CENTER for PRODUCE SAFETY

CPS 2017 RFP
FINAL PROJECT REPORT

Project Title
Scientifically valid corrective actions for multiple harvest shade-house production systems

Project Period
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Objectives

1. *Determine the die-off kinetics of E. coli, ~~EHEC~~*, Salmonella, ~~and indicator Listeria~~*, in controlled research protected culture, on cucumber, roma-type tomato, and jalapeño peppers following simulated leaf and fruit contamination from irrigation and soil surface and other shadehouse environmental sources, including trellising and harvest activities.*
2. *Comparatively evaluate methods, sampling protocols, sample processing protocols, and sensitivity of detection of bacterial pathogens in preharvest testing designed for shadehouse EMPs.*
3. *Evaluate the efficacy of various corrective actions** to minimize the risk of transference and minimize persistence of bacterial pathogens within and on the standing crop.*

NOTE: Objectives 1 and 3 were modified in spring 2019 due to reductions in technical staff and farm management challenges with this project.

* For Objective 1, enterohemorrhagic *E. coli* (EHEC) and indicator *Listeria* spp. were removed from the inoculation's matrix.

** For Objective 3, all corrective action experiments focus on shadehouse crops and exclude field trials.

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FINAL REPORT

Abstract

The domestic and global production of fruits and vegetables under various forms of protected culture has grown rapidly and provides substantial market volume to year-round availability of fresh produce. Seven events of foodborne illness associated with raw cucumber consumption have been reported (1996 to 2016). Six of the outbreaks were attributed to *Salmonella* and one to *E. coli*, resulting in over 1,200 illnesses and 260 hospitalizations. In this project, 25 experiments were conducted to determine die-off kinetics of *Salmonella* and *E. coli* on slicing cucumber, roma-type tomatoes and jalapeño peppers growth in both high tunnel hoop-house structures and the open environment at UC Davis open-field plots. Bacteria recovery from crops growing in open-field plots showed a rapid decline within the first day after inoculation; however surviving bacteria may establish and persist at very low cell numbers that require concentration or enrichment for their detection. *E. coli* and *Salmonella* inoculated onto cucumbers, tomato and jalapeño plants in hoop houses cannot only survive but grow on the crops, given the conditions (temperature and humidity). Three different preharvest sanitizer treatments were evaluated as part of corrective actions to minimize contaminant persistence and transference in the preharvest environment on tomato and jalapeño plants. Treatment with 1% Oxidate 5.0 for 24-h contact time resulted in significantly lower numbers of viable bacteria on the treated plants. Circumstances that possibly contribute to the amplification of *Salmonella* contamination, such as transfer from soil, drip lines, early season fruit imperfections, wash-line injury, waxing, and modified atmosphere packaging (MAP) shipping bags, were evaluated. *Salmonella* Poona population increases of approximately 1.0 and 2.8 log were observed in 6 days at 15 and 20°C, respectively, following immersion of cucumbers in contaminated wash water. Results from the microbiological assessments in this study indicated that waxing and MAP bags can increase the persistence and growth of *Salmonella*, depending on storage temperature. This project provided science-based die-off kinetics data of *E. coli* and *Salmonella* under field and protected produce production (hoop houses) on three different crops. If contamination occurs in protected environments such as shadehouses, cross-contamination of produce is likely to continue to be a food safety concern.

Background

From 1996 to 2016, seven events of foodborne illness associated with the consumption of raw cucumbers were reported (3, 4, 21). Six of the outbreaks were attributed to *Salmonella* and one to *E. coli*, resulting in over 1,200 illnesses and 260 hospitalizations. Outbreaks in the five most recent incidents were attributed to cucumbers grown in protected culture (three *Salmonella* outbreaks and one *E. coli* O157:H7 outbreak) and in an open farm environment (one *Salmonella* outbreak (2, 3, 21). Illnesses from consumption of cucumber have included *Salmonella enterica* serovars Saintpaul, Oslo, Newport, and Poona. In the final update about the large multistate outbreak of *Salmonella enterica* serovar Poona associated with consumption of fresh cucumber, CDC reported that more than 907 people from 40 states had been infected with the outbreak strains of *Salmonella* Poona (4); of these, 204 ill people were hospitalized and six deaths were directly attributed to the infection (4). Epidemiology, trace-back, and isolation of multiple isolates of *S*. Poona from specific shipper labeled cucumbers at warehouse and in distribution channels, indistinguishable from case isolates, implicated a single grower and handler. The implicated cucumbers were grown in protected shadehouses (4, 21).

The domestic and global production of fruits and vegetables under various forms of protected culture has grown rapidly and provides substantial market volume to year-round availability of fresh produce. It is estimated that there are over 25,000 hectares (~61,700 acres) of protected culture production in the U.S. and Mexico alone. Much of this production is in soil-based or plastic row–covered greenhouses and shadehouses, which offer several advantages in crop protection from environmental and pest stressors, but have been shown to be intermediary in the level of risk potential for crop contamination with foodborne pathogens compared to fully open field and more sophisticated, soilless glasshouse production. Similar to open-field poled or staked production systems, most protected culture involves crops with multiple harvests over a few months period. At this time, there is very sparse science-based guidance for systematically assessing the risk of contamination, persistence, and potential for cross-contamination of fresh produce grown and harvested under protected culture. Closing this knowledge gap is critical to decision-making and application of validated corrective actions in the case of presumptive or confirmed pathogen detection in preharvest product or environmental samples. Equally, any presumptive or confirmed pathogen detection resulting from postharvest product testing or environmental monitoring in packing facilities would naturally implicate the farm source. A common response to positive postharvest pathogen detection may be to destroy the remaining shadehouse production as the practical economic loss containment decision; however, personal conversations with industry in several regions have indicated that a better knowledge foundation for die-off expectations and systematic sampling regime is needed. This knowledge may shift the risk burden to packing operations as the more plausible 'tipping-point' for outbreak-level contamination (8). There is a great deal of uncertainty regarding the immediate and long-term implications and appropriate corrective actions in the case of pathogen detection in finished product testing, or due to a mandated recall or outbreak incident, in protected culture units.

Irrespective of the details and outcomes of the root-cause investigation for the individual companies involved in the *Salmonella enterica* sv. Poona outbreak associated with fresh cucumbers (mentioned above), this incident dramatically revealed the challenges to design or define effective corrective actions and implement validated preventive controls for shadehouse fruit and vegetable producers. These would include producers of regular, roma-type, and heirloom tomatoes, sweet and pungent peppers, and cucumber. Our participation in the root-cause investigation and response to the outcomes of the outbreak environmental investigation of *S*. Poona on cucumber, conducted by FDA with the Secretariat of Agriculture, Livestock, Rural Development, Fisheries and Food in Mexico (SAGARPA) and National Agro-Alimentary Health, Safety and Quality Service (SENASICA) boldly underscored the need to develop a specific science-basis for the critical decisions necessary by any shadehouse producer and affiliated shipper to prevent, or respond and recover in the event of a detected contamination on a multiple harvest crop.

Subsequent to the recent series of outbreaks attributed to cucumber, the FDA began a surveillance testing assignment on domestic and imported cucumbers in November 2015 (21). Both cucumbers and hot peppers were tested for *Salmonella* and *E. coli* O157:H7, and hot peppers were also being tested for the broader group of Shiga toxin–producing *E. coli*. As of 2016, the FDA had collected and tested 352 domestic samples and 976 import samples (21). Eleven of 341 domestic cucumber samples were positive for *Salmonella* and none of the 352 domestic samples were positive for *E. coli* O157:H7. For the import sampling assignment, 16 of 960 cucumber samples were positive for *Salmonella* and none for *E. coli* O157:H7.

Limited literature specifically describes field-based assessments and data-based development of corrective actions, mitigations, response to positive findings, and standardization of Environmental Monitoring Programs (EMPs) under shadehouse crop production systems.

Several papers describe research outcomes in related production systems (5, 6, 12, 15, 16, 17). Côté and Quessy (2005) describe the persistence in soil and attachment of *E. coli* and *Salmonella* to pickling cucumbers under field trial conditions following application of contaminated hog manure. Interestingly, neither indicator *E. coli* nor *Salmonella* was detected from the fruit at harvest. In a study focused only on *Listeria monocytogenes,* growth up to 1.5 log on whole cucumber was reported to occur at 10–30°C and high relative humidity (5). An equally limited number of journal papers address postharvest disinfection of cucumber during washing. Yuk et al. (2006) reported on three different postharvest treatments for removal of *Salmonella* from cucumber and bell pepper fruit surfaces, including washes with chlorinated water (HOCl; 200 ppm), acidified sodium chlorite (ASC; 1200 ppm), and peroxyacetic acid (PAA; 75 ppm). Fully recognizing the sound scientific approach by the authors, the selected doses are exceptionally high for these commodities and categorically impractical and unacceptable based on personal discussions with industry. For example, test conditions reported involved using distilled water at a pH of 6.5, which would have resulted in free chlorine concentrations essentially equal to total chlorine added as fruit were purchased from a retail outlet and would have been previously washed before experimental uses. Contact times under these study conditions were 60 and 120 seconds, atypically long durations for both commodities. However, the limited log reduction at these dose conditions on fruit inoculated at wounds and stem scar tissue may provide some insights into possibilities for postharvest contributing factors in the *S.* Poona outbreak of cucumbers.

Aiming to quantify fresh produce microbial contamination factors, Ailes et al. (2008) reported significantly higher concentrations from packing shed samples compared to samples taken on-farm, further illuminating a need for contamination intervention. In relation to post-contamination survival, outcomes of EMPs conducted in the shadehouse or in the packing operation and positive detection in finished product testing would immediately prompt a whole-crop disposition decision-path. These decisions have substantial economic impact and are known to have spill-over effects to other shadehouse crops on the same farm or under the same management. Unfortunately, there is a paucity of practical, field-based and publicly available data upon which to base sound decisions, which both protect public safety and support the integrity and sustainability of the impacted producers, handlers and retailers. Our goal in this project was the development of data predicting die-off and the practical potential for post-contamination transference for shadehouse crops in the preharvest environment.

Research Methods and Results

1. Methods

Plants

Slicing cucumber, roma-type tomatoes and jalapeno pepper plants were grown from commercial seeds in University of California, Davis (UCD) greenhouses, moved to a lathe house for hardening-off, and later transferred to open-field plots at the UCD Plant Sciences Field Research Facility (**Figure 1**). Two high tunnel hoop houses were installed at the UCD Plant Sciences Field Research Facility, and plants were transplanted for subsequent inoculation of fruit (**Figure 2**). Additionally, a set of cucumber and tomato plants were transferred to a greenhouse in San Rafael, CA, at the USDA Special Facility National Ornamentals Research Site at the Dominican University of California (NORS-DUC). The trellising and trimming of plants, simulating commercial practice to the best of our situation, was conducted at least three times per week (**Figure 3**).

Objective 1

Challenge inoculation

Die-off kinetics of avirulent *Salmonella enterica* and commensal *E. coli* were determined on slicing cucumber, roma-type tomatoes and jalapeno peppers grown in both high tunnel hoop houses and the open environment at the UCD open-field plots.

Two avirulent *Salmonella* rifampicin-resistant mutants (80 mg L^{-1}) *att*PTVS 337 (*S. enterica* sv Typhimurium χ3895), attPTVS 354 (*S. enterica* sv Typhimurium LT2 MHM112) and three non-pathogenic *E. coli* rifampicin-resistant strains (TVS 353, 354 and 354) were used in all persistence/die-off inoculation studies. Strains were cultured separately and combined into a cocktail. Bacteria solutions were diluted at target inoculation concentrations of the experiment (log 6, 4 and 2 CFU/ml). Tagged fruit was inoculated using a fine sprayer bottle simulating a splash-spot contamination event. Soil-transference contamination was simulated by spot inoculation using ten 10-20 µl droplets of a mix of 1 ml of inoculum with 1 mg of Yolo silt-loam soil (native UCD field soil) applied onto the fruit/leaves.

Fruit and leaves were aseptically removed from each plant using sterilized clippers at 0, 1, 3 and 8 days post inoculation (DPI). At 0 DPI, inoculum was allowed to dry at ambient conditions, depending on the weather; 20 min to 1 hour was the interval between inoculation and harvesting. Inoculated fruit was processed as follows: 0 and 1 DPI, five fruits were processed separately; 3 DPI, four replicates of 5 fruits were processed in composite (n=20); and 8 DPI, six replicates of 5 (n=30). Five non-inoculated fruits were processed separately at 0 and 1 DPI, ten replicates of 5 fruits were processed at 3 and 8 DPI (n=50). Leaves were processed in composites of around 20 grams of plant material (3 to 6 leaves, depending on size). Five replicates were processed on 0 and 1 DPI and ten replicates on 3 and 8 DPI.

No special effort was made to prevent cross-contamination within a given plot or treatment with a specific purpose to simulate transfer potential from farm labor involved in stringing, tying, harvest, or handling. Cross-contamination assessment was conducted by harvesting and comingling inoculated and non-inoculated fruit simultaneously. Inoculated and non-inoculated fruit/leaves were harvested and placed into the same bin, as had been observed in commercial operations.

