



## **CPS RAPID RESPONSE FINAL PROJECT REPORT**

### **Project Title**

CPS Rapid Response – Yuma Valley

### **Project Period**

January 1, 2019 – June 30, 2019

### **Principal Investigator**

Channah Rock  
University of Arizona  
Maricopa Agricultural Center  
37860 W. Smith-Enke Road  
Maricopa, AZ 85138  
P: 480-628-9794  
E: Channah@ag.arizona.edu

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### **Objectives**

The overall objective of this rapid response research effort was to capitalize on the unique opportunity to study romaine production under real world conditions within close proximity to potentially implicated fields from the 2018 outbreak of *E. coli* O157:H7 in romaine lettuce. This venture allowed the research and extension team to generate new knowledge that is useful to industry in order to bridge the gap created by the recent outbreak. The team focused on the areas of water treatment, persistence of microbial contamination on romaine, as well as sampling protocols appropriate for the detection of indicators and pathogens in commercial scale agriculture.

### **Funding for this project provided by the Center for Produce Safety through:**

CPS Campaign for Research

## FINAL REPORT

### Abstract

Our research team evaluated commercial scale produce production practices in order to provide enhanced clarity on the potential role of 1) agricultural water, 2) freeze damage, and 3) harvesting practices in contaminant transfer and harborage of *E.coli* TVS353 on Romaine lettuce. Results indicate that while both 12% PAA and 12.5% Sodium Hypochlorite at 4ppm were effective at producing over a 2-log reduction in *E.coli* in irrigation water, that bacteria remained at detectable levels on plant tissue (0 - 38%) and in soil (33-66%) samples collected post treatment. With respect to freeze damage, while both freeze-damaged and non-damaged romaine leaves indicated the presence of bacteria at 7 days post-inoculation, when leaves were analyzed at 30 days, 100% of the freeze-damaged romaine at the high bacterial dose maintained detectable levels of *E. coli* TVS353 and only 33% of freeze-romaine inoculated at a low dose had recoverable amounts of bacteria. While this indicates that bacterial die-off is occurring over the course of the 30 days on the surface of romaine leaves, results provide evidence that freeze-damage may extend the period at which bacteria are able to survive post-inoculation even at low doses. Additionally, data indicates that harvesting practices aid in contaminant transfer from field contamination to harvest equipment and back to harvested product with 100% of harvested product indicating the bacteria of interest from 1-acre plots that were aerial contaminated and 75% for animal intrusion contamination. For plots mimicking furrow irrigation contamination, the spread of bacteria to harvesting tools and surfaces were detected however, post-harvest sampling neglected to find positive indications of bacterial contamination. Overall, these results shed light on what industry practices and conditions may have played a role in the 2018 outbreak of *E. coli* O157:H7 in Romaine lettuce while highlighting the impact of harvest practices on the spread of contamination. A closer look at both pre and post-harvest sampling strategies and recommended best practices for harvesting and equipment sanitation is warranted to enhance industry capacity to detect and prevent contaminated product making it into the marketplace and ultimately enhance food safety.

### Background

Recent outbreaks in fresh produce coupled with heightened media coverage, have elevated produce safety into the forefront of public attention. More specifically, the 2018 outbreak involving romaine lettuce grown in the Yuma region, was linked to *Escherichia coli* (*E. coli*) O157:H7, in which agricultural water was suspected as the source, yet the origin and

environmental distribution and potential reservoir(s) of the outbreak strain remains unknown. In response to the outbreak, the California and Arizona Leafy Greens Marketing agreements adopted revised metrics in an effort to address the possible role of atmospheric deposition (dust) and water quality on crop contamination. Revised metrics now require growers to treat their irrigation water if when overhead irrigating within 21 days of harvest. Additionally, the set-back distance from CAFO's of 80,000 head or more was extended to 1,200 feet, from a previous 400 feet. Exacerbating these concerns, new Federal regulations released by the U.S. Food and Drug Administration (FDA) as part of implementation of the FDA Food Safety Modernization Act (FSMA), impose new requirements on growers to improve food safety and include testing of agricultural water quality for generic *E. coli*. Many of these rapid changes within a reduced time frame for implementation as well as the findings included in the FDA Environmental Assessment (FDA 2018), which questioned the value of indicator *E. coli* testing of agricultural water, have left industry with many unresolved questions as to how best to improve their food safety standards and practices.

