & Hygiene

The USA has approximately 3 million farm workers (2 million are family members and 1 million are hired). Of the 1 million hired workers 50% are settled, 20% are 'shuttler' (itinerant) workers, 12% are migrants and 15% are 'newcomers' to the farm labor force. Hired farm workers are a greater risk of spreading pathogens because of poor health and food safety and hygiene knowledge and skills.

Significant challenges are encountered with language, literacy and cultural issues when attempting to communicate food safety, health and hygiene messages and requirements. Issues raised in this session that require further research or improved communication of the successful strategies for managing worker health and hygiene include:

- are there better ways to "reach" workers with health and hygiene training and change behavior? These need to be tailored to the culture, language and educational level of the audience;
- industry find it difficult to communicate messages to workers with low literacy and language skills - especially for a "risk" that workers cannot see with their own eyes and do not understand. Many farmers are uncomfortable or lack confidence in being the 'teacher' for worker health and hygiene, particularly when there are cultural and language issues involved;
- health and hygiene education should be tailored to address the needs that exist, be results based and reinforced through multiple outlets/channels;
- are "worker health" expectations scalable to different sized operations?;
- there is a need to evaluate the efficacy of glove use in preventing pathogen transfer from worker to produce;
- need to determine best management practices BMP for teaching and training workers to model appropriate food safety behaviors and evaluate the effectiveness of training programs and the trainers;
- industry need to develop a better understanding of the health status of farm workers and extend this back to home life practices. Poverty is the driving cause for most of the poor health outcomes of workers;
- what is the role /effectiveness of hand sanitiser use?;
- what role does hand grooming play in regards to transfer of pathogens from workers o produce? e.g. some workers grow and maintain their finger nails ('little pinky') for use as a tool for cutting, grabbing and/or digging;
- there have been no recorded outbreaks from Shigella or Hepatatis from US-grown produce since 2003 - is this evidence of improved practices across the industry? Need to evaluate the broader impacts of change in industry practices on food safety outcomes.
- most human-to-produce contamination occurs post-farm gate, not from agricultural workers.

Data Mining

The management and sharing of the significant and escalating quantity of produce, water and soil testing data generated by industry, academia and regulatory agencies continues unresolved due to concerns about confidentiality and potential legal and financial penalties on industry.

Key issues and discussion in this session focused on questions of how this data could be better managed and accessed for research purposes, to extract more meaningful information that industry and regulators could use. Key points raised included:

- the existing data sets include: seed, water, soil amendments, product testing, in-process tests, receiving samples, finished products and research sampling;
- the drivers behind testing are customer/buyer demand, providing consumer confidence, process verification, lot-acceptance criteria and surveillance to increase knowledge of pathogens in the product/processes;
- indicator organisms and pathogens tested by producers vary, with sampling methods different depending on who is testing and where. Testing varies by company or sometimes even within a company if they have multiple locations.
- can product testing data be used to develop risk models for products and agro-ecological regions? How and when should testing be conducted and how do we sample?;
- confidentiality is a paramount concern, but perhaps one of the most simple to accomplish. There are a number of mechanisms to blind data if a universal format for sharing data could be developed? Need to develop a format, repository, and rules about who would manage it/owns the data and ensure its availability to all stakeholders
- industry need to be able to share data without eliciting a regulatory response because of adverse results. Should there be a 'safe harbor' if you supply data that is subsequently available to regulatory agencies?
- How do you compare analytical methodologies? How do you compare historical data when the testing methods change so rapidly? Need a clear transparency of methods being used to give perspective.
- audit data can also be valuable. How do we use this type of data for training and education programs?;
- Can we compare methods used in product testing and the historical actions we have taken as an industry? What have our actions been and what has worked? What are the basic protocols?
- How should we sample? What are the sample sizes?
- pathogen prevalence in produce or water samples is usually so low, how do we develop measures that let us measure any improvement in food safety practices?;
- the presence of pathogens is not necessarily the same as the risk of illness. What data needs to be collected to support this assertion? What knowledge gaps do we have concerning these relationships and how do we address them?;