E. coli and *Salmonella* quantification and detection

For each inoculation treatment, at each time-point, inoculated and non-inoculated plant materials were individually collected in Whirl-Pak bags (Nasco, Fort Atkinson, WI) holding between 50-100 mL of potassium phosphate buffer (3.9 mM KH2PO4 and 6.1 mM K2HPO4) supplemented with 0.05% Tween 20 to facilitate bacterial removal. Sample bags were individually massaged by hand for 1 min. For enumeration, ten-fold dilutions were spread on the appropriate recovery media, including CHROMagar ECC (ECC; CHROMagar, Paris, France) and CHROMagar Salmonella supplemented with 80 mg/L rifampicin (R) and incubated for 48 h at 37°C. Membrane filtration was also performed on samples with no count or low numbers using the washate passed through a 0.45-µm IsoGrid membrane filter, and then plated on ECC+R and incubated at 37°C for 18-24 h.

Qualitative analysis of surviving populations below the normal limit of detection was carried out. Briefly, 40 mL of the remaining potassium phosphate buffer was added to 20 mL of double strength (2X) buffered peptone water (BPW; Difco) supplemented with 80 mg/L rifampicin. Samples were incubated for 24 h at 37°C. After incubation, enrichments were spot inoculated onto ECC and XLT4 supplemented with rifampicin. Plates were incubated for 24-48 h at 37°C.

Cross-contamination during manipulation of inoculated fruit and leaves at harvest

Cross-contamination risk assessment was evaluated by collecting and processing disposable nitrile gloves, clippers and bins used for harvesting. After harvesting, gloves and clippers were

put into sterile bags. Once fruits and leaves were processed, sterile sponges were used to sample binds and clippers. Sponges and gloves were processed for quantitative detection of *E. coli* and *Salmonella* as described below.

Objective 2

The sampling studies of indicator bacteria in commercial shadehouses in San Quintin, Baja (MX) could not be carried out. Coordination of the experiment's logistics and assessment of availability of crops were done in October 2019 in Baja MX; however, delays in getting official permits for entry of non-pathogenic surrogate *E. coli* to Mexico forced the team to suspend the planned schedule. The next crops available for the study growing in shadehouses of the commercial partners are available at the beginning of May 2020. The team was planning to carry out this part of the study with support of the industry. (Note: These plans are currently on hold due to work and travel restrictions associated with the ongoing COVID-19 pandemic.)

Objective 3

Preharvest assessment of registered food contact surface sanitizers

Preharvest experiments assessing two registered antimicrobial formulations were conducted twice under containment conditions: PURE® Hard Surface, an EPA registered food contact surface sanitizer and disinfectant, containing the patented Silver Dihydrogen Citrate (SDC) molecule; and Oxidate 5.0 (BioSafe Systems, East Hartford, CT), a hydrogen peroxide formulation widely used in high tunnel protected culture for bacterial disease control. Data were subjected to JMP 12.0 (SAS Institute, Cary, NC) for analysis of variance and Duncan's multiple range tests to determine significant differences (α=0.05) between mean values.

Experiment 1: Tomato plants growing at the San Rafael greenhouse (NORS DUC at Dominican University) were spray inoculated with attenuated *Salmonella* and generic *E. coli* cocktail (described above) at 7.70 log CFU/ml. Inoculum was allowed to dry, and fruit and leaves were collected. After 2 DPI, sanitizer treatments were applied following the manufacture instructions. Ten replicates of 5 fruits (n=50) and five of 5 leaves (n=25) were used for each treatment. Five replicates of 5 fruits and leaves (n=25) not treated with sanitizers were used as controls.

PURE® Hard Surface was fine-misted onto fruit and leaves using an airbrush and after 2-minute contact the samples were collected and placed into a sterile bag containing neutralizing buffer. Two different concentrations of Oxidate 5.0 were tested (0.39% and 1%). Solutions were freshly prepared before each experiment using sterile nanopure water, and plants were treated using a fine sprayer. After around 45 min (sanitizer completely dried as recommended by manufacturer), samples were collected and placed into sterile bags and processed immediately for bacteria quantification-detection, as described above (**Figure 4**). An average of ten replicates was taken for calculating log reduction achieved by the disinfectant for each organism. Log reduction was calculated by using the following formula:

Estimated Log10 Reduction Factor (ELR) = Log10 control – Log10 post-treatment

Experiment 2: Jalapeno pepper plants planted in high tunnel hoop houses installed at the UCD Plant Sciences Field Research Facility were used for this experiment. (**Figure 5**). Plants were inoculated with cocktail of attenuated *Salmonella* and generic *E. coli* (described above) at 7.40 log CFU/ml. Plants at 24 h post-inoculation were treated as described above; the only difference with Experiment 1 was that the contact period was 24 hours.

Evaluation of conditions contributing to potential amplification of contamination in distribution

Assessment of presumptive conditions contributing to potential amplification of contamination was conducted. Evaluation of bacteria transfer from soil, drip lines, and harvest totes was

carried out through spot inoculation. Additionally, dip inoculation was performed to evaluate early season fruit imperfections, handling and wash-line injury, waxing, and MAP shipping bags during temperature shifts (**Figure 6**). This assessment was conducted twice.

Spot inoculation
Cucumbers were harvested from the UCD field at the Research Facility and transferred to room temperature (20 or 30°C) for tempering prior to use. Fruits were individually spot-inoculated by circling three areas along the length of the cucumber and adding 30 µl of 4.0 log CFU/ml of an outbreak isolate of rifampicin-resistant *Salmonella* Poona (PTVS 026) per area (90 µl total per fruit), as shown in **Figure 7A**. Cucumbers were left to dry at room temperature for 3-4 hours and fruits were then stored at 15 and 29°C. At designated times during the storage (0, 1, 5 DPI) fruits were sampled for *Salmonella* quantification/detection and the entire experiment was conducted twice (4 cucumbers per time point for each temperature).

Dip inoculation
Cucumbers were harvested from the UCD field and transferred to room temperature (20 or 30°C) for tempering prior to use. Inoculation was performed by submerging each cucumber in a water bath containing 3.0 log CFU/ml of *S*. Poona PTVS 026 for 30 seconds (**Figure 7B**). Cucumbers were left to dry at room temperature for 2-3 hours. The fruits were divided into three main groups: the first group was sprayed with undiluted Decco Vegetable Lustr® 277f coating wax and placed into sealed MAP bags (**Figure 7C**); the second group was treated with wax but not bagged; and the third group was not waxed or bagged. Fruits from each group were further divided into replicates of nine and stored at 10, 15, 20, and 29°C. At designated times during the storage (0, 2, 6 DPI), fruits were sampled for *Salmonella* quantification/detection. The experiment was conducted twice (3 cucumbers per time point for each temperature). Additionally, a subset of 6 inoculated fruits were waxed and bagged, and stored at 10°C for 14 days. Also, an experiment was conducted with fruits brushed before the inoculation step to simulate possible wash-line injury.

2. Results

Objective 1
Die-off assessments of *Salmonella* and *E. coli* in cucumbers, roma-type tomatoes and jalapeno peppers were conducted in the open-field environment and hoop shadehouses at the UCD Plant Sciences Field Research Facility. In total, 25 separate experiments simulating two possible contamination routes, irrigation (spray inoculation) and soil transfer (spot inoculation), and spanning three different contamination levels (around 6, 4 and 2 log CFU/ml) were carried out. Thirteen experiments were conducted on plants in the open-field environment, and 11 experiments in hoop shadehouses at UCD Plant Sciences Field Research Facility. Most plants were exposed to the sun, although various tomato experiments were conducted on partially shaded plants (**Table 1**).

A. Open-field experiments:
Quantitative and qualitative survival of *E. coli* and *Salmonella* in the open-field environment was assessed in 13 separate experiments, with five, six and two experiments conducted on cucumber, tomato and jalapeno plants, respectively.
Cucumber plants
Five experiments on cucumbers were conducted between July and August. Fruits and leaves were spray inoculated at 5.6 (Exp 1), 4.5 (Exp 3), and 2.7 (Exp 5) log10 CFU ±0.5/ml. Spot

inoculation (soil) at 5.2 (Exp 2) and 3.5 (Exp 4) log10 CFU ±0.5/ml was carried out. Plants were exposed to direct sun most of the day. Recovery data are presented in **Table 2A**.

In Experiment 1, *E. coli* and *Salmonella* populations at inoculation (0 DPI) were 2.68 and 2.99 log10 CFU per cucumber, respectively. Around 3.0 log10 CFU in leaves was recovered for both *Salmonella* and *E. coli*. In addition, both bacteria were detected in non-inoculated fruit at similar levels (1.21 log10 CFU). A considerable decrease in bacteria population on fruit (0.8 log10 CFU) and leaves (~1.0 log10 CFU) was observed at 1 DPI. From 3 DPI, neither *E. coli* nor *Salmonella* could be quantified on fruits, leaves, and non-inoculated cucumbers; nonetheless 75% and 25% of enrichments from inoculated cucumbers were positive for *E. coli* and *Salmonella*, respectively, and 30% of enrichments from leaves were positive for both bacteria. *Salmonella* was detected in 10% of enrichments of non-inoculated cucumbers. At 8 DPI, 83% of enrichments from inoculated fruit and 50% of enrichments from leaves were still positive for *E. coli*, but all non-inoculated fruit were negative.

In Experiment 2, counts of *E. coli* (2.46 log10 CFU) and *Salmonella* (3.0 log10 CFU) from inoculated fruit at 0 DPI were similar to those observed in experiment 1, however counts almost 1.0 log10 CFU higher were observed on non-inoculated fruits. In contrast, recovery from leaves was around 1.0 log10 CFU lower than found in experiment 1: *E. coli* (2.16 log10 CFU) and *Salmonella* (1.97 log10 CFU). Similarly, at 3 DPI only some enrichments were positive (20 to 50%) and cross contamination was not observed.

For Experiment 3, as expected, lower bacterial counts and fewer positive enrichments were observed from inoculated fruits and leaves than in experiments 1 and 2. At 0 DPI bacteria was quantifiable only on leaves. Additionally, cross-contamination of non-inoculated fruit was not observed. At 8 DPI all enrichments were negative for both *E. coli* and *Salmonella*.

In Experiment 4, at inoculation day (0 DPI) around 1.0 log10 CFU of both *E. coli* and *Salmonella* were recovered from inoculated fruit and close to 3.0 log10 CFU from leaves. Additionally, cross contamination was detected on non-inoculated fruit enrichments; 20% were positive for *E. coli* and 60% were positive for *Salmonella*. At 1 DPI all samples were negative, however at 3 DPI 10% of enrichments from leaves were positive for *E. coli.*

In Experiment 5, the lower inoculation challenge dose was used. At inoculation day (0 DPI) *E. coli* and *Salmonella* counts from fruits were under the limit of detection (0.65 log10 CFU), nonetheless 20% of enrichments were positive for both bacteria, and leaves had counts slightly above the limit of detection (0.87 log10 CFU). Moreover, 10% of enrichments were positive for *E. coli* and 40% were positive for *Salmonella*. Even with lower survival at 1 DPI (end point of this experiment) 20% of enrichments from fruit and leaves were positive for *Salmonella*.

Tomato plants

Quantitative and qualitative survival of *E. coli* and *Salmonella* on inoculated tomato plants in the open-field environment was assessed in six separate experiments. All experiments were carried out in August. Four of the six experiments were conducted through spray inoculation at different levels of inoculation: 5.74 (Exp 6), 6.49 (Exp 7), 3.77 (Exp 9) and 4.5 (Exp 10) log10 CFU ±0.5/ml. Spot inoculation was applied in two experiments at 6.49 (Exp 8) and 4.50 (Exp 11) log10 CFU ±0.5/ml. Various tomato plants growing at the UCD field were under continuous direct sunlight while other plants were only partially exposed due to orientation shading of the 1-m staked and strung vines. Recovery data are presented in **Table 2B**.

Experiment 6 was conducted on the side of plants exposed to the sun. *E. coli* and *Salmonella* populations at inoculation (0 DPI) were lower than expected at 1.26 and 1.49 log10 CFU per tomato, respectively. Lower bacteria recovery from leaves was also observed, around 1.50 log10 CFU. At 1 DPI bacteria, counts were just above the limit of detection and all non-

inoculated fruits were negative. At 3 DPI between 30-75% of enrichments from inoculated fruits and leaves were positive for *E. coli* and *Salmonella*. At 8 DPI only 50% of enrichments from inoculated fruit were positive for *E. coli* and enrichments from other samples were negative.

In Experiment 7, the same challenge inoculation as in Experiment 6 was carried out, however plants were partially shaded by the vine. Recovery at 0 DPI was higher than in Experiment 6 and similar to Experiment 1 (cucumbers). Cross contamination on non-inoculated fruit was observed at 0 DPI in 100% of enrichments for *E. coli* and in 40% of enrichments for *Salmonella*. At 3 DPI, cross contamination was detected only in 10% of *Salmonella* enrichments. Only *Salmonella* enrichments from leaves at 8 DPI were negative.

For Experiment 8, spot inoculation was applied on partially shaded plants. Counts of *E. coli* (3.48 log10 CFU) and *Salmonella* (3.43 log10 CFU) from inoculated fruit at 0 DPI were higher than observed in experiments 6 and 7. Bacteria recovery from leaves was similar to experiment seven (~3.6 log10 CFU). High cross-contamination was observed, around 2.0 log10 CFU from both *E. coli* and *Salmonella* was detected on non-inoculated tomatoes. At 1 DPI 1.56 (*E. coli*) and 1.80 (*Salmonella*) log10 CFU per fruit was recovered, 20% of non-inoculated enrichments were positive for *E. coli,* and in leaves samples both bacteria were quantifiable ~2.3 log10 CFU. At 3 DPI *E. coli* recovery on fruit and leaves were ~2.0 log10CFU, however Salmonella counts and positive enrichments on inoculated fruits and leaves were considerably lower than *E. coli*. Surprisingly at 8 DPI *E. coli* and *Salmonella* were quantifiable on fruit and leaves, but no cross contamination was observed.