Because of generous industry support, our research and extension team were provided the unique opportunity to study romaine production under real world conditions within close proximity to potentially implicated fields from the 2018 outbreak of *E. coli* O157:H7 in romaine lettuce. This venture allowed the research and extension team to generate new knowledge that is useful to industry in order to bridge the gap created by the recent outbreak. The team focused on the areas of water treatment, persistence of microbial contamination on romaine, as well as sampling protocols appropriate for the detection of indicators and pathogens in commercial scale agriculture.

### **Research Methods and Results**

As stated above, the research and extension team aimed to evaluate commercial scale growing conditions in-order to better understand microbial ag water treatment technologies available to industry, the impact of harvest practices on contaminant transfer, and finally sampling protocols commonly used by industry to assess microbial contamination. Throughout the study, the framework outlined by Harris et al 2012 and Suslow et al 2011; using established SOPs and shared protocols for the evaluation of microbial hazards and controls during production that pertain to the quality of agricultural water contacting fresh produce was followed. The strain of generic *E. coli* (which is rifampicin resistant), used in this study was TVS353 *E. coli* W778, isolated from irrigation water (Salinas Region, CA, USA). The bacterial strain selected is

currently used in CPS funded field research with spinach and baby romaine, and was provided by the Suslow laboratory archives to maintain consistency across previous environmental contamination studies conducted across the country.

Before the target organisms were transferred to the water source or plant, the microbial population density was determined at various stages using an appropriate selective medium specific for the target microorganism(s), e.g., (i) after the initial growth of bacteria in culture medium, (ii) immediately before and after blending with the water or carrier (phosphate buffered saline solution), and (iii) after application to the environmental water matrix or plant tissue. Final levels of the target organism(s) in the inoculated water/soil/fecal slurry reflects the upper end of the expected range even when the frequency of occurrence of these levels is rare. The levels of “spiking solution” were established based on historical data collected by the PI and knowledge of microbial distribution in the environment in the southwest.

***I. Water Treatment.*** Our research and extension team evaluated the use of two US EPA approved water treatment sanitizers for the reduction of both naturally occurring and spiked microbiological indicators in irrigation water, on product, and in soil. The team compared 12% Peracetic Acid (PAA) to industry standard of 12.5% Sodium Hypochlorite for the reduction of natural index microbes found in irrigation water and established microbes on romaine plants under sprinkler irrigation settings. Peracetic Acid (PAA) has recently been gaining attention as a “green” alternative for both irrigation water and wastewater treatment. PAA is an organic peroxide compound with the formula  $\text{CH}_3\text{CO}_3\text{H}$ . It is generally considered as environmentally-friendly as it hydrolyzes to acetic acid and hydrogen peroxide in water. It is also known as a broad-spectrum biocide with a fast reaction time (minutes rather than hours), leaving no harmful by-products behind. PAA is also a strong oxidant and disinfectant with oxidation potential higher than that of chlorine or chlorine dioxide. PAA is generally available in the form of liquid mixture which may contain hydrogen peroxide, acetic acid (vinegar) and water.

For this study, the bacterial inoculum was incorporated into irrigation water through direct injection from an external reservoir followed by baffling in the pump. Irrigation water samples were collected when target ppm (parts per million) disinfectant residuals were detected at the last sprinkler head of each test plot. The target ppm for each sanitizer was between 4 and 6, and took roughly one hour after sprinkler irrigation commenced before appropriate sanitizer residual was detected in the field. Water samples (water pre and post-treatment) were collected

in 1-L sterile wide-mouth polypropylene containers directly from sprinkler heads at the first and last sprinkler head of each test plot as well as from the spiking reservoir, and transported on ice back to the laboratory to begin analysis with 6 hours of collection. Appropriate quenching reagents were used for each sanitizer; Sodium Metabisulfite (SMBS) for PAA and Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>) and Sodium Thiosulfate for Sodium Hypochlorite treatments. Water treatment trials were conducted in triplicate and included both positive (1-acre plots where no sanitizer was used) and negative fields (1-acre plots where no bacteria was applied) for control purposes.