Food and Drug Administration data

- FDA data is biased as they mostly test where they suspect there is a problem. FDA data can be obtained via FOIA (Freedom Of Information) but they cannot share data with the industry until an investigation in concluded. FDA data collected for research purposes can be shared;
- how does the industry share data with FDA? - publish the data in a peer-reviewed journal? This data can then be cited and used in rule making. Industry could potentially collect data and provide it to a third party and have them 'blind' it before giving it to FDA;
- FDA cannot get the diversity of data the industry can. FDA need raw industry data with contextual information (summaries do not help) and data needs to be presented in a way that FDA can use to craft rules. Note: there are so few positive results for pathogens among the quantity of tests conducted;
- if industry data could be used to identify control points and action levels for specific scenarios we need to identify the appropriate parameters to use when collecting and reporting data.
- USDA NASS could develop a confidential survey that can be used as a mechanism to collect 'confidential' data.

Profile of Research Organisations and Funding - USA

Representatives from US research funding agencies and organisations in attendance were asked to provide a brief overview of their programs and opportunities for fresh produce food safety research and extension funding.

USDA - Key Organizational Contact Points

- Fruit and Vegetable Advisory Committee
- Specialty Crop Subcommittee of National Agricultural Research, Extension, Education and Economics Advisory Board
- Research Program Leaders and Directors

USDA Agriculture and Food Research Initiative (AFRI) Food Safety Program

- Food Safety Challenge Research Funding Applications (RFA) $20 Million – 2011 Release of next RFA
- Foundation RFA – $9 Million – funds basic research
- Fellow's RFAs. Other programs - more specific threats - emerging threats, e.g. CAPS Grants & educational efforts
- Setting research priorities is both formal and informal. Stakeholder listening sessions. 5 minute presentations. In addition, each RFA asks for input. The informal process happens in meetings throughout the year. Key to our process: Communicate with national program leaders and project directors – they appreciate feedback.
- Engage Program Leaders while the RFAs are being developed e.g. 2011 CAP Grant RFA development will begin within next 6 weeks.
- Challenges. SCRI 100% match. Requirement for integrated proposals, i.e. integration of extension/educational component. Congressional requirements re: the type of researcher who can apply for the grant (govt. vs. university vs. private) that may limit pool of qualified researchers.

USDA National Integrated Food Safety Initiative (NIFSI)

- $15 Million – Education and Extension/ Intervention Based. 100-140 proposals annually; 35-40 grants issued. Special emphasis areas including fresh produce food safety identified here.
- Interagency Collaboration. NIFSI annually meet with FDA and FSIS to integrate their priorities.
- Input from stakeholders. Formal/informal stakeholder input – consensus process at Listening Sessions, annual meetings, etc. Research questions – pertinent questions. Outside influence includes political.
- Research priorities not spelled out, but are hinted at in the RFA. Review panels make recommendation what should be funded annually. It's the review panel that ultimately makes the decision and they're typically focused on short term results.
- Challenges include required impact analysis of intervention strategies in proposals and the accomplishment of longer term objectives given the short term funding of projects.

USDA Agricultural Research Service (ARS)

- Intramural Research Agency for the USDA. Conducts research in multiple research areas, including food safety to meet the needs of regulatory and other federal agencies and other stakeholders, such as industry.
- Total annual funding for food safety research is $105 million; $16 million is for produce. Of that about 7% reaches the bench for actual research (about $1.2 million).
- Leverages resources through cooperative agreements with industry and grants. Also have initiated international collaborations and research.
- Research priorities developed through 5 year strategic plan. Plan is available on the internet. Look at food safety issues nationally/internationally, stakeholder input, staff. Draft produced for official input; will be updated soon.

- Major challenge: ARS scientists are not eligible to compete for some research grant programs (SCRI?). Federal appropriations process is always a challenge to maintain or increase funding.