In Experiments 9 and 10, plants were spray inoculated with around 4.0 log10 CFU, however plants used for Experiment 9 were exposed to direct sun whereas plants for Experiment 10 were partially shaded. Bacteria counts from Experiment 9 were lower than those from Experiment 10. At 0 DPI all inoculated fruit and leaves were under the limit of detection and only a small percentage of the enrichments were positive. Similar outcomes were observed at 1 DPI, and all samples were negative at 3 and 8 DPI. No cross contamination was detected. In contrast, from Experiment 10, bacterial counts at 0 DPI were ~1.50 log10 CFU and 20% of enrichments from non-inoculated tomatoes were positive. At 1 DPI, 60% of enrichments from inoculated fruits were positive for *E. coli,* nonetheless, at 3 DPI 30% of enrichments from leaves showed a positive outcome for both *E. coli* and *Salmonella*. Enrichments of inoculated fruits and leaves from 8 DPI were positive, while cross contamination to non-inoculated fruit was not observed.

Experiment 11 evaluated spot inoculation at ~4.0 log10 CFU on partially shaded plants. At 0 DPI around 1.70 and 2.30 log10 CFU of both *E. coli* and *Salmonella* were recovered from inoculated fruit and leaves, respectively. Cross contamination of non-inoculated fruit was detected from 100% of enrichments for *E. coli* and 20% of enrichments for *Salmonella*. At 1 DPI, counts of *E. coli* and *Salmonella* on inoculated fruit were ~1.40 log10 CFU while 0.83 and 1.13 log10 CFU, respectively, were detected on inoculated leaves. Twenty percent of enrichments from non-inoculated fruit were positive for *Salmonella*. Inoculated fruits at 3 DPI were negative for *E. coli* but 20% of inoculated leaves were positive, while 25% of enrichments from inoculated fruit were positive for *Salmonella* but all inoculated leaves were negative. At 8 DPI, 16% and 10% of enrichments were positive for *E. coli* from inoculated fruit and leaves, respectively. Enrichments from inoculated fruit were negative for *Salmonella* but 10% of leaves were still positive.

Jalapeno pepper plants

Two experiments were conducted on jalapeno pepper plants with no shading, as these are shorter stature plants, in the open-field environment during late October to early November. Recovery data are presented in **Table 3**.

In Experiment 12, plants were sprayed with 6.92 log10 CFU. At 0 DPI, bacterial counts were ~3.22 log10 CFU and ~4.13 log10 from inoculated fruit and leaves, respectively. High cross-contamination was observed from non-inoculated peppers, around 1.5 log10 CFU from both *E. coli* and *Salmonella*. At 1 DPI, bacteria recovery from inoculated fruit and leaves was around 1.0 log10 CFU; from non-inoculated fruit, 20% of enrichments were positive for *Salmonella*. At 3 DPI, bacteria were below the limit of quantification, however enrichments from inoculated fruit were positive and 60% of non-inoculated fruit were positive for *Salmonella*. At the end point of the experiment (8 DPI), enrichments were positive for all samples except for *Salmonella* from non-inoculated fruit.

Experiment 13 evaluated spray inoculation at 4.0 log10 CFU. At inoculation day (0 DPI), *E. coli* and *Salmonella* counts from inoculated fruit and leaves were ~1.0 to 1.8 log10 CFU and non-inoculated fruit showed 20% and 60% of enrichments positive for *E. coli* and *Salmonella*, respectively. At 1 DPI, only 20% of enrichments from inoculated samples were positive for *Salmonella*; in contrast, at 3 DPI enrichments were positive only for *E. coli.* At 8 DPI, enrichments from inoculated samples were positive for both *E. coli* and *Salmonella*.

B. Hoop-house experiments

Quantitative and qualitative survival of *E. coli* and *Salmonella* in hoop houses was assessed in 12 individual experiments, with three, four and five experiments conducted on cucumbers, tomato and jalapeno plants, respectively.

Cucumber plants

Three experiments were conducted on cucumber plants growing in hoop houses between September and November, evaluating spray inoculation at 6.11 (Exp 16), 4.63 (Exp 19) and 4.17 (Exp 24) log10 CFU. Recovery data are presented in **Table 4.**

In Experiment 16, counts of *E. coli* and *Salmonella* at inoculation (0 DPI) were around 3.79 and 4.07 log10 CFU per cucumber, respectively. Approximately 3.7 log10 CFU of both *Salmonella* and *E. coli* were recovered from inoculated leaves, and both bacteria were detected in non-inoculated fruit at similar levels (~1.70 log10 CFU). At 1 DPI, around 2.5 log10 CFU bacteria was recovered from inoculated fruit and leaves and 1.7 log10 CFU from non-inoculated fruit. At day 3 DPI, bacteria counts from inoculated fruit and leaves were ~2.0 and 0.96 log10 CFU, respectively. Cross contamination from non-inoculated fruit was observed (~0.77 log10 CFU). In contrast, at the end point of the experiment at 8 DPI, counts from inoculated fruit were below the limit of quantification while ~1.51 (*E. coli)* and 1.04 (Salmonella) log10 CFU were recovered from leaves and 40% of enrichments from non-inoculated fruit were positive for *Salmonella*.

In Experiments 19 and 24, plants were sprayed at around 4.0 log10 CFU. Experiment 19 was conducted in September and experiment 24 in November. In both experiments, higher counts of *E*. *coli* and *Salmonella* were observed at the end point of the experimental period. At 0 DPI, inoculated fruit and leaves had counts between 2.62 to 3.31 log10 CFU and non-inoculated fruit had counts around 0.65 log10 CFU. In experiment 19 at 1 DPI, bacteria recovery from inoculated fruit and leaves was ~ 1.0 and 0.89 log10 CFU, respectively, and only 60% of enrichments from non-inoculated fruit were positive for *Salmonella*. At 3 DPI, *E. coli* and *Salmonella* levels from inoculated fruit were 1.20 and 2.05 log10 CFU, respectively, and only enrichments from leaves and non-inoculated fruit were positive. At 8 DPI, however, considerable growth was observed on inoculated fruit, with counts ~4.11 and 2.91 log10 CFU for *E. coli* and *Salmonella*, respectively, and high levels of cross contamination were detected at ~1.73 (*E. coli*) and 0.76 (*Salmonella*) log10 CFU. Bacteria recovery was below the level of quantification from leaves, but enrichments were positive.

In Experiment 24 at 0 DPI, ~3.0 log10 CFU was observed from inoculated fruit and leaves. Bacteria counts were ~2.0 log10 CFU at 1 and 3 DPI. At 8 DPI, *E. coli* counts from inoculated fruit and leaves were 4.83 and 3.47 log10 CFU, respectively. Similarly, *Salmonella* counts were 3.87 and 3.71 log10 CFU from fruits and leaves, respectively. And a high occurrence of cross contamination was observed in non-inoculated fruit: 3.28 log10 CFU *E. coli* and 2.28 log10 CFU *Salmonella* was detected.

Tomato plants

Quantitative and qualitative survival of *E. coli* and *Salmonella* on inoculated tomato plants in hoop houses was assessed in four experiments conducted at the same time. All experiments were carried out during October. Two experiments were conducted through spray inoculation at levels 5.86 (Exp 20) and 4.46 (Exp 21) log10 CFU, and two additional experiments evaluating spot inoculation at levels 5.97 (Exp 22) and 4.28 (Exp 23) log10 CFU. Recovery data are presented in **Table 5**.

In Experiments 20 and 22, plants were inoculated at a similar level but with a different type of inoculation (20, spray: and 22, spot). In general, similar results were observed. At 0 DPI, *E. coli* and *Salmonella* populations were ~4.0 log10 CFU from inoculated fruit and leaves. Bacteria recovery from non-inoculated fruit was close to 3.0 log10 CFU per fruit. In both experiments at 1 DPI the bacterial counts were comparable, around 2.78 to 4.07 log10 CFU from inoculated samples. Higher counts of *E. coli* and *Salmonella* were observed in Experiment 22 from non-inoculated fruit (~1.20 and 1.54 log10 CFU, respectively) in comparison to experiment 20 (~0.67 and 0.90 log10 CFU, respectively). At 3 DPI, similar bacteria recovery from inoculated fruit (~3.4 log10 CFU) and leaves (2.3 log10 CFU) was observed in both experiments; however from non-inoculated fruit in experiment 20 the bacteria counts were ~2.0 log10 CFU whereas in Experiment 22 cross contamination was below the limit of quantification and only enrichments were positive. At the end point of the experiment at 8 DPI, similar recovery was observed from inoculated fruit and leaves in both experiments, ~ 2.0 and 1.0 log10 CFU for *E. coli* and *Salmonella*, respectively. Despite the relatively high bacteria counts from inoculated samples, cross contamination in non-inoculated fruit was only detected through enrichments. More enrichments were positive from Experiment 20 than from 22.

Experiments 21 (spray) and 23 (spot) evaluated ~4.0 log10 CFU inoculation. Overall, similar outcomes were observed in both experiments although bacteria seemed to survive/grow better when spot inoculation was conducted. At 0 DPI, ~2.2 log10 CFU were recovered from inoculated fruit and leaves. Cross contamination from non-inoculated fruit was observed between 0.67 and 0.76 log10 CFU per fruit. In both experiments at 1 DPI, around 1 log reduction was observed in inoculated fruit, however in Experiment 23 lower bacteria counts were observed on inoculated leaves (~0.85 log10 CFU) than in Experiment 21 (~1.90 log10 CFU). Cross contamination in non-inoculated fruit was detected through enrichments in both experiments, but only *E. coli* was recovered from Experiment 23 (30%). At 3 DPI, bacteria recovery from inoculated fruit and leaves was slightly lower than at the previous time point in Experiment 21, however recovery from leaves was below the limit of quantification for Experiment 23 and only enrichments were positive. Cross contamination in non-inoculated fruit was detected through enrichments in both experiments. At 8 DPI in Experiment 21, bacteria counts from inoculated fruit were very close to the limit of quantification and only a portion of enrichments were positive. In contrast, ~3.86 and 2.24 log10 CFU of *E. coli* and *Salmonella* were recovered in Experiment 23. In both experiments, only enrichments were positive from the leaves. Cross contamination was observed in both experiments but only *Salmonella* was detected through enrichment in Experiment 21 while *E. coli* was observed in experiment 23.

Jalapeno pepper plants

Five experiments were conducted on jalapeno plants growing in hoop houses between September and November. Spray inoculation was assessed at 6.63 (Exp 14), 4.54 (Exp 15) and 2.72 (Exp 25) log10 CFU, and spot inoculation at levels 6.64 (Exp 17) and 4.64 (Exp 18) log10 CFU. Recovery data are presented in **Table 6**.

In Experiments 14 (spray) and 17 (spot), plants were inoculated at a similar level ~6.60 log10 CFU. At 0 DPI, bacteria populations were between 3.22 to 4.37 log10 CFU from inoculated fruit and leaves, while almost 2.0 log10 CFU were detected in non-inoculated fruit. In both experiments at 1 DPI, bacteria counts were comparable: around 1.09 and 1.75 log10 CFU of *E. coli*, and between 1.19 and 2.54 log10 CFU of *Salmonella* were recovered from inoculated fruits and leaves. Cross contamination was only observed in Experiment 17, where 40% of enrichments were *Salmonella* positive. At 3 DPI, *E. coli* levels (~3.00 log10 CFU) and *Salmonella* (2.40 log10 CFU) from inoculated fruit were similar in both experiments. Nonetheless, lower bacteria counts from leaves were observed in Experiment 14, and for *Salmonella* only 30% of enrichments were positive. No cross contamination was observed in non-inoculated fruit in samples from Experiment 14, while 40% (*E. coli*) and 20% (Salmonella) of enrichments were positive in Experiment 17. At 8 DPI in both experiments, *E. coli* levels (~4.10 and 3.64 log10 CFU) from inoculated fruit were slightly higher than *Salmonella* (~2.82 and 2.98 log10 CFU). In leaf samples, bacteria were only detectable through enrichment; *Salmonella* transference was not detected in Experiment 14. Cross contamination was observed in both experiments; however, levels were lower in Experiment 14, as only 10% of enrichments were *E. coli* positive and *Salmonella* was not detected.