It should also be noted that all sampling events include the proper number of both positive and negative controls in order to ensure confidence in sample results. For irrigation water samples, in order to calculate log reductions of bacteria from each sanitizer, 100 ul of collected water sample was direct plated in-duplicate on selective media and 100 mls of each water sample was filtered in duplicate onto a 0.45 micron filter (Millipore SAS, 67120 Molsheim, France) and then placed on selective media followed by incubation at 37 degrees Celsius for 18-24 hours before counting. Resulting colonies were reported as CFU/100ul or CFU/100ml respectively. Additionally, to increase the sensitivity of the analysis, 250ml water samples collect at 1 hour and 2 hours post irrigation start, were filtered in duplicate and placed into 90 mLs of enrichment broth. All sample enrichments were incubated at 37 degrees Celsius for 18 to 24 hours. After incubation, approximately 100ul of sample enrichments were assayed by spread plate technique onto selective media ChromAgar ECC supplemented with 80mg/L Rifampicin (ChromAgar, Paris, France) in duplicate. Samples resulting in blue colonies on selective media were counted as positive for the bacteria of interest.

Table 1. Recovery of TVS353 from Irrigation Water Samples Post Treatment (inoculum dose of 492 CFU/100ml irrigation water)

<b>Treatment</b>	<b>100ml (%; Log Reduction)</b>	<b>mMS</b>	<b>250ml - 1hr</b>	<b>250ml - 2hr</b>
PAA	0%; 2.69	13%	25%	50%
NaOCl	0%; 2.69	60%	17%	33%
Positive Control	100%; 0	100%	100%	100%

For non-irrigation water sample collection, the research team employed direct plate count as well as enrichment of either soil, plant tissue, sponge-sticks, glove wash water, or modified Moore Swab (m-MS) followed by plating on selective media for the detection of the applied *E.*

*coli*. The research team also employed a variety of industry standard pre-harvest sampling protocols to determine which methods evaluated work “best” at detecting the various contamination events.

Results from raw-product romaine lettuce samples collected 7 days after irrigation in each of the treatment plots indicate detectable levels (38%; n=18/48) of *E. coli* TVS353 in plots treated with PAA, while plots treated with Sodium Hypochlorite resulted in non-detectable levels of bacteria (0%; n=0/48). Similar results were seen when evaluating soil samples collected in five zones per treatment plot post treatment. Plots treated with PAA resulted in 66% positive detections in soil versus 33% positive detections in soil collected from Sodium Hypochlorite treatment plots.

***II. Crop Contamination.*** Simulated crop contamination events were also be conducted in order to investigate how the differences in the three already established varieties of romaine and the mechanism of contamination (aerial application, furrow irrigation, simulated animal intrusion “hot spot”) affect survival and persistence of spiked indicator bacteria in-field on individual plants and their distribution through commercial harvest. For this subset of experiments the research and extension team had the unique opportunity to evaluate the use of commercial scale harvest equipment on the acquisition and spread of microbial contamination.

For the contamination event simulating **aerial application**, research backpack sprayers were calibrated to known precise application rates and volumes of 200ml per minute to apply bacteria to plants. Single-head manifolds fitted with TeeJet nozzles were selected to provide a uniform droplet size distribution and pattern to minimize drift. Irrigation water collected on-site was used as the diluent for *E. coli* TVS353 applied in the field. The bacterial inoculum was created the day of experimentation by diluting known concentrations of TVS353 bacteria with collected surface water. Water and bacteria were homogenized by stirring for approximately 5 minutes prior to application. Sub-samples of water and bacteria mix were collected at the time of application in-order to calculate exact concentration of bacteria + water being applied to the field and were assayed within 6 hours of collection. All samples were stored on ice until transport to the laboratory for further processing. On average 100ml of water + bacteria inoculum contained  $5.0 \times 10^5$  *E. coli* TVS353.