FDA CFSAN

- Priorities developed through strategic plan process. That links Public Health Objectives/Priorities to the research objectives
- Strategic Goal linked to Outcomes
- Developed with Program Offices – e.g. Eggs, Cosmetics, Fresh Produce. Facilitated discussion to id research to support mission.
- Presentation shows how CFSAN approaches fresh produce data gaps and research goals.
- Typically driven by Regulatory Goal. Id Knowledge Gap (Project outcomes generate one or more research projects). Then they are prioritized. [Missed how they are prioritized].
- CFSAN Goes to FDA Centers, other government agencies to build partnerships for funding of research.

Center for Produce Safety CPS

- Funding: $3 million/annually; goal $4 million/annually. 5 RFA's in ~2 years. Going forward single RFA. Timing release rfa early spring; review; announce early fall.
- Priorities based in the technical committee. Initial compilation of multiple risk assessments on multi crops. 22 different topics in common. Used technical committee (cross section of members). Asked the committee to id knowledge gaps; which led to priorities.
- Each RFA – resulted in a refinement of the priorities. Partners in research helped. Research priorities changed as we receive feedback from stakeholders, partners in research. Also very important to this spring's RFA was sitting down with the FDA to learn about their specific needs.
- Initial challenge in reaching the research community that has changed after multiple RFAs.
- Also initially, appeared from review of responses to our early RFAs that there was a lack of understanding of the produce industry's existing practices within the broader research community. Made a focused effort of outreach to ensure that researchers who were proposing projects.
- Also challenge for all programs – how to reach out to multi-disciplinary researchers to respond to RFAs.

<u>Private Company Interaction with Research Results</u>

- Where do private companies get exposed to research results? Connection to Associations and Professional Societies for new scientific information; independent literature search/review, regulatory bodies and most importantly, suppliers.

<u>Association Interaction with Congress/Administration Re: Produce Safety Research</u>

What are the important areas and challenges that an association faces working to educate and influence Congress/Administration in the area of produce safety research?

- Make sure that Congress understands the research programs in their context, including which program is working best regarding the industry's needs.
- Constant give and take between commodity specific needs. Multi-commodity needs are approached differently vs. a single commodity's issue.
- Budgetary environment makes it tough to fund "new" programs.
- Need to overlay the diversity of needs in the political context.
- However, food safety is a high priority – politically right now. It is an opportunity to capture funding for research.

Field Tour – Taylor Farms Salinas Valley

An invitation was received for a guided on-farm visit to Taylor Farms in the Salinas Valley, in Monterey County on the Central Coast of California.

Due to the intensity of local agriculture the area has earned itself the nickname "America's Salad Bowl, for the production of lettuce, broccoli, peppers and numerous other crops. The climate is also ideal for the floral industry and grape vineyards planted by world-famous vintners.

Supplying Salinas Valley farms is an underground water supply fed, in part, by the large watershed in surrounding mountains. Groundwater is used to irrigate about 275,000 acres (approx. 111,000 ha) of fruits and vegetables and to supply the valley cities.

The industry was impacted significantly by the 2006 outbreak of *E.coli* O157:H7 on spinach leaves and is at the forefront of adoption of the new on-farm food safety practices under the California Leafy Greens Marketing Agreement (CLGMA).

Taylor Farms are a significant producer and processor of salads and other vegetables for the USA food service sector. With nine major processing plants spread across the USA and one in Mexico they are specialty fresh produce service provider to major fast food companies. Taylor Farms also have linkages to the Australian fresh produce processing sector *via* their holdings in GSF Australia.

The on-farm tour was hosted by Andrew Fernandez, VP Raw Product, and the tour was to a number of farms in the Salinas Valley area supplying a variety of leafy greens to their Salinas processing facility. The tour participants included some undergraduate agriculture students on work experience and a postgraduate student recently employed by Taylor Farms.

The following photographs and captions illustrate some of the key on-farm food safety practices and issues for Taylor Farms.