Experiments 15 (spray) and 18 (spot) evaluated ~4.5 log10 CFU inoculation. At 0 DPI, ~1.68 and 1.65 log10 CFU of *E. coli* and *Salmonella,* respectively, were recovered from inoculated fruit in Experiment 15. Slightly higher counts were observed from Experiment 18, as 2.58 (*E. coli*) and 2.66 (*Salmonella*) log10 CFU were recovered. Moreover, in Experiment 15, inoculated leaves were observed to have lower counts 2.12 (*E. coli*) and 1.86 (*Salmonella*) log10 CFU in comparison to Experiment 18 which had ~2.90 log10 CFU recovery for both bacteria. Cross contamination in non-inoculated fruit was observed at ~1.00 log10 CFU per fruit in both experiments. At 1 DPI in both experiments, ~ 1 log reduction was observed in inoculated fruit; however, lower bacteria counts were observed on inoculated leaves in Experiment 15 (~0.92 log10 CFU) than in Experiment 18 (~1.40 log10 CFU). Cross contamination in non-inoculated fruit was detected through enrichments only in Experiment 18 (20% of enrichments). At 3 DPI, higher bacteria recovery from inoculated fruit and leaves was observed in Experiment 18; leaves were below limit of quantification in Experiment 15 and only 20% (*E. coli*) and 40% (*Salmonella*) of enrichments were positive. Similarly, greater cross contamination was observed in Experiment 18. At 8 DPI, similar levels of bacteria were observed from inoculated fruit in both experiments, with ~2.71 and 1.8 log10 CFU of *E. coli* and *Salmonella* recovered in Experiment 15, while ~1.48 and 2.02 log10 CFU were observed in Experiment 18. Nevertheless, leaf samples from Experiment 15 were below the limit of quantification and only 10% of enrichments were positive for *Salmonella*. In contrast, 0.93 (*E. coli*) and 1.63 (*Salmonella*) log10 CFU were recovered from leaves in Experiment 18. As expected, greater cross contamination at ~40% for both bacteria was observed in Experiment 18, while Experiment 15 samples were negative.

Experiment 25 assessed low level of contamination at ~2.72 log10 CFU. At 0 DPI, ~2.31 and 1.35 log10 CFU of *E. coli* and *Salmonella* were recovered from inoculated fruit. Bacteria counts from leaves were ~1.15 log10 CFU, and a low level of cross contamination was observed in non-inoculated fruit, with only of 10% of enrichments positive for *E. coli*. At 1 DPI (end point of this experiment), bacteria survival declined to very low levels; 0.87 log10 CFU of *E. coli* was detected from inoculated fruit and 0.71 log10 CFU of *Salmonella* from leaves. Also 60% and

20% of enrichments from inoculated fruit were positive for *E. coli* and *Salmonella*, respectively. Cross contamination was detected through enrichments, with 30% positive for *Salmonella*.

C. Cross contamination during manipulation of inoculated fruit and leaves at harvest
Cross-contamination risk assessment was evaluated by collecting and processing disposable nitrile gloves, clippers and bins used for harvesting. Samples were processed for quantitative detection of *E. coli* and *Salmonella.*

Results obtained from qualitative enrichment analyses are shown in **Table 7**. As expected at 0 DPI, a considerable number of samples, including bins, gloves and clippers, showed positive outcomes. Overall, more enrichments were positive for *E. coli* than for *Salmonella*, which could be a product of nutrition competition during enrichment. A decrease in the number of positive enrichments was observed from 1 DPI, especially in experiments conducted in the field. *E. coli* recovery from bins was observed until 8 DPI in experiments conducted in hoop houses.

Objective 3
Efficacy of various corrective actions to minimize the risk of transference and minimize persistence of bacterial pathogens within and on the standing crop was assessed. Roma-type tomato plants were grown in a containment greenhouse in San Rafael, CA (NORS DUC).

A. Preharvest assessment of registered food contact surface sanitizers
Two separate preharvest experiments assessing two registered antimicrobial formulations (PURE® Hard Surface and Oxidate 5.0) were conducted**.**

Tomato plants (*Experiment 1*) growing at the San Rafael greenhouse, and jalapeno peppers (*Experiment 2*) growing at hoop houses at the UCD Facility were spray inoculated with attenuated *Salmonella* and generic *E. coli* cocktails (described above) at 7.70 log10 CFU/ml. In the first experiment the sanitizer contact times recommended by the manufacturers were assessed: 2 and 45 minutes for PURE® Hard Surface, and 45 min for Oxidate 5.0. In the second experiment, the sanitizer contact time was 24 hours.

Mean counts of surviving *E. coli* and *Salmonella* from tomato (*Experiment 1*) and jalapeno pepper (*Experiment 2*) leaves after sanitizer application are presented in **Table 8** and **Table 9**, respectively The initial load of bacteria used for inoculation in the experiments was determined to be 7.7 log CFU/ml. The control data show the numbers of *E. coli* and *Salmonella* on non-treated fruit and leaves at the time of sanitizer treatments.

Compared with results from the controls, inoculated tomato fruit and leaves treated with PURE Hard Surface had no reduction in the amount of *E. coli* and *Salmonella* bacteria recovered (Table 8). Treatment with Oxidate 5.0 resulted in some reductions of *E. coli* and *Salmonella* in comparison to both the control and PURE Hard Surface treated samples. Compared with results for controls, 0.39% Oxidate application resulted in *E. coli* and *Salmonella* ELRs (estimated log reductions) of 1.5 and 2.3 log10 CFU on tomato leaves ($P \leq 0.05$). However, this application did not seem to have a significant effect on additional log reduction of bacterial populations on tomato fruit. Interestingly, the application of 1% Oxidate 5.0 resulted in measurable *Salmonella* ELR on fruit and leaves (3.1 and 1.9 log10 CFU, respectively) but did not have an effect on *E. coli*.

Populations of *E. coli* and *Salmonella* were reduced by less than 1 log on jalapeno peppers and leaves treated with PURE Hard Surface and 0.39% Oxidate for 24 h compared with the results from controls (Table 9). In contrast, 1% Oxidate application resulted in ELRs for *E. coli* and

Salmonella of 1.3 and ~1.8 log10 CFU on jalapeno fruit and leaves, respectively ($P \leq 0.05$). Bacteria reductions may be attributed to the longer contact time (24 h).

B. Evaluation of conditions that can contribute to potential amplification of contamination in distribution

Two separate experiments assessing presumptive conditions contributing to potential amplification of contamination were conducted.

Spot inoculation experiments

In both experiments, actual inoculum concentration was around 3.9 log10 CFU/ml. Inoculated cucumbers were left to dry at room temperature for 3-4 hours, and the fruits were stored at 15 and 29°C for up to 5 days. Overall, similar results were observed in both experiments, however slightly higher *Salmonella* Poona populations were observed at 0 DPI in experiment 1 (**Table 10**). Populations of *Salmonella* were below the detection limit by the end of five days of storage at both storage temperatures. No viable bacteria were detected through enrichment at 5 DPI.

Dip inoculation experiments

Two different experiments evaluated survival of *Salmonella* Poona (at two inoculation levels) on dip inoculated non-brushed cucumbers after 6 days storage (**Figure 8**). Normal variation between both experiments was observed, with *Salmonella* populations around 1 log lower in experiment 2 than in experiment 1. Waxed cucumbers and those also kept in modified atmosphere packaging (MAP) bags showed higher *Salmonella* populations.

The average surviving *S*. Poona populations on dip-inoculated cucumber at 0 DPI were ~1.83 and 0.85 log10 CFU/fruit for experiments 1 and 2, respectively. Lower populations were observed on waxed cucumbers at ~1.39 and 0.68 log10 CFU/fruit from experiments 1 and 2, respectively. After 2 days storage at three different temperatures, *S*. Poona counts on the unwaxed cucumbers (No Wax + No bag) decreased to levels below or close to the detection limit of the plate count method (**Figure 8A** and **C**). However, by the end of the 6 days of storage at 20°C, almost 2.0 log10 CFU/fruit of *S*. Poona was observed on these unwaxed cucumbers in Experiment 1 (**Figure 8B**).

In Experiment 1, populations of *S*. Poona on waxed but not bagged (Wax + No bag) cucumbers were higher after 6 days storage at 20 and 29°C (2.69 log10 CFU/fruit) than at 15°C (Figure 8B). However, in Experiment 2 at 6 DPI, *Salmonella* populations were under limit of detection.

Regardless of storage temperature, cucumbers waxed and kept in MAP bags (Wax + MAP bag) showed considerable *S*. Poona growth. After 6 days at 20°C, 4.2 log10 CFU/fruit of *S.* Poona was recovered from these cucumbers, which represented a 2.80 log10 CFU/fruit increase (Experiment 1). Although the number of viable cells was not as high in Experiment 2, stable populations of *Salmonella* (1.5 log10 CFU/ fruit) were observed from 2 DPI to 6 DPI. For storage at 29°C, populations of *S*. Poona on cucumbers (Wax + MAP bag) were around 3.77 and 3.10 log10 CFU/fruit at 2 DPI for experiments 1 and 2, respectively. At 6 DPI, 2.7 and 1.59 log10 CFU/fruit were observed. As expected, *Salmonella* recovery after 6 days at 15°C was lower than at higher temperatures, but nonetheless notable at 2.4 and 1.2 log10 CFU/fruit from Experiment 1 and 2, respectively (Figure 8A).

Comparison of survival of *Salmonella* Poona on dip inoculated non-brushed (Experiment 1) vs brushed cucumbers after 6 days storage is presented in **Figure 9**. The average surviving *S*. Poona populations on dip inoculated brushed cucumbers at 0 DPI were 2.44 and 1.76 log10 CFU/fruit on non-waxed and waxed fruit, respectively. Similar *Salmonella* counts were observed from non-brushed cucumbers in Experiment 1. In general, slightly lower *S*. Poona survival/growth was observed on non-brushed cucumbers at 20°C. Nonetheless, results from

No Wax + No Bag cucumbers after 2 days storage showed *S*. Poona counts slightly higher on brushed cucumbers (1.50 log10 CFU/fruit) (**Figure 9A**) than on non-brushed cucumbers (0.65 log10 CFU/fruit). At 6 days of storage, counts from these brushed cucumbers stored at 20 and 29°C were higher than those observed on non-brushed cucumbers.

Levels of *S*. Poona on waxed but not bagged brushed cucumbers (Wax + No MAP bag) after 6 days storage at 15°C were 1.64 log10 CFU/fruit, while at 20 and 29°C the counts were below the detection limit of the plate count method (**Figure 9B**).

After 6 days of storage, *S*. Poona counts on waxed and bagged brushed cucumbers (Wax + MAP bag) were 2.4 log10 CFU/fruit at 15°C and 3.27 log10 CFU/fruit at 20°C, but at 29°C the counts were below the detection limit (**Figure 9C**).

Outcomes and Accomplishments

This project faced substantial difficulties due to Research Farm management by the UC Davis Plant Sciences Department and repeated turn-over in Research Farm and greenhouse supervisory and assistant superintendent staffing during 2018 that effectively prevented the opportunity to complete any objectives that year. The inadequate research plot land preparation, most significantly pre-plant weed management, and insufficient pest control during crop development did not support enough fruit quality or numbers to conduct research during 2018. Given the circumstances, the original project had to be modified to achieve as much as possible in one year against the originally proposed objectives.

Despite the challenges, this project managed to achieve and provide science-based die-off kinetics data of *E. coli* and *Salmonella* under field and protected produce production (hoop houses) on three different crops. Collectively in these studies, the potential for post-contamination transference for shadehouse crops in the preharvest environment and, especially, during harvest was assessed in 25 experiments conducted within a very narrow time window during 2019 by relying on great team effort from academic, technical and laboratory assistant personnel available. This UCD research team gained considerable transferable knowledge on crop management and increased expertise in the execution of controlled open-field and protected production research projects applying attenuated bacterial pathogens.

Assessment of the efficacy of corrective actions to minimize contaminant persistence and transference in preharvest environment was greatly hampered by limited follow-through support from the original industry cooperators but managed to evaluate two different experiments conducted on tomato and jalapeno plants. Three different preharvest sanitizer treatments were evaluated, and 1% Oxidate 5.0 treatment with 24-h contact time was shown to significantly lower the number of viable bacteria on treated plants. Although PURE Hard Surface sanitizer has shown to be effective on food contact surfaces it did not seem to have a significant effect on *E. coli* and *Salmonella* population reduction on tomato and jalapeno pepper plants under the conditions tested. Additional studies and delivery systems are worth pursuing.

Postharvest circumstances, such as transfer from soil, drip lines, early season fruit imperfections, wash-line injury, waxing and MAP shipping bags, can contribute to the amplification of *Salmonella* contamination. Results from the microbiological assessments in this study indicated that waxing and MAP bags can increase the persistence and growth of *Salmonella*, depending on storage temperature.

This study provides field-based assessments and data-based development of corrective actions, mitigations, response to positive findings under shadehouse crop production systems. However, assessment of monitoring programs and EMP standardization are planned to be

conducted in May, once crops growing in commercial shadehouses are available for the study. Industry collaborators in Baja MX recognize the scientific and practical value of information that objective 2 will provide to the sector and have stated that will do as much as possible to support our efforts to complete this part of the study.

Summary of Findings and Recommendations

- This study provides science-based die-off kinetics data of *E. coli* and *Salmonella* under field and protected production (hoop houses) on cucumbers, tomatoes and jalapeno peppers.
- Recovery of both *E. coli* and *Salmonella* from crops growing in the open-field environment showed a rapid decline within the first day after inoculation; however surviving bacteria may establish and persist at very low cell numbers that require concentration or enrichment for their detection (**Figure 10**).
- Results from studies on the behavior of *E. coli* and *Salmonella* inoculated onto cucumber, tomato and jalapeno plants in hoop houses showed that given the conditions (temperature and high humidity in a closed system resulting in condensation) bacteria cannot only survive but may grow on the crops (**Figure 11**).
- Transfer of *E. coli* and *Salmonella* from utensils (bins and clippers) and human hands (gloves) to the fruit was observed frequently on inoculation day; however, in experiments conducted within hoop houses the transference was observed until 8 days post inoculation. While the risk of contamination in commercial shadehouses will likely be reduced as compared to this study, these findings show the potential for pathogens to survive/transfer in protected environments.
- Preharvest treatment can reduce significantly the number of viable bacteria, as observed using 1% Oxidate 5.0 with a 24-h contact time.
- *Salmonella* contamination surviving to postharvest phases can be increased by waxing and MAP bags. Wash-line injuries (brushed cucumbers) were shown to have the potential to enhance *Salmonella* survival and growth on non-waxed and non-MAP bagged cucumbers.
- If contamination occurs in protected environments, such as shadehouses, cross-contamination of produce is likely to continue to be a food safety concern.