Figure 1. Example Aerial Application Contamination



For the contamination event simulating **animal intrusion** and subsequent fecal contamination, the research team followed protocols selected from published literature related to the creation of a fecal slurry (Chase et al. 2019). Fecal material was collected within 24 h of slurry construction from three species: horse, goat, and pig. These three sources of fecal material were selected for inclusion in this study because growers and researchers have consistently observed some of these wildlife species (feral pigs) intruding into agricultural fields of the southwest as well as ease of access to the species and their fecal matter. Feces used in the experiments were confirmed negative for *E. coli* O157:H7 and rifampicin resistant non-O157 *E. coli*. Fecal slurries (horse, goat, and pig) were constructed as described in Chase et al. (2019), modified using a fecal-PBS ratio of 1 g:4 mL. Briefly, feces and PBS were stomached in a 55-oz Whirl-Pak filter bag (Nasco, Fort Atkinson, WI, USA) using a Seward Stomacher 400 Circulator (Seward Laboratory Systems Inc., Bohemia, NY, USA) for 5 min at 230 rpm; the liquid portion was dispensed into a sterile flask, vortexed for 1 min, then inoculated with the target concentration of

*E. coli* TVS353. Prior to use, the fecal slurries were homogenized by vigorous shaking for 1 min. Slurries were stored at 4 degrees C for approximately 48-h prior to field inoculation. *E. coli* concentrations were confirmed using serial dilution spread plate method in duplicate; the concentration in each slurry was estimated immediately prior to field inoculation to confirm no significant growth or death of TVS353 occurred during the 48-h refrigeration hold. During each trial, romaine heads, furrows and beds were inoculated with 25 mLs of the composite fecal slurry per contamination. The slurry was applied directly onto the surface of each head of romaine, the furrow in the field, and or the bed in-between romaine heads. On average 0.5 mL of slurry inoculum contained  $6.0 \times 10^7$  *E. coli* TVS353 and 55 heads of romaine, furrow locations, or beds were contaminated across twenty beds of two-line romaine on a one-acre plot.

Figure 2. Example Animal Intrusion Contamination



For the field contamination event mimicking **furrow irrigation** contamination, approximately 350ml of irrigation water spiked with known concentrations of *E. coli* TVS353 were applied to the axial leaves (outer) of the first three heads of romaine lettuce on each line of a one-acre plot. This application allowed a known concentration of bacteria and volume of water to be applied to each head of romaine in a precise manner. As stated above, the bacterial inoculum was created the day of experimentation by diluting known concentrations of TVS353 bacteria with collected surface water. Water and bacteria were homogenized by stirring for approximately 5 minutes prior to application. Sub-samples of water and bacteria mix were collected at the time of application in-order to calculate exact concentration of bacteria + water being applied to romaine heads and were assayed within 6 hours of collection. All samples were stored on ice until transport to the laboratory for further processing. On average 100ml of water + bacteria inoculum contained  $1 \times 10^5$  *E. coli* TVS353. A total of 120 heads of romaine were contaminated across twenty beds of two-line romaine on a one-acre plot.

Figure 3. Example Furrow Irrigation Water Contamination



For each of the field scale contamination events, approximately seven days post contamination, the research team solicited support from industry to conduct standard pre-harvest sampling of raw product in each field. Accordingly, both an “S” (or serpentine pattern), and “Z” pattern were used to collect raw product samples from the field. An n of 60 was used to create a composite sample of approximately 1500 grams of romaine lettuce from each one-acre plot, collected in an 4L Whirl-Pak bag (Nasco, Fort Atkinson, WI, USA) by sample collectors. Sterile technique was used to prevent cross contamination and each sample collector changed all Personal Protective Equipment (PPE) in-between sample collections. This included changing and replacing gloves, Tyvek suits, and face shields for each sample collector. From each 1500 gram composite sample, four individual 100g sub-samples were assayed for *E. coli* TV353 by enrichment followed by direct spread plating of 100ul of sample enrichment onto ChromAgar ECC + 80ug/mL Rifampicin in duplicate. Positive results were recorded as blue colonies on each plate.