Mobile toilets and hand-washing stations are now compulsory in the field and follow every work crew. They are used at the start of each shift and hygiene compliance is monitored by the field supervisor

Prominent signs in English and other appropriate languages (e.g. Spanish) are used to reinforce worker training. Because workers often come from lower socio-economic backgrounds they do not have basic health knowledge about disease transmission. Worker training takes a holistic approach to improving their hygiene knowledge and practices in the home, as well as the field. Also use prominent graphics because of low literacy.

Andrew Fernandez (left) explains to tour participants the quality required and signs of pest, disease and nutritional disorders that pickers are trained to identify and remove at harvest

Hand disinfectant/sanitiser cream attached to the harvester for workers to use in the field when not near the hand-wash station

Harvesting Romaine (Cos) lettuce using high-pressure water jet cutting technology

Outer leaves are stripped by the harvester crew wearing gloves, arm protectors and hair/beard protection

Note that some cut stems (lettuce in middle) still have residual soil on cut surface using this type of harvest technology

All water used on the harvester for high pressure water cutting jets and wash is treated to a potable standard

Coring-in-field (CIF) iceberg lettuce harvester with a harvest knife wash and sanitation container attached. Knives are company controlled and any loss is recorded.

Cored lettuce on conveyor belt pass through shower with sanitized water to minimize equipment cross-contamination

Mini-greens mowing system being cleaned down between shifts/fields

Flags indentify the beds where samples have been taken 5-7 days pre-harvest for microbial testing under the LGMA

Taylor Farms crop microbial testing site flag

Workers weeding the mini-greens beds just prior to harvest

Flags identify sites where customer-specified microbial test samples have been collected

Dissemination of Information

- A summary from this report on the conference will be circulated to Freshcare and members of the horticulture food safety network.
- Summary articles from this report will be prepared and provided to industry journals with a view to publishing relevant information (e.g. Vegetables Australia).
- Ideas for future research and technologies will also be discussed directly with industry at food safety meetings, events, workshops, etc,.
- Industry specific information will be discussed directly with industry with a view to adoption of the research outcomes.
- Discussion regarding outcomes from these conferences will be held more widely with research and industry colleagues at national conferences.

Recommendations

Attending the inaugural CPS symposia proved very informative in terms of gaining insights into emerging research on food safety management in fresh produce, particularly for the pathogen *E.coli*.

The information gathered will be used to inform and guide developments in Australian fresh produce food safety systems. Standards owners, certification bodies and fresh produce supply chains can incorporate improved risk management practices as a consequence of these research outcomes.

The information gathered in this project may also lead to opportunities for collaborative research projects between the USA and Australia, utilising the Produce Marketing Association's organisational linkages with CPS.

It is recommended that attendance at future CPS symposia by Australian delegates should be a high priority and that this be supported by industry and Horticulture Australia Limited. It is also recommended that appropriate CPS-funded researchers be invited to participate in Australian fresh produce food safety conferences.

This inaugural CPS event has established a leading platform for sharing fresh produce food safety research outcomes that Australia needs to participate in. To maintain Australian fresh produce food safety systems in their leading development position, strong linkages are needed to the world-leading food safety science being created through the CPS.

Acknowledgements

Funding for this project was provided by Frontline Services Australia Pty Ltd and Horticulture Australia Limited. Without this support it would not have been possible to attend these important food safety events.

Appendix I Itinerary

DATE	ACTIVITY
Sunday 20th	Depart Gosford – Sydney – Los Angeles
Monday 21st	Travel Los Angeles - Salinas - San Francisco
Tuesday 22nd	San Francisco – UC Davis/CPS
Wednesday 23rd	CPS Symposium UC Davis
Thursday 24th	CPS/JIFSAN/WCFS Workshop UC Davis – travel to Salinas
Friday 25th	Taylor Farms Salinas – travel to Los Angeles
Saturday 26th	Los Angeles – Sydney – arrive Gosford (arrive Monday 28th)