APPENDICES

Publications and Presentations

The PI presented preliminary results of this project at the CPS Research Symposium in:

- Austin, TX – June 2019
- Charlotte, NC – June 2018

Budget Summary

The total funds awarded to this project were $249,143. The team expects to spend all the project funds by the end of the project agreement.

Figures 1–11 and Tables 1–10 (see below)

Figure 1. UCD Plant Sciences Field Research site

Figure 2. Tunnel hoop houses at UCD Plant Sciences Field Research site

Figure 3. Tomato and cucumber plants at San Rafael greenhouse

Figure 4. Sanitizer treatment on inoculated tomato plants

Figure 5. Jalapeno plant marked for sanitizer treatment experiment in UCD hoop houses

Figure 6. Experimental diagram flow of assessment of possible conditions that contribute to amplification of contamination in distribution

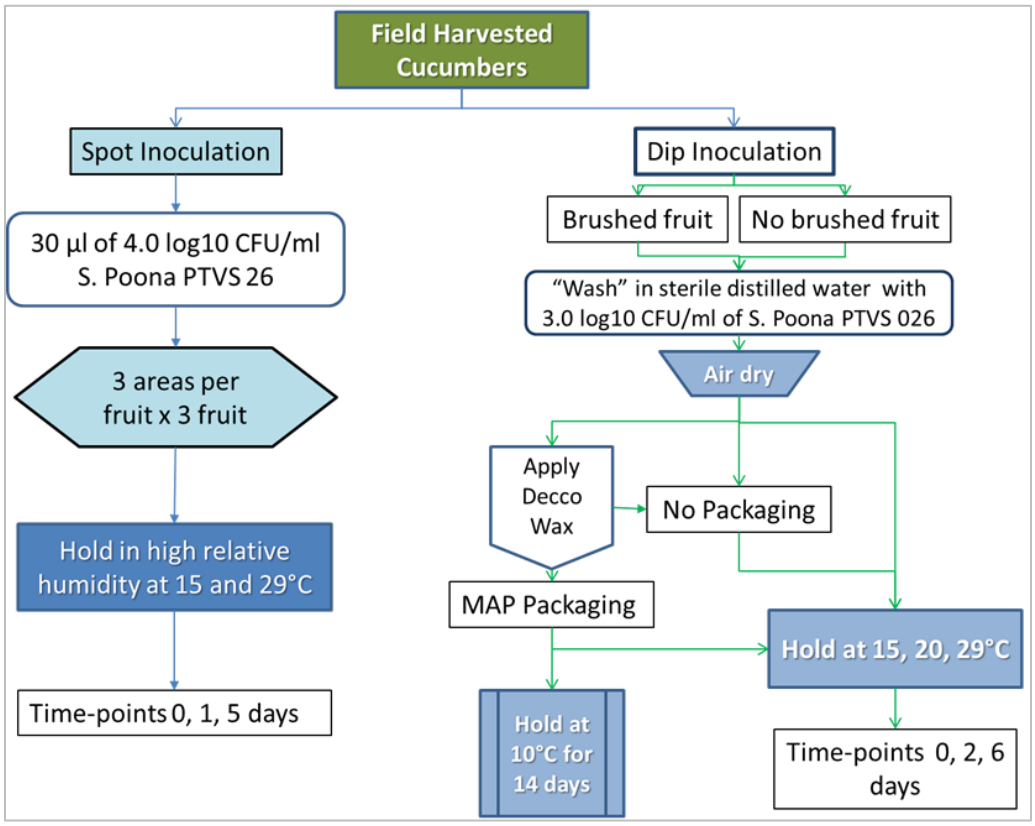


Figure 7. Field harvest cucumber spot and dip inoculation with *Salmonella* Poona PTVS 026

A. Spot inoculation

B. Dip inoculation

C. Cucumbers in MAP bags (top), and waxed cucumbers

Figure 8. Survival of *Salmonella* Poona (mean log10 CFU per fruit ± SD) of dip inoculated non-brushed cucumbers during storage at 15°C (A), 20°C (B), and 29°C (C)

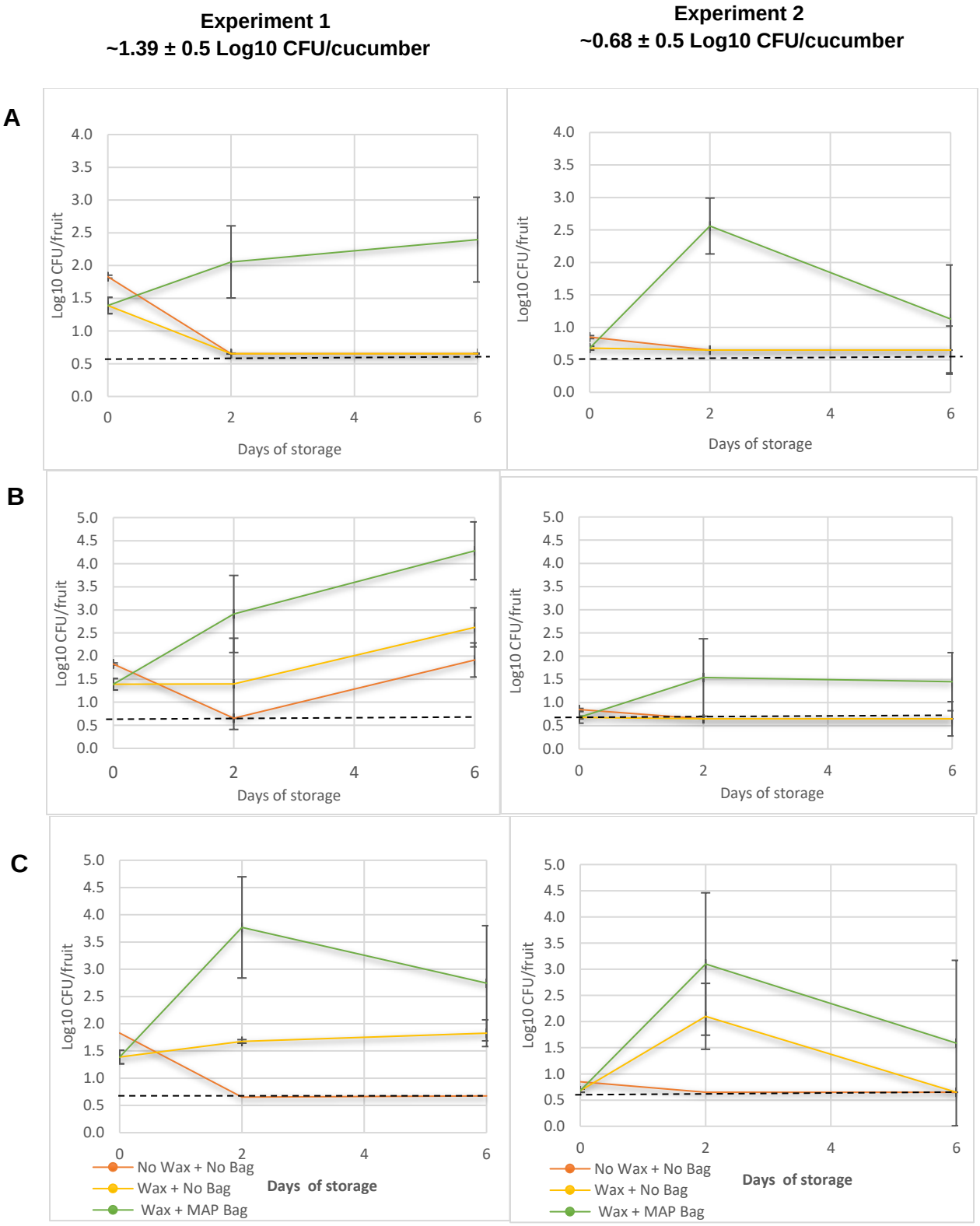


Figure 9. Survival of *Salmonella* Poona (mean log10 CFU per fruit ± SD) of dip inoculated non-brushed vs brushed cucumbers during storage conditions: No waxed-No MAP bag (A), Waxed-No MAP bag (B), and Waxed-MAP bag (C)

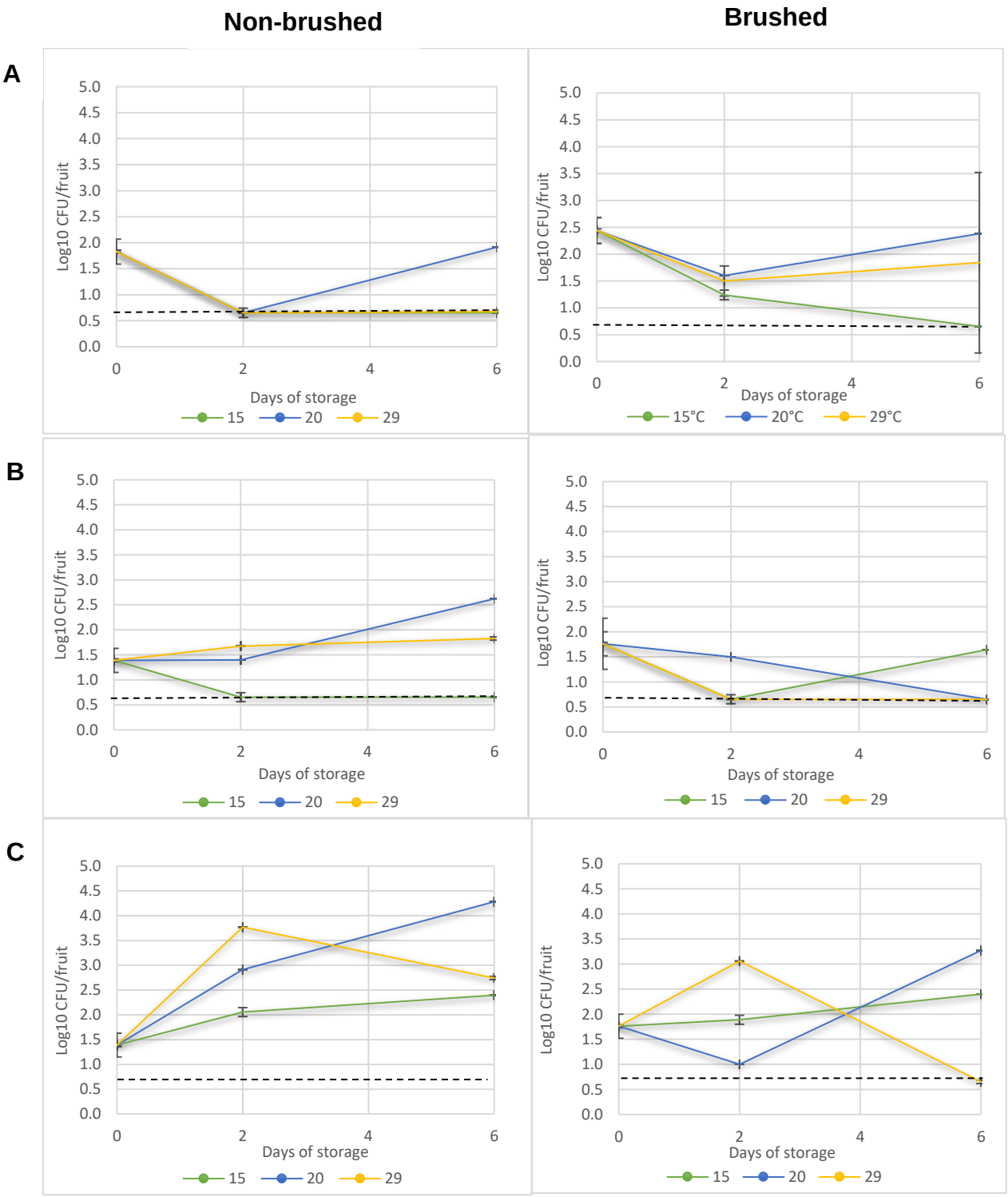
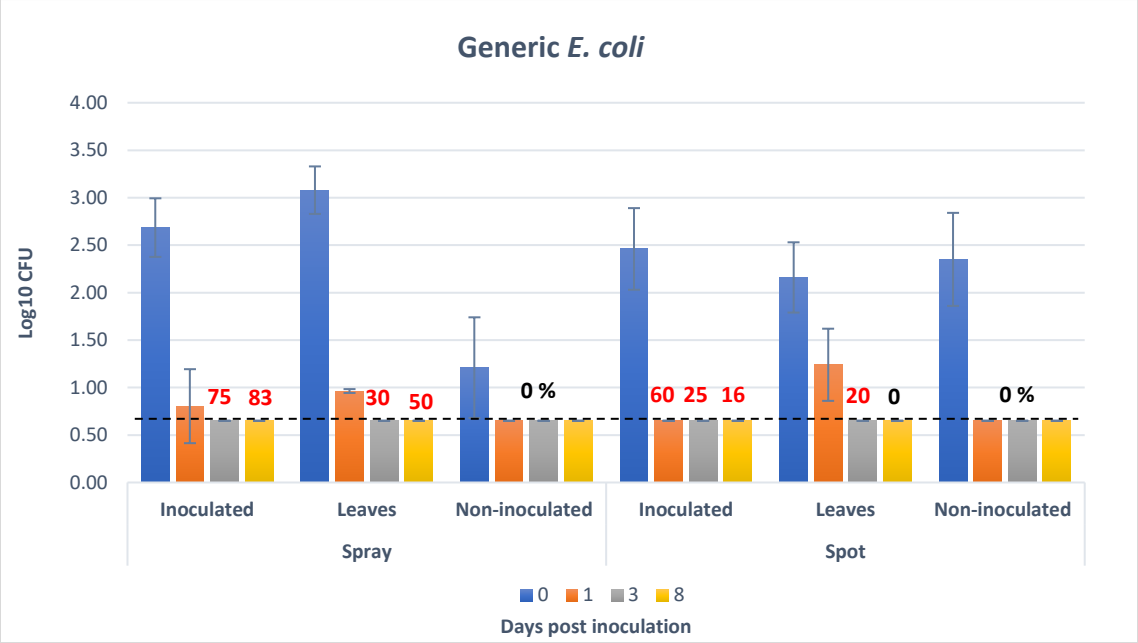
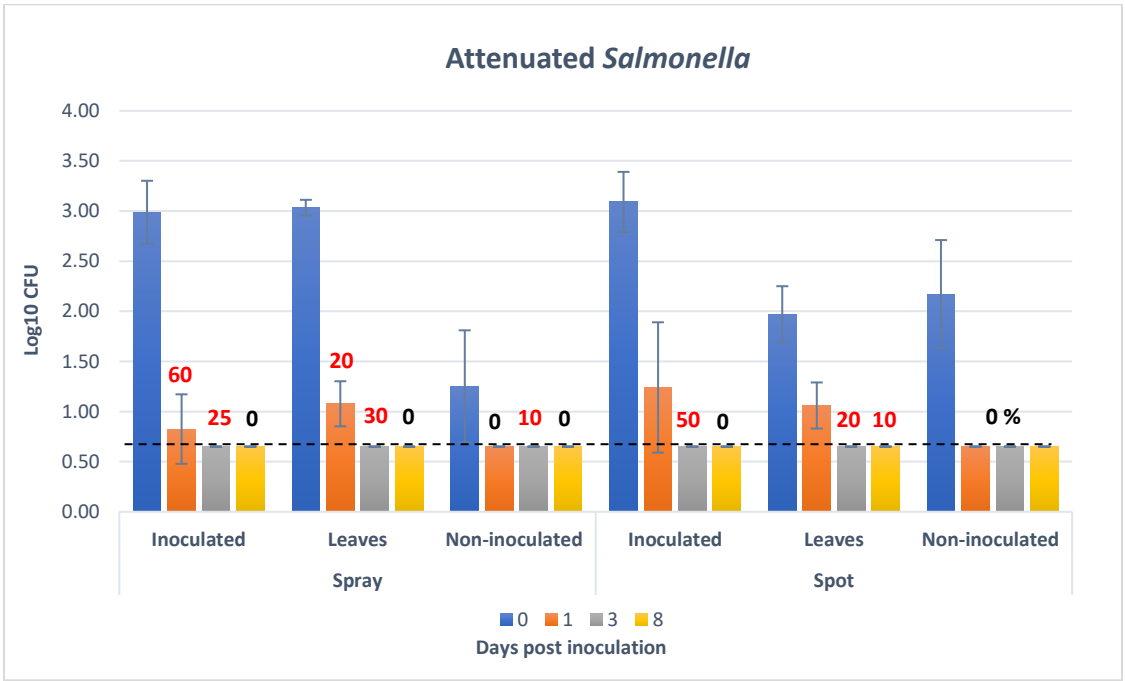