Results indicate that for the furrow contaminated field, samples collected in the Z pattern show 4/4 (100%) positive for the bacteria of interest, while samples collected in the S pattern resulted in 0/4 (0%) positive. Alternatively, the animal intrusion field indicated 0/4 (0%) for both the S and the Z patterns, while the aerial application field resulted in 4/4 (100%) for both the S and Z patterns.

Once pre-harvest samples had been collected, all test fields were subject to commercial scale harvest. For the harvest, a 40-person harvest crew along with a 9-station harvester was deployed. Prior to harvest, harvest crews were provided PPE including Tyvek suits, face shields, arm shields, gloves and foot protection. They will also verbally informed about the research being conducted and asked to sign a waiver in accordance with the University of Arizona policy. Five sampling teams were positioned in-front of the harvest crews and were tasked with swabbing of the following locations; workers gloves, knives, cutting table, conveyer belt, and elevator/down spout. Harvest crews and foreman were instructed to begin harvest and were asked to stop every 12 to 15 minutes for a total of 5 stops per acre. During each “stop”, sampling teams conducted swabs of the aforementioned surfaces. Harvesting resumed after all swabs had been collected for each “stop”. Swabs were stored on ice until transport back to the laboratory for further processing within 24 hours. Upon processing, swabs were placed into 90 mLs of TSB + RIF (80mg/L Rifampicin) media and hand massaged for 1 minute. Massaged samples were then placed in the incubator for 18 hours at 37 degrees Celsius. After incubation, sample enrichments were assayed for TVS353 by direct spread plating of 100ul of sample enrichment onto ChromAgar ECC + 80ug/mL Rifampicin in duplicate. Positive results were recorded as blue colonies on each plate.

Table 2. Animal Intrusion

<b>Swab Location</b>	<b>Stop 1</b>		<b>Stop 2</b>		<b>Stop 3</b>		<b>Stop 4</b>		<b>Stop 5</b>	
	<b>(n;</b>	<b>%)</b>								
Gloves	0/10	0%	4/10	40%	6/10	60%	4/10	40%	4/10	40%
Knife	0/10	0%	4/10	40%	6/10	60%	0/10	0%	2/10	20%
Table	0/10	0%	6/10	60%	8/10	80%	6/10	60%	6/10	60%
Belt	0/10	0%	2/10	20%	0/10	0%	0/10	0%	0/10	0%
Elevator	0/2	0%	2/2	100%	2/2	100%	2/2	100%	2/2	100%

Table 3. Aerial Application

<b>Swab Location</b>	<b>Stop 1</b>		<b>Stop 2</b>		<b>Stop 3</b>		<b>Stop 4</b>		<b>Stop 5</b>	
	<b>(n; %)</b>									
Gloves	0/10	0%	8/10	80%	8/10	80%	8/10	80%	10/10	100%
Knife	0/10	0%	8/10	80%	8/10	80%	8/10	80%	8/10	80%
Table	0/10	0%	8/10	80%	8/10	80%	8/10	80%	8/10	80%
Belt	0/10	0%	10/10	100%	8/10	80%	8/10	80%	0/10	0%
Elevator	0/2	0%	2/2	100%	2/2	100%	2/2	100%	2/2	100%

Table 4. Furrow Irrigation

<b>Swab Location</b>	<b>Stop 1</b>		<b>Stop 2</b>		<b>Stop 3</b>		<b>Stop 4</b>		<b>Stop 5</b>	
	<b>(n; %)</b>									
Gloves	0/10	0%	2/10	20%	0/10	0%	0/10	0%	0/10	0%
Knife	0/10	0%	2/10	20%	2/10	20%	0/10	0%	0/10	0%
Table	0/10	0%	2/10	20%	0/10	0%	0/10	0%	0/10	0%
Belt	0/10	0%	0/10	0%	0/10	0%	0/10	0%	0/10	0%
Elevator	0/2	0%	0/2	0%	0/2	0%	0/2	0%	0/2	0%