Appendix II Program: Produce Research Symposium

AGENDA

Wednesday, June 23, 2010

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Location	**Jackson Hall Stage, Mondavi Center, University of California Davis**
7:00 – 8:00 am	**Registration / Continental Breakfast**
8:00 – 8:15 am	**Welcome** – Tim York, Markon Cooperative; Chairman CPS Advisory Board
8:15 – 8:30 am	**CPS Research Selection Process** Bonnie Fernandez-Fenaroli, Executive Director CPS
8:30 – 10:10 am	**SESSION I. Survivability of *E. coli* in field conditions**
	Survival of attenuated Escherichia coli O157:H7 ATCC 700728 in field-inoculated lettuce. Linda Harris, Ph.D., University of California, Davis
	Contribution of phyllosphere microbiota to the persistence of Escherichia coli O157:H7 ATCC700728 on field-grown lettuce. Maria Marco, Ph.D., University of California, Davis
	Comparison of surrogate *E. coli* survival and epidemiology in the phyllosphere of diverse leafy green crops. Trevor Suslow, Ph.D., University of California, Davis
	Examination of the survival and internalization of E.coli on spinach under field production environments. Steven T. Koike, University of California Cooperative Extension
	Discussion
	Moderator: Bob Whitaker, Produce Marketing Association; Chairman CPS Technical Committee
	Panellists: James Gorny, FDA – Center for Food Safety and Applied Nutrition
	Cortney Parker, Chiquita Brands International
	Joe Pezzini, Ocean Mist Farms
	Casey Barton Behravesh, Center for Disease Control and Prevention
	Vic Smith, JV Farms
10:10 – 10:40 am	**Break**
10:40 - 12:00 am	**SESSION II. Enhanced testing methods for pathogens in produce**
	A high-throughput, culture-independent approach to identify index and indicator species for *E. coli* O157:H7 contamination. Gitta Coaker, Ph.D., University of California, Davis
	Enhancing the effectiveness of human pathogen testing systems for the advancement of practical produce safety research and commercial management. Carol D'Lima Ph.D., University of California, Davis
	A sensitive and specific molecular testing method for live Salmonella in produce. Beilei Ge, Ph.D., Louisiana State University
	Discussion
	Moderator: Bob Whitaker, Produce Marketing Association; Chairman CPS Technical Committee
	Panellists: Robert Mandrell, USDA – Agricultural Research Service
	Drew McDonald, Taylor Farms
	Stephen Patricio, Westside Produce
	Martha Roberts, University of Florida
	Stacy Stoltenburg, Primus Labs
	Don Zink, FDA – Center for Food Safety and Applied Nutrition
12:00 – 1:30pm	**Lunch – UC Davis Conference Center**

1:30 – 3:10 pm **SESSION III. Potential vectors for pathogen transfer during field production**

Fly reservoirs of *E. coli* O157:H7 and their role in contamination of leafy greens. Astri Wayadande, PhD, Oklahoma State University

Food safety risks associated with sheep grazing in vegetable stubble fields. Bruce Hoar, DVM, PhD, University of California, Davis

Environmental effects on the growth or survival of stress-adapted Escherichia coli 015:H7 and Salmonella spp. in compost. Xiuping Jiang, PhD, Clemson University

Minimizing pathogen transference during lettuce harvesting by optimizing the design of the harvesting device and operation practices. Yaguang Luo, PhD, USDA-ARS

Discussion

Moderator: Bob Whitaker, Produce Marketing Association; Chairman CPS Technical Committee

Panellists: Rob Atwill, Western Institute for Food Safety and Security, UC Davis
Hank Giclas, Western Growers
Ana Hooper, Darden
Bill Pool, Wegmans

3:10 – 3:40 pm **Break**

3:40 – 4:40 pm **SESSION IV. Food Industry / Government Discussion**

Discussion

Moderator: Bryan Silbermann, President and CEO Produce Marketing Association

Panellists: Mary Ellen Burris, Wegmans
Alec Leach, Taylor Farms
Michael Taylor, US Food and Drug Administration