Figure 10. Bacteria recovery from open-field environment cucumber plants that were spray or spot inoculated (~5.6 ± 0.5 log10 CFU) alongside with uninoculated fruit

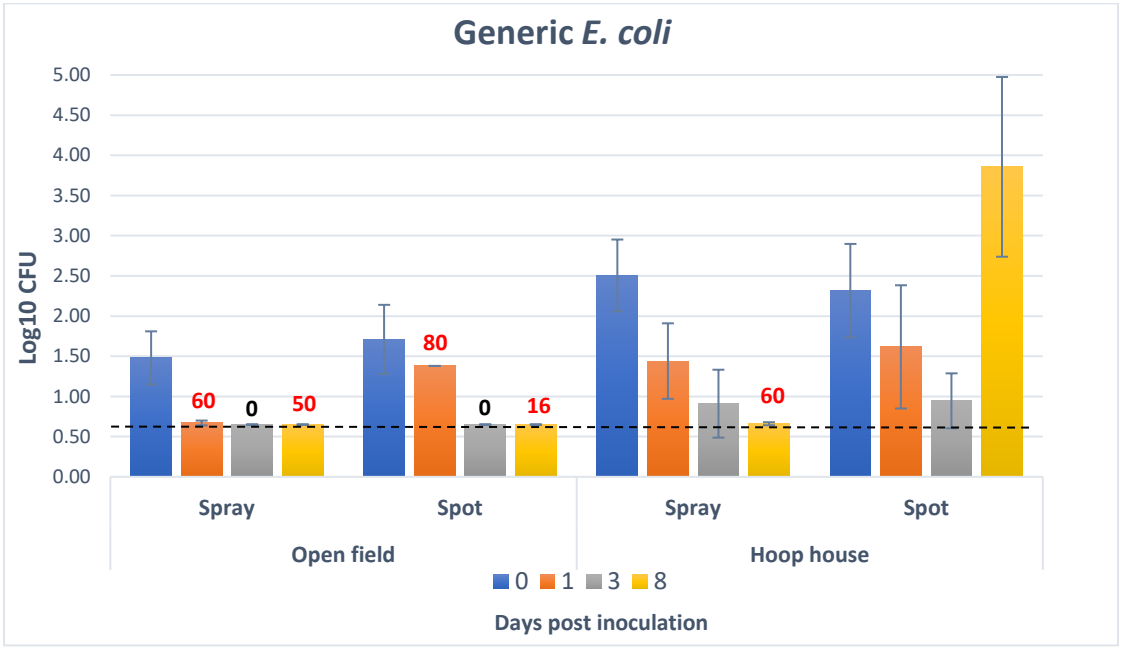


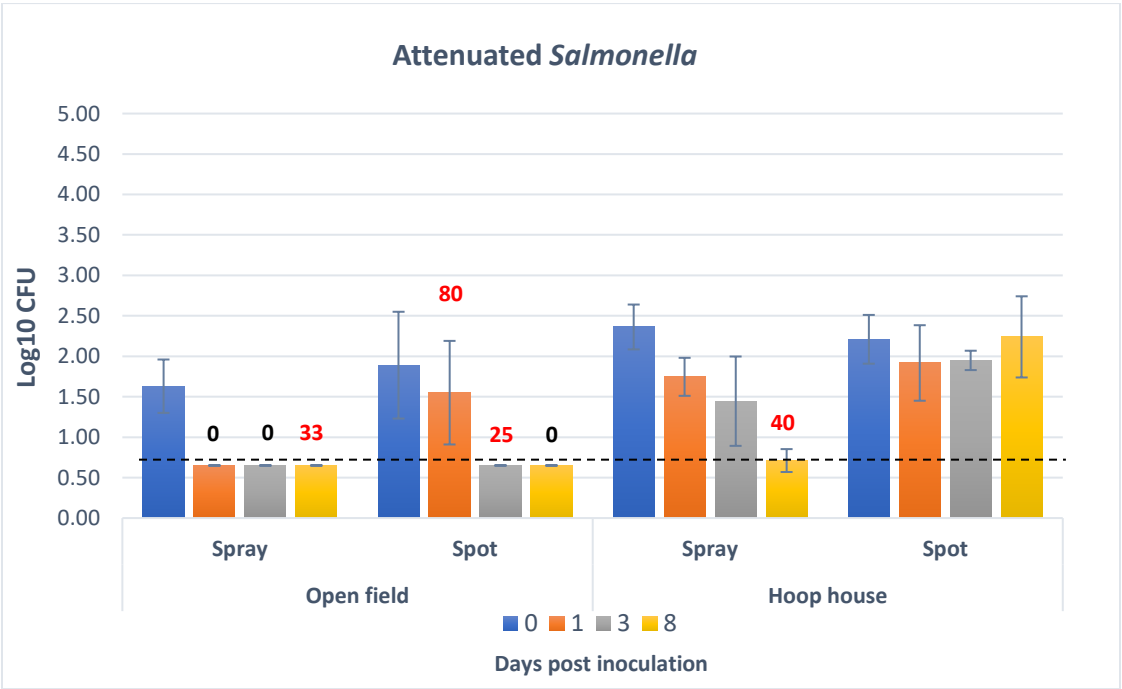


Numbers in the graphs are percentage of enrichments positive

----Quantification limit, 0.65 log 10 CFU/fruit-composite

Figure 111. Bacteria recovery from open-field environment and hoop-house tomato plants that were spray or spot inoculated (~4.2 ± 0.5 log10 CFU) alongside with uninoculated fruit





Numbers in the graphs are percentage of enrichments positive

----Quantification limit, 0.65 log 10 CFU/fruit-composite

Table 1. *E. coli* and *Salmonella* die-off experiments description

Experiment ID	Location	Fruit type	Inoculum level (log CFU ±0.2)	Inoculum type	Sun exposure	Weather conditions	Temperature High - Low	Wind High (mph)
1	UCD Open field	Cucumber	5.60	Spray	Exposed	Scatter clouds - clear	94-59	15
2		Cucumber	5.23	Soil	Exposed	Scatter clouds - clear	101-61	17
3		Cucumber	4.57	Spray	Exposed	Scatter clouds - clear	100-61	18
4		Cucumber	3.55	Soil	Exposed	Scatter clouds - clear	101-61	17
5		Cucumber	2.72	Spray	Exposed	Clear	100-63	14
6		Tomato	5.74	Spray	Exposed	Clear	101-62	12
7		Tomato	6.49	Spray	Partially shaded	Cloudy - clear	97-59	15
8		Tomato	6.49	Soil	Partially shaded	Cloudy - clear	97-59	15
9		Tomato	3.77	Spray	Exposed	Clear	96-63	14
10		Tomato	4.50	Spray	Partially shaded	Cloudy - clear	97-59	15
11		Tomato	4.50	Soil	Partially shaded	Cloudy - clear	97-59	15
12		Peppers	6.92	Spray	Exposed	Scatter clouds - clear	87-42	24
13		Peppers	4.92	Spray	Exposed	Scatter clouds - clear	87-42	24
14	Hoop houses	Peppers	6.63	Spray	NA	Scatter clouds - clear	91-51	16
15		Peppers	4.54	Spray	NA	Scatter clouds - clear	91-51	16
16		Cucumber	6.11	Spray	NA	Clear	100-43	16
17		Peppers	6.64	Soil	NA	Scatter clouds	87-42	24
18		Peppers	4.64	Soil	NA	Scatter clouds	87-42	24
19		Cucumber	4.63	Spray	NA	Scatter clouds - cloudy	86-44	24
20		Tomato	5.86	Spray	NA	Scatter clouds - clear	77-35	30
21		Tomato	4.46	Spray	NA	Scatter clouds - clear	77-35	30
22		Tomato	5.97	Soil	NA	Scatter clouds - clear	77-35	30
23		Tomato	4.28	Soil	NA	Scatter clouds - clear	77-35	30
24		Cucumber	4.17	Spray	NA	Scatter clouds - clear	77-41	7
25		Peppers	2.65	Spray	NA	Scatter clouds - cloudy	73-45	20

NA: Not applicable

Table 2A. *E. coli* and *Salmonella* recovery (mean log10 CFU ± SD) from inoculated cucumber plants alongside with uninoculated fruit in open environment