Following harvest, a total of (3) 1500 gram grab samples were collected from four bins per one-acre plot of harvested romaine. Carboard bins were placed on the field and allow to sit for three hours to mimic transport from field to processing/distribution center. Bins and plastic liners were cut open using sterile box-cutter blades from the side of each of the sampled bins. Harvested product was collected from the bottom, middle and top of each of the assayed bins. From each 1500 gram composite sample, four individual 100g sub-samples were assayed for *E. coli* TV353 by enrichment followed by direct spread plating of 100ul of sample enrichment onto ChromAgar ECC + 80ug/mL Rifampicin in duplicate. Positive results were recorded as blue colonies on each plate.

Harvested romaine samples collected from each of the three contamination event types resulted in varying results. Post-harvest bin sampling from the furrow contaminated field resulted in zero detectable bacteria in 12 of the sample collected from the four bins. Alternatively, three out of the four bins resulted in 9 out of 12 positive detections from the animal intrusion contaminated fields and 100% or 12/12 of the aerial application contaminated field resulted in positive detections.

Table 5. Percent Positive Bin Sampling of Harvested Product

	Bin Samples (n=)	% Positive
Furrow Irrigation	0/12	0%
Animal Intrusion	9/12	75%
Aerial Application	12/12	100%

***III. Freeze and Injury.*** In order to assess the impact of freeze/frost on the susceptibility of three varieties of romaine to harbor *E. coli* O157:H7, the research team used local weather stations with the University of Arizona AZMET network to track temperatures resulting in freeze or frost damage to romaine at the test plots. A total of twelve freeze events, temperatures dropping below 32 degrees at crop height, occurred during the rapid response investigation period. Approximately 12 days post freeze injury, individual plants were inoculated with *E. coli* TVS353 bacteria at a high and a low concentration, and tracked over time through harvest in order to better understand the impact of plant tissue damage and epidermal peel on retention of bacteria in the plant or on the plant surface. Two concentrations of bacteria were inoculated on both freeze damaged and non-damaged romaine lettuce,  $1 \times 10^4$  and  $1 \times 10^7$ . Approximately 7 and 30 days post inoculation, 18 heads of romaine were harvested from the research field plots and transported on ice back to the laboratory for processing within 24 hours. Once at the laboratory, 6 freeze damaged and non-freeze damaged leaves of three variety types (R1, R2, and R3) were removed. 25 grams of each lettuce variety and damage type were then separated and placed into 710 mL Whirl-Pak bags containing 90 mL of enrichment broth. Lettuce/broth samples were then stomached for 2 minutes at 230 rpm before incubating at 37 degrees Celsius for 18 hours. After incubation, sub-samples were assayed for *E. coli* TV353 by direct spread plating of 100ul of sample enrichment onto ChromAgar ECC + 80ug/mL Rifampicin in duplicate. Positive results were recorded as blue colonies on each plate.

Figure 4. Freeze Damaged Romaine



Figure 5. Recovery of *E. coli* TVS 353 on damaged (green) and un-damaged (blue) Romaine 7-days post inoculation