5:00 – 7:00 pm **Reception:** Robert Mondavi Institute for Wine and Food Science, Courtyard and Gardens

Appendix III Program: Produce Safety – Research Priorities

June 24, 2010
UC Davis Conference Center
UC Davis, California

Presented by: **Center for Produce Safety, University of California, Davis**
Joint Institute for Food Safety and Applied Nutrition, University of Maryland
Western Center for Food Safety, University of California, Davis

7:00 **Registration and continental breakfast buffet**

8:00 **Introductory Remarks**

Bonnie Fernandez Fenaroli, Center for Produce Safety, UC Davis

Joe Pezzini, Ocean Mist Farms, California Leafy Greens Marketing Agreement

Bryan Silbermann, Produce Marketing Association

8:30 – 9:45 **Compost & Soil Amendments:** Soil amendments, including commonly used animal manure containing soil amendments, increase soil tilth and fertility for production of fresh fruits and vegetables. It is well established that animal manures have the potential for containing enteric human pathogens such as *E. coli* O157:H7 and Salmonella spp. Composting plays an important role in enhancing the availability of nutrients essential to plant growth and reducing the presence of human pathogens in manure. This session will explore the role of key variables that impact the reduction of human pathogens during the composting process as well as how risk can be further reduced during subsequent handling. Topics to be explored regarding further research will include agro-ecological considerations, development and measurement of key compost process metrics to ensure process efficacy and the role of organic standard/requirements.

- Moderator for session – Jim Gorny, FDA Center for Food Safety & Applied Nutrition
- Overview speaker – Mike Doyle, University of Georgia
- Discussion panel
  - ✓ Pat Millner, USDA Agriculture Research Service
  - ✓ Xiuping Jiang, Clemson University
  - ✓ Johnny Massa, Comgro
  - ✓ Matt Cotton, Past President – US Composting Council
- Session Summary: Michelle Danyluk, University of Florida

9:45 – 10:00 **Break**

10:00 - 11:15 **Water (Field and Processing):** Agricultural water is used extensively during the production, harvest and postharvest handling of fresh fruits and vegetables. Water is a potential vector to spread the contamination of enteric human pathogens to fresh produce. This session will discuss research needs associated with means of detecting, eliminating and/or reducing the potential risk of human pathogens being associated with agricultural water and postharvest water use.

- Moderator for session – Steve Patricio, Westside Produce
- Overview speaker – Trevor Suslow, University of California, Davis
- Discussion Panel
  - ✓ Charles Gerba, University of Arizona
  - ✓ Nick Ashbolt, Environmental Protection Agency
  - ✓ Ken Tate, University of California, Davis
  - ✓ Rob Mandrell, USDA Agricultural Research Service
  - ✓ Joe Pezzini, Ocean Mist Farms
- Session Summary: TBA

11:15 - 12:30 **Co-Management: Food Safety and the Environment:** On-farm food safety practices have been implemented by many growers to reduce potential contamination of fresh fruit and vegetable crops by wildlife fecal contamination. Some on-farm food safety practices to reduce wildlife intrusion into production fields may be detrimental to environmental conservation best practices. This session will explore conflicts of produce food safety and environmental co-management as well as research to balance these two

needs.

- Moderator for session – Hank Giclas, Western Growers Association
- Overview speaker – Michele Jay-Russell , University of California, Davis
- Discussion panel
  - ✓ Chris Fischer, The Nature Conservancy
  - ✓ Jeff LeJuene, The Ohio State University
  - ✓ Daniel Mountjoy, USDA Natural Resources Conservation Service
  - ✓ Ken Stearns, D'Arrigo Bros.
- Session Summary : Devon Zagory, Devon Zagory & Associates

12:30 – 1:30 **Lunch (Buehler Alumni and Visitors Center)**

1:30 – 2:45 **Worker Health & Hygiene (Farm to Fork):** Attention to worker health and hygiene is an important aspect of ensuring produce safety from farm for fork as it is well established that humans can be a significant source of human pathogens on fresh produce. This session will review what best practices are currently regarding worker health and hygiene programs and discus innovative means of assuring compliance to best practices. The session will identify obstacles to the implementation of effective health and hygiene programs and the research needed to significantly reduce the risk of contamination by poor health and hygiene practices throughout the supply chain.