Experiment ID (Log ±0.2) Inoculation		DPI	*E. coli*						*Salmonella*					
			Inoculated Fruit		Non-Inoculated Fruit		Leaves		Inoculated Fruit		Non-Inoculated Fruit		Leaves	
			n	Log CFU/Fruit ± SD (% Positive) *	n	Log CFU/Fruit ± SD (%Positive) *	n	Log CFU/4 Leaves ± SD (% Positive) *	n	Log CFU/Fruit ± SD (% Positive) *	n	Log CFU/Fruit ± SD (% Positive) *	n	Log CFU/4 Leaves ± SD (% Positive) *
1	5.60 Spray	0	5	**2.68 ± 0.31**	5	**1.21 ± 0.53**	5	**3.08 ± 0.25**	5	**2.99 ± 0.31**	5	**1.25 ± 0.56**	5	**3.03 ± 0.08**
		1	5	**0.8 ± 0.39 (60%)**	5	0%	5	**0.96 ± 0.02 (80%)**	5	**0.82 ± 0.35 (60%)**	5	0%	5	**1.08 ± 0.22 (20%)**
		3[a]	20	**75%**	50	0%	10	**30%**	20	**25%**	50	**10%**	50	**30%**
		8[a]	30	**83%**	50	0%	10	**50%**	30	0%	50	0%	50	0%
2	5.23 Spot Soil	0	5	**2.46 ± 0.43**	5	**2.35 ± 0.49**	5	**2.16 ± 0.37**	5	**3.09 ± 0.31**	5	**2.17 ± 0.54**	5	**1.97 ± 0.28**
		1	5	**0.65 ± 0 (60%)**	5	0%	5	**1.24 ± 0.38 (100%)**	5	**0.65 ± 0 (0%)**	5	0%	5	**1.06 ± 0.23 (0%)**
		3[a]	20	**25%**	50	0%	10	**20%**	20	**50%**	50	0%	50	**20%**
		8[a]	30	**16%**	50	0%	10	0%	30	0%	50	0%	50	**10%**
3	4.57 Spray	0	5	**0.65 ± 0 (20%)**	5	0%	20	**1.40 ± 0.42 (60%)**	5	**0.73 ± 0.15 (60%)**	5	0%	5	**1.55 ± 0.34 (40%)**
		1	5	**20%**	5	0%	20	0%	5	**0%**	5	0%	5	**80%**
		3[a]	20	0%	50	0%	10	0%	20	**25%**	50	0%	50	**30%**
		8[a]	30	0%	50	0%	10	0%	30	0%	50	0%	50	0%
4	3.55 Spot Soil	0	5	**1.08 ± 0.43**	5	**20%**	20	**2.90 ± 0.13**	5	**1.32 ± 0.25**	5	**60%**	5	**2.70 ± 0.16 (40%)**
		1	5	0.65 ± 0 (0%)	5	0%	20	0.65 ± 0 (0%)	5	0.65 ± 0 (0%)	5	0%	5	0.65 ± 0 (0%)
		3[a]	20	0%	50	0%	10	**10%**	20	0%	50	0%	50	0%
		8[a]	30	0%	50	0%	10	0%	30	0%	50	0%	50	0%
5	2.72 Spray	0	30	0.65 ± 0 (**20%***)	50	0%	10	**0.87 ± 0.26 (10%)**	30	0.65 ± 0 (**20%**)	50	0%	5	**0.81 ± 0.15 (40%)**
		1	30	0%*	50	0%	10	0%	30	**20%**	50	0%	5	**20%**

*Enrichments in 2X TSB + RIF for 24 h at 37°C; **Limit of Detection = 0.65 log10 CFU

[a] From day 3, fruit were processed in composite of five

Table 2B. *E. coli* and *Salmonella* recovery (mean log10 CFU ± SD) from inoculated tomato plant alongside with uninoculated fruit in open environment

Experiment ID (Log ±0.2) Inoculation		DPI	*E. coli*						*Salmonella*					
			Inoculated Fruit		Non-Inoculated Fruit		Leaves		Inoculated Fruit		Non-Inoculated Fruit		Leaves	
			n	Log CFU/Fruit ± SD (% Positive) *	n	Log CFU/Fruit ± SD (%Positive) *	n	Log CFU/4 Leaves ± SD (% Positive) *	n	Log CFU/Fruit ± SD (% Positive) *	n	Log CFU/Fruit ± SD (% Positive) *	n	Log CFU/4 Leaves ± SD (% Positive) *
6	5.74 Spray	0	5	**1.26 ± 0.51 (40%)**	5	0.65 ± 0 (0%)	5	**1.55 ± 0.45 (80%)**	5	**1.49 ± 0.37 (40%)**	5	0.65 ± 0 (0%)	5	**1.76 ± 0.53 (20%)**
		1	5	**0.98 ± 0.48 (40%)**	5	0%	5	**0.90 ± 0.36 (80%)**	5	**1.18 ± 0.71 (40%)**	5	0%	5	**1.19 ± 0.48 (80%)**
		3[a]	20	**50%**	50	0%	50	**60%**	20	**75%**	50	0%	50	**30%**
		8[a]	30	**50%**	50	0%	50	0%	30	0%	50	0%	50	0%
7	6.49 Spray	0	5	**2.59 ± 0.53**	5	**0.81 ± 0.28 (100%)**	5	**3.69 ± 0.17**	5	**3.09 ± 0.31**	5	0.96 ± 0.30 (40%)	5	**3.54 ± 0.26**
		1	5	0.65 ± 0 (40%)	5	0%	5	**0.95 ± 0 (25%)**	5	0.65 ± 0 (**0%**)	5	0%	5	**0.85 ± 0.45** (0%)
		3[a]	20	**75%**	50	0%	50	**50%**	20	**50%**	50	**10%**	50	**40%**
		8[a]	30	**67%**	50	0%	50	**30%**	30	**33%**	50	0%	50	0%
8	6.49 Spot Soil	0	5	**3.48 ± 0.45**	5	**2.06 ± 0.98 (100%)**	5	**3.62 ± 0.60**	5	**3.43 ± 0.58**	5	**2.13 ± 0.75**	5	**3.60 ± 0.54**
		1	5	**1.56 ± 0.98**	5	**20%**	5	**2.31 ± 0.94**	5	**1.80 ± 1.03**	5	0%	5	**2.34 ± 0.59**
		3[a]	20	**2.25 ± 1.16 (100%)**	50	**10%**	50	**2.12 ± 1.22 (90%)**	20	0%	50	0%	50	**1.61 ± 0.81 (10%)**
		8[a]	30	**2.08 ± 1.40 (83%)**	50	0%	50	**1.48 ± 1.28 (40%)**	30	**1.34 ± 0.79 (16%)**	50	0%	50	**0.75 ± 0.32 (0%)**
9	3.77 Spray	0	5	0.65 ± 0 (0%)	5	0%	5	0.68 ± 0.03 (40%)	5	0.67 ± 0.03 (40%)	5	0%	5	0.67 ± 0.03 (20%)
		1	5	0.65 ± 0 (0%)	5	0%	5	**20%**	5	**20%**	5	0%	5	**40%**
		3[a]	20	0%	50	0%	50	0%	20	0%	50	0%	50	0%
		8[a]	30	0%	50	0%	50	0%	30	0%	50	0%	50	0%
10	4.50 Spray	0	5	**1.48 ± 0.33**	5	0.65 ± 0 (20%)	5	**1.33 ± 0.79**	5	**1.63 ± 0.33**	5	0.65 ± 0 (20%)	5	**1.56 ± 0.78**
		1	5	**0.67 ± 0.03 (60%)**	5	0%	5	0%	5	0.65 ± 0 (0%)	5	0%	5	0%
		3[a]	20	0%	50	0%	50	**30%**	20	0%	50	0%	50	**30%**
		8[a]	30	**50%**	50	0%	50	**10%**	30	**33%**	50	0%	50	**10%**
11	4.50 Spot Soil	0	5	**1.71 ± 0.77**	5	**0.88 ± 0.46 (100%)**	5	**2.49 ± 0.75**	5	**1.89 ± 0.66**	5	**1.08 ± 0.52 (20%)**	5	**2.29 ± 0.79**
		1	5	**1.38 ± 0.60 (80%)**	5	0%	5	**0.83 ± 0.40 (80%)**	5	**1.55 ± 0.64 (80%)**	5	**20%**	5	**1.13 ± 0.64 (20%)**
		3[a]	20	0%	50	0%	50	**20%**	20	**25%**	50	0%	50	0%
		8[a]	30	**16%**	50	0%	50	**10%**	30	0%	50	0%	50	**10%**

*Enrichments in 2X TSB + RIF for 24 h at 37°C; **Limit of Detection = 0.65 log10 CFU

[a] From day 3, fruit were processed in composite of five

Table 3. *E. coli* and *Salmonella* recovery (mean log10 CFU ± SD) from inoculated jalapeno pepper plant alongside with uninoculated fruit in open environment

Experiment ID (Log ±0.2) Inoculation		DPI	*E. coli*						*Salmonella*					
			Inoculated Fruit		Non-Inoculated Fruit		Leaves		Inoculated Fruit		Non-Inoculated Fruit		Leaves	
			n	Log CFU/Fruit ± SD (% Positive) *	n	Log CFU/Fruit ± SD (%Positive) *	n	Log CFU Leaves ± SD (% Positive) *	n	Log CFU/Fruit ± SD (% Positive) *	n	Log CFU/Fruit ± SD (% Positive) *	n	Log CFU Leaves ± SD (% Positive) *
12	6.92 Spray	0	5	**3.22 ± 0.47**	5	**1.55 ± 0.25**	5	**3.71 ± 0.42**	5	**3.61 ± 0.43**	5	**1.94 ± 0.22**	5	**4.13 ± 0.27**
		1	5	0.67 ± 0.03 (**40%**)	5	0%	5	**1.07 ± 0.32**	5	**0.82 ± 0.32 (60%)**	5	**20%**	5	**1.37 ± 0.44**
		3[a]	20	**100%**	50	0%	50	**40%**	20	0%	50	**60%**	50	**10%**
		8[a]	30	**67%**	50	**20%**	50	**60%**	30	**33%**	50	0%	50	**20%***
13	4.92 Spray	0	5	**1.25± 0.46**	5	0.65 ± 0.0 **(20%)**	5	**1.00 ± 0.46**	5	**1.86 ± 0.25**	5	0.66 ± 0.02 **(60%)**	5	**1.56 ± 0.54**
		1	5	0%*	5	0%	5	0%	5	**20%**	5	0%	5	**20%**
		3[a]	20	**40%**	50	0%	50	**10%**	20	0%	50	0%	50	0%
		8[a]	30	**16%**	50	0%	50	**10%**	30	**33%**	50	0%	50	**10%**

*Enrichments in 2X TSB + RIF for 24 h at 37°C

**Limit of Detection = 0.65 log10 CFU

[a] From day 3, fruit were processed in composite of five

Table 4. *E. coli* and *Salmonella* recovery (mean log10 CFU ± SD) from inoculated cucumber plants alongside with uninoculated fruit grown in hoop houses

Experiment ID (Log ±0.2) Inoculation		DPI	*E. coli*						*Salmonella*					
			Inoculated Fruit		Non-Inoculated Fruit		Leaves		Inoculated Fruit		Non-Inoculated Fruit		Leaves	
			n	Log CFU/Fruit ± SD (% Positive)*	n	Log CFU/Fruit ± SD (%Positive)*	n	Log CFU Leaves ± SD (% Positive)*	n	Log CFU/Fruit ± SD (% Positive)*	n	Log CFU/Fruit ± SD (% Positive)*	n	Log CFU Leaves ± SD (% Positive) *
16	6.11 Spray	0	5	**3.79 ± 0.37**	5	**1.63 ± 0.22**	5	**3.85 ± 0.96**	5	**4.07 ± 0.28**	5	**1.88 ± 0.09**	5	**3.61 ± 0.70**
		1	5	**2.43 ± 0.40**	5	**1.61 ± 0.30 (100%)**	5	**2.69 ± 0.34**	5	**2.66 ± 0.15**	5	**1.94 ± 0.10 (100%)**	5	**2.79 ± 0.26**
		3[a]	20	**2.03 ± 0.48 (100%)**	50	**0.77 ± 0.18 (67%)**	50	**0.96 ± 0.42 (100%)**	20	**2.01 ± 0.15 (100%)**	50	**0.76 ± 0.21 (17%)**	50	**0.91 ± 0.22 (100%)**
		8[a]	30	0.65 ± 0.0 **(60%)**	50	0%	50	**1.51 ± 1.23 (60%)**	30	0.65 ± 0.0 **(40%)**	50	**40%**	50	**1.04 ± 0.53 (20%)**
19	4.63 spray	0	5	**2.79 ± 0.25**	5	**0.69 ± 0.02**	5	**2.81 ± 0.16**	5	**2.91 ± 0.13**	5	**0.79 ± 0.21**	5	**2.62 ± 0.08**
		1	5	**1.17 ± 0.48**	5	0%	5	**0.89 ± 0.36**	5	**1.62 ± 0.67**	5	**60%**	5	**0.84 ± 0.31**
		3[a]	20	**1.20 ± 0.72**	50	**20%**	50	**40%**	20	**2.05 ± 0.37**	50	**10%**	50	**30%**
		8[a]	30	**4.11 ± 1.15**	50	**1.73 ± 1.17 (90%)**	50	**80%**	30	**2.91 ± 1.29**	50	**0.76 ± 0.28 (10%)**	50	**20%**
24	4.17 spray	0	5	**2.96 ± 0.56**	5	0.65 ± 0 **(20%)**	5	**3.31 ± 0.25**	5	**2.97 ± 0.32**	5	0.66 ± 0.02 **(40%)**	5	**3.08 ± 0.14**
		1	5	**2.05 ± 0.98**	5	**0.78 ± 0.22 (80%)**	5	**1.56 ± 0.44**	5	**2.32 ± 0.55**	5	**1.01 ± 0.60 (80%)**	5	**2.19 ± 0.26**
		3[a]	20	**2.44 ± 0.70**	50	**1.16 ± 0.63 (80%)**	50	**2.34 ± 1.29**	20	**2.50 ± 0.42**	50	**1.01 ± 0.60 (60%)**	50	**1.75 ± 1.17**
		8[a]	30	**4.83 ± 0.87**	50	**3.28 ± 0.56 (100%)**	50	**3.47 ± 0.75**	30	**3.87 ± 0.98**	50	**2.28 ± 0.70 (100%)**	50	**3.71 ± 0.90**

*Enrichments in 2X TSB + RIF for 24 h at 37°C

**Limit of Detection = 0.65 log10 CFU

[a] From day 3, fruit were processed in composite of five

Table 5. *E. coli* and *Salmonella* recovery (mean log10 CFU ± SD) from inoculated tomato plants alongside with uninoculated fruit grown in hoop houses