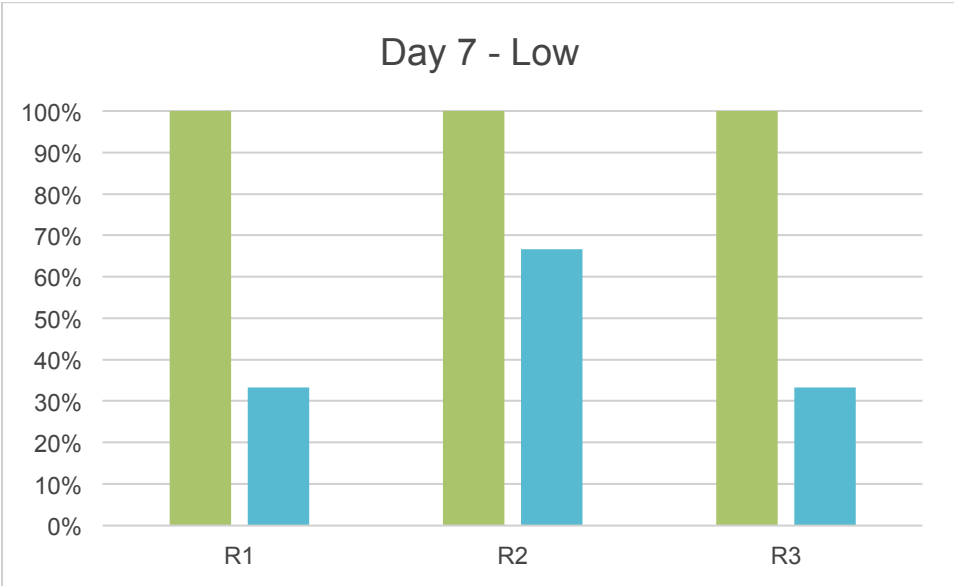
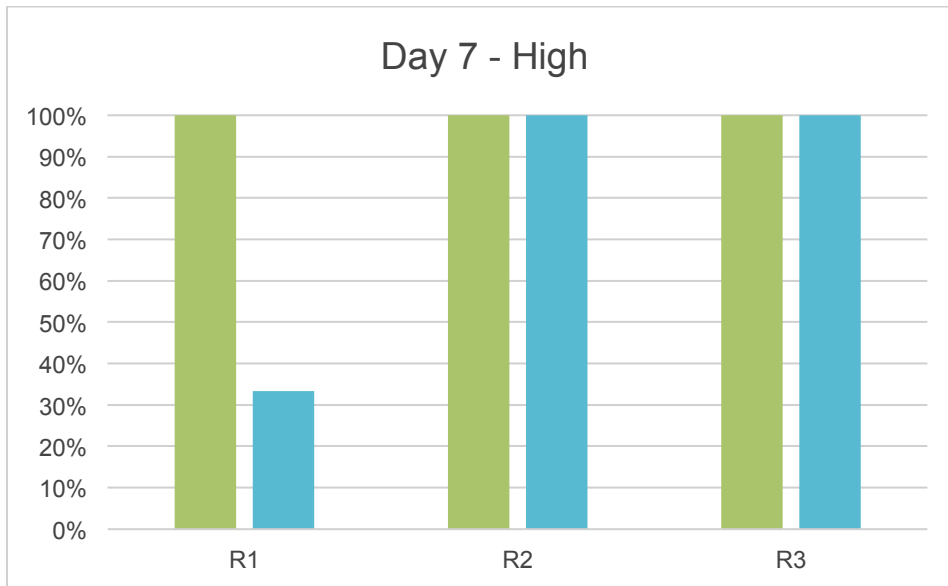


Figure 6. Recovery of *E. coli* TVS 353 on damaged (green) and un-damaged (blue) Romaine 7-days post inoculation



While both freeze-damaged and non-damaged romaine leaves indicated the presence of inoculated bacteria at 7 days (Figures 5 and 6), when leaves were analyzed at 30 days post inoculation, 100% of the freeze-damaged romaine still maintained detectable levels of *E. coli* TVS353 at the high concentration while only 33% of freeze-romaine had recoverable amounts of bacteria from the low inoculum dose. While this indicates that bacterial die-off is occurring over the course of the 30 days, results do provide evidence that freeze-damage romaine may extend the period at which bacteria are able to survive post-inoculation.