- Moderator for session – Linda Harris, University of California, Davis
- Overview speaker – Bob Gravani , Cornell University
- Discussion panel
  - ✓ Marc Schenker, University of California, Davis
  - ✓ Chris Loss, Culinary Institute of America, St Helena
  - ✓ Lisa Fuentes, The Nunes Company
  - ✓ Walter Ram, Giumarra Companies
- Session Summary: Dave Gombas, United Fresh Produce Association

2:45 – 3:00 **Break**

3:00 – 4:15 **Data Mining:** The produce industry and regulators have recently implemented a wide array of microbial testing regimes that allow for quantitative verification of produce food safety best practices. Also, buyer demands for production agriculture input and product testing, as well as FDA and USDA routine surveillance sampling of produce at various points throughout the supply chain, are occurring with greater frequency. Additionally, grower/shippers and fresh"cut produce processors frequently perform raw and finished product testing for human pathogens as a standard part of their food safety program and buyer requirements. The net effect has been a proliferation of data sets which provide insight regarding the microbial safety of agricultural inputs, raw agricultural commodities and finished products. These data sets have the potential to be leveraged to identify important trends regarding the prevalence of human pathogens in produce over time, provide insights regarding the effective use of risk reducing best practices, and help direct future research efforts. One barrier to pooled utilization of these data sets is their confidential nature in that they are owned by numerous competing business entities. Additionally, the systematic sharing of data among private companies and regulatory agencies has been hampered by the proprietary nature of the data. This session will identify the types of data currently being collected by produce industry companies and government agencies and report on efforts made to date on "data mining" these data sets. Also to be explored are effective means of facilitating data sharing among produce industry companies and regulatory agencies to enhance produce food safety via a data"driven risk and science"based approach.

3:00 – 4:15 **Data Mining (cont)**

- Moderator for session – Martha Roberts, University of Florida
- Overview speaker – Drew McDonald, Taylor Farms
- Discussion panel
  - ✓ Courtney Parker, Chiquita / Fresh Express
  - ✓ Rob Atwill, University of California, Davis
  - ✓ Martha Lamont, USDA Agriculture Marketing Service
  - ✓ Casey Barton Behravesh, Centers for Disease Control

- ✓ Scott Horsfall, California Leafy Greens Marketing Agreement
- Session Summary: Bob Whitaker, Produce Marketing Association

4:15 – 5:30 **How do you define research priorities in your organization?** Produce research that can be used to assist all stakeholders in the produce farm to fork continuum to reduce, control or eliminate hazards associated with fresh produce has become a high priority. Industry, government and academic research leaders will come together in this session to discuss the means by which they are identifying and ranking produce food safety priority research needs. This session will also summarize currently funded on-going produce safety research efforts to facilitate cross pollination of research ideas/techniques and minimize duplication of efforts.

- Moderator – Bonnie Fernandez"Fenaroli, Center for Produce Safety
  - ✓ Bob Whitaker , Produce Marketing Association,
    Chair – CPS Technical Committee
  - ✓ Don Zink, FDA Center for Food Safety & Applied Nutrition
  - ✓ Jeanette Thurston, USDA National Institute For Food & Agriculture
  - ✓ Jan Singleton, USDA National Integrated Food Safety Initiative
  - ✓ James Lindsay / Mary Torrence, USDA Agricultural Research Service
  - ✓ Robert Guenther, United Fresh Produce Association
  - ✓ Ana Hooper, Darden
- Session summary: Tad Bell, Velo Consulting Group

5:30 **Closing Remarks**

James Gorny, FDA Center for Food Safety & Applied Nutrition

5:45 – 6:45 **Reception**