Experiment ID (Log ±0.2) Inoculation		DPI	*E. coli*						*Salmonella*					
			Inoculated Fruit		Non-Inoculated Fruit		Leaves		Inoculated Fruit		Non-Inoculated Fruit		Leaves	
			n	Log CFU/Fruit ± SD (% Positive) *	n	Log CFU/Fruit ± SD (%Positive) *	n	Log CFU Leaves ± SD (% Positive) *	n	Log CFU/Fruit ± SD (% Positive) *	n	Log CFU/Fruit ± SD (% Positive) *	n	Log CFU Leaves ± SD (% Positive) *
20	5.86 Spray	0	5	4.19 ± 0.23	5	2.90 ± 0.14	5	4.65 ± 0.04	5	4.02 ± 0.20	5	2.60 ± 0.07	5	4.07 ± 0.06
		1	5	2.78 ± 0.46	5	0.67 ± 0.03 (100%)	5	3.06 ± 0.47	5	3.28 ± 0.58	5	0.90 ± 0.23 (60%)	5	3.09 ± 0.41
		3[a]	20	3.42 ± 0.12	50	2.24 ± 0.34 (100%)	50	2.39 ± 0.46	20	3.60 ± 0.20	50	2.31 ± 0.32 (100%)	50	2.41 ± 0.44
		8[a]	30	2.20 ± 0.62	50	50%	50	1.69 ± 0.97	30	2.45 ± 0.55	50	50%	50	1.92 ± 0.81
21	4.46 Spray	0	5	2.51 ± 0.45	5	0.76 ± 0.23 (40%)	5	2.59 ± 1.11	5	2.36 ± 0.28	5	0.73 ± 0.15 (20%)	5	2.22 ± 0.26
		1	5	1.44 ± 0.47	5	20%	5	1.89 ± 0.19 (100%)	5	1.75 ± 0.23	5	40%	5	1.93 ± 0.31 (100%)
		3[a]	20	0.91 ± 0.42	50	70%	50	1.02 ± 0.35	20	1.44 ± 0.55	50	20%	50	1.02 ± 0.48
		8[a]	30	0.66 ± 0.02 (60%)	50	0%	50	70%	30	0.71 ± 0.14 (40%)	50	20%	50	30%
22	5.97 Spot	0	5	4.15 ± 0.44	5	2.92 ± 0.74	5	4.34 ± 0.43	5	3.99 ± 0.30	5	2.75 ± 0.75	5	4.04 ± 0.32
		1	5	3.83 ± 0.43	5	1.20 ± 0.53 (100%)	5	3.09 ± 1.03	5	4.07 ± 0.15	5	1.54 ± 0.56 (100%)	5	3.02 ± 1.08
		3[a]	20	3.25 ± 1.02	50	70%	50	2.14 ± 0.32	50	3.67 ± 0.46	50	20%	50	2.36 ± 0.24
		8[a]	30	2.32 ± 1.16	50	10%	50	1.05 ± 0.54 (60%)	50	2.03 ± 0.97	50	0%	50	0.74 ± 0.19 (20%)
23	4.28 Spot	0	5	2.32 ± 0.58	5	0.68 ± 0.03 (100%)	5	2.26 ± 0.62	5	2.21 ± 0.30	5	0.65 ± 0.03 (80%)	5	2.03 ± 0.44
		1	5	1.62 ± 0.77	5	30%	5	0.87 ± 0.49 (80%)	5	1.92 ± 0.47	5	0	5	0.85 ± 0.45 (100%)
		3[a]	20	0.95 ± 0.34	50	20%	50	50%	20	1.95 ± 0.12	50	0%	50	20%
		8[a]	30	3.86 ± 1.12	50	40%	50	90%	30	2.04 ± 0.49	50	0%	50	100%

*Enrichments in 2X TSB + RIF for 24 h at 37°C

**Limit of Detection = 0.65 log10 CFU

[a] From day 3, fruit were processed in composite of five

Table 6. *E. coli* and *Salmonella* recovery (mean log10 CFU ± SD) from inoculated jalapeno pepper plants alongside with uninoculated fruit grown in hoop houses

Experiment ID (Log ±0.2) Inoculation		DPI	*E. coli*						*Salmonella*					
			Inoculated Fruit		Non-Inoculated Fruit		Leaves		Inoculated Fruit		Non-Inoculated Fruit		Leaves	
			n	Log CFU/Fruit ± SD (% Positive) *	n	Log CFU/Fruit ± SD (%Positive) *	n	Log CFU Leaves ± SD (% Positive) *	n	Log CFU/Fruit ± SD (% Positive) *	n	Log CFU/Fruit ± SD (% Positive) *	n	Log CFU Leaves ± SD (% Positive) *
14	6.63 Spray	0	5	3.22 ± 0.20	5	1.98 ± 0.47	5	3.98 ± 0.34	5	3.50 ± 0.22	5	1.94 ± 0.33	5	4.12 ± 0.28
		1	5	1.75 ± 0.63	5	0%	5	1.22 ± 0.50	5	2.04 ± 0.14	5	0%	5	1.19 ± 0.48 (80%)
		3[a]	20	3.00 ± 1.33	50	0%	50	0.91 ± 0.79 (60%)	20	2.42 ± 0.84	50	0%	50	30%
		8[a]	30	4.10 ± 0.29	50	10%	50	0.76 ± 0.25 (20%)	30	2.82 ± 0.23	50	0%	50	0%
15	4.54 Spray	0	5	1.68 ± 0.52	5	1.00 ± 0.50	5	2.12 ± 0.22	5	1.65 ± 0.61	5	0.90 ± 0.36	5	1.86 ± 0.33
		1	5	1.07 ± 0.63 (80%)	5	0%	5	0.92 ± 0.57 (80%)	5	1.15 ± 0.62 (20%)	5	0%	5	0.98 ± 0.68 (25%)
		3[a]	20	1.02 ±0.20 (75%)	50	0%	50	20%	20	1.05 ±0.1 (25%)	50	10%	50	40%
		8[a]	30	2.71 ± 0.81 (100%)	50	0%	50	0%	30	1.80 ± 0.33 (100%)	50	0%	50	10%
17	6.64 Spot	0	5	4.28 ± 0.51	5	2.04 ± 1.06	5	3.73 ± 0.51	5	4.37 ± 0.36	5	1.99 ± 0.96	5	4.01 ± 0.27
		1	5	1.96 ± 0.67	5	0%	5	1.09 ± 0.41	5	2.54 ± 0.67	5	40%	5	1.58 ± 0.55
		3[a]	20	3.62 ± 0.32	50	40%	50	1.91 ± 1.86	20	2.71 ± 0.15	50	20%	50	1.62 ± 0.87
		8[a]	30	3.64 ± 0.93	50	20%	50	20%	30	2.98 ± 0.76	50	10%	50	50%
18	4.64 Spot	0	5	2.58 ± 0.76	5	0.80 ± 0.28	5	2.92 ± 0.18	5	2.66 ± 0.60	5	0.93 ± 0.38	5	2.95 ± 0.06
		1	5	1.39 ± 0.72 (80%)	5	20%	5	1.32 ± 0.78 (80%)	5	1.98 ± 0.38	5	20%	5	1.45 ± 0.91 (100%)
		3[a]	20	1.95 ± 0.54 (100%)	50	60%	50	1.82 ± 1.45 (90%)	20	2.75 ± 0.23	50	40%	50	1.90 ± 0.76 (90%)
		8[a]	30	1.48 ± 1.00 (50%)	50	40%	50	0.93 ± 0.40 (100%)	30	2.02 ± 0.50	50	40%	50	1.63 ± 0.38 (100%)
25	2.72 Spray	0	30	2.31 ± 1.01	50	10%	10	1.26 ± 0.79 (30%)	30	1.35 ± 0.25	50	0%	50	1.14 ± 0.34 (40%)
		1	30	0.87 ± 0.53 (60%)	50	0%	10	0%	30	20%	50	30%	50	0.71 ± 0.16 (30%)

*Enrichments in 2X TSB + RIF for 24 h at 37°C

**Limit of Detection = 0.65 log10 CFU

[a] From day 3, fruit were processed in composite of five

Table 7. Qualitative enrichment analyses of elements used during manipulation of inoculated fruit and leaves at harvest

Experiment ID	Location	Fruit type	Inoculum level (log CFU ±0.2)	Inoculum type	DPI *E. coli*				DPI *Salmonella*			
					0	1	3	8	0	1	3	8
1	UCD Open field	Cucumber	5.60	Spray	B, G	-	-	-	G	-	-	-
2		Cucumber	5.23	Soil	B, C, G	-	-	-	G	-	-	-
3		Cucumber	4.57	Spray	-	-	-	-	-	-	-	-
4		Cucumber	3.55	Soil	-	-	-	-	B	-	-	-
5		Cucumber	2.72	Spray	-	-	NA	NA	-	-	NA	NA
6		Tomato	5.74	Spray	B, C	B	-	-	B, G	B	-	-
7		Tomato	6.49	Spray	B	-	-	B, C	G	-	-	-
8		Tomato	6.49	Soil	B, C, G	B	-	-	G	B	-	-
9		Tomato	3.77	Spray	-	-	-	-	-	-	-	-
10		Tomato	4.50	Spray	B	-	-	-	-	-	-	-
11		Tomato	4.50	Soil	B	-	-	-	-	G	-	-
12		Peppers	6.92	Spray	B, C, G	B	-	-	C	B	-	-
13		Peppers	4.92	Spray	B	B	-	-	-	-	-	-
14	Hoop houses	Peppers	6.63	Spray	B, C, G	-	-	-	-	G	-	-
15		Peppers	4.54	Spray	B, C	-	-	-	-	-	-	-
16		Cucumber	6.11	Spray	B, C, G	B	-	-	G	-	B, C, G	B
17		Peppers	6.64	Soil	B, C, G	B	-	B	-	-	C	-
18		Peppers	4.64	Soil	B, C	B	B	B	G	-	-	G
19		Cucumber	4.63	Spray	B	B	C	B, C, G	C	C	-	-
20		Tomato	5.86	Spray	B, C, G	B, C	B, G	B, G	-	G	-	-
21		Tomato	4.46	Spray	B, C	B, C	-	-	G	G	G	-
22		Tomato	5.97	Soil	B, C, G	B, C, G	B, G	B	-	-	-	-
23		Tomato	4.28	Soil	B, C, G	B, C	B	B	-	G	-	-
24		Cucumber	4.17	Spray	B, G	B, C	B, C, G	B, C, G	C	G		
25		Peppers	2.65	Spray	B	-	NA	NA	-	-	NA	NA

DPI, day(s) post-inoculation
B: Bin, C: Clippers, G: Gloves

Table 8. Effect of different sanitizing solutions on the populations of *E. coli* and *Salmonella* on tomato fruit and leaves

	Tomatoes				Leaves			
Treatment[a]	***E. coli***	**ELR[b]**	***Salmonella***	**ELR**	***E. coli***	**ELR**	***Salmonella***	**ELR**
Control	2.66 ± 0.7$_A$	NA	3.14 ± 0.5$_A$	NA	3.67 ± 0.9$_A$	NA	3.83 ± 1.5$_A$	NA
Pure Hard Surface	4.30 ± 1.0$_A$	-1.6	3.95 ± 0.9$_A$	0.7	3.65 ± 0.8$_A$	0.0	2.57 ± 0.7$_A$	**1.3**
0.39% Oxidate 5.0	3.18 ± 1.4$_A$	-0.5	2.48 ± 1.1$_A$	2.2	2.17 ± 0.2$_B$	**1.5**	1.56 ± 0.7$_B$	**2.3**
1% Oxidate 5.0	2.15 ± 0.9$_A$	0.5	0.93 ± 0.4$_B$	**3.1**	2.99 ± 0.4$_A$	0.7	1.52 ± 0.9$_B$	**1.9**

[a] Control, were not subjected to any treatment

[b] ELR, estimated log reduction (obtained by subtracting the log CFU after each treatment from the log CFU for control)

Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$)

Table 9. Effect of different sanitizing solutions on the populations of *E. coli* and *Salmonella* on jalapeno pepper fruit and leaves after 24-h contact time

	Peppers				Leaves			
Treatment[a]	***E. coli***	**ELR[b]**	***Salmonella***	**ELR**	***E. coli***	**ELR**	***Salmonella***	**ELR**
Control	3.22 ± 0.3$_A$	NA	3.25 ± 0.3$_A$	NA	3.28 ± 1.1$_A$	NA	3.55 ± 0.7$_A$	NA
Pure Hard Surface	2.46 ± 0.5$_A$	0.8	3.03 ± 0.6$_A$	0.2	2.19 ± 0.9$_A$	0.9	2.65 ± 0.7$_A$	0.9
0.39% Oxidate 5.0	2.96 ± 1.0$_A$	0.3	3.09 ± 0.6$_A$	0.2	2.94 ± 0.8$_A$	0.3	3.23 ± 0.7$_A$	0.3
1% Oxidate 5.0	1.94 ± 1.1$_B$	**1.3**	1.96 ± 0.7$_B$	**1.3**	1.48 ± 0.3$_B$	**1.8**	1.68 ± 0.7$_B$	**1.9**

[a] Control, were not subjected to any treatment

[b] ELR, estimated log reduction (obtained by subtracting the log CFU after each treatment from the log CFU for control)

Means within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test ($P < 0.05$).

Table 10. Survival of *Salmonella* Poona