### **Outcomes and Accomplishments**

Over the course of this study, the UA research and extension team worked side-by-side with industry to conduct an intensive assessment of industry practices surrounding the contamination of Romaine lettuce in close proximity to the 2018 Outbreak. In total our team assessed 6-acres of romaine lettuce, assayed over 2000 samples for the presence and concentration of indicator bacteria, involved over 50 faculty, students and staff towards the completion of the project, and produced a series of presentations both in-person and online that reached a record number of stakeholder participants. Additionally, the findings of this rapid response project can be used by industry to enhance food safety practices today to reduce the likelihood of contaminant transfer and aid in the protection of public health.

## **Summary of Findings and Recommendations**

Overall, our data indicated that both sanitizers evaluated, PAA and Sodium Hypochlorite, in the rapid response study were effective at reducing microbial loading in irrigation water. However, it should be noted that when larger sample volumes were assayed beyond the 100ml standardized metric, water samples that were assumed to be negative for bacteria resulted in positive detections. This indicates that while the 100ml sample volume is useful for its ease in assessment, that industry should not assume 100% efficiency with water treatment and be aware that bacteria still may persist in treated water. This finding is also exemplified in the results of detectable bacteria on raw-product and soil samples collected post treatment.

With respect to the simulation and evaluation of contamination events and the impact of commercial scale harvest on bacteria transfer, results indicate that harvest practices do aid in contamination transfer. Both the animal intrusion and aerial application contamination events resulted in pervasive contamination and shed light on the likelihood or improbability of these types of contamination being the cause of the 2018 Outbreak. Surprisingly, furrow irrigation contamination simulations were detectable through harvest and point to the ability of furrow contaminated water to transfer to harvestable romaine lettuce. Resulting best management practices include raised bed height, omission of harvest at the head of the field, as well as improved or increased frequency in knife cleaning practices.

While current industry standards used for pre-harvest assessment, S and Z, seem to be sufficient for detecting pervasive contamination, research indicated that they could be improved upon. While pre-harvest assessment has been the “go to” to understand field level contamination, results indicating the impact of harvest practices on the spread of contamination warrants a closer look at post-harvest sampling as a mechanism to enhance our capacity to detect contaminated product prior to distribution into the marketplace.

Finally, with respect to the ability of bacteria to survive on “freeze-damaged” romaine, our data indicates an increased propensity for bacteria to be recovered from damaged product. This result was seen at both high and low inoculum concentrations. Our data also indicates that variety type had minimal impact on our ability to recover bacteria. Overall, this data shows proof-of-concept that “freeze damaged” romaine may have the ability to harbor bacteria for extended periods of time and may have played a role in the 2018 Outbreak.

## Literature Cited/Bibliography

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5. Tomás-Callejas, A. G. López-Velasco, A. Camacho, F. Artés, F. Artés-Hernández, T. V. Suslow. 2011. Survival and distribution of *Escherichia coli* on diverse fresh-cut baby leafy greens under preharvest through postharvest conditions, *I. J. Food Micro.*, 151: 216-222.

## **APPENDICES**

### **Publications and Presentations (required)**

- CPS Rapid Response- Yuma Valley, JV Smith and Co., Yuma, AZ, May 24<sup>th</sup>, 2019
- CPS Rapid Response- Yuma Valley, Center for Produce Safety Annual Meeting, Austin, TX, June, 18<sup>th</sup>, 2019
- CPS Rapid Response-Yuma Valley, Center for Produce Safety Webinar, online, August 1<sup>st</sup>, 2019
- CPS Rapid Response-Yuma Valley, ONE Health Conference, Flagstaff, AZ, August 6<sup>th</sup>, 2019
- CPS Rapid Response-Yuma Valley, Yuma Safe Produce Council, Yuma, AZ, August 20<sup>th</sup>, 2019

### **Budget Summary (required)**

Total request: \$81, 472.10

*Salaries: \$23,700.00*

*ERE @ 34.9%: \$8,271.30*

*Supplies and Consumables: \$20,000.00*

*UA Travel: \$19,500.80*

*UC Davis Travel: \$10,000.00*

### **Tables and Figures (optional)**

See above.

### **Suggestions to CPS (optional)**

Not suggestions at the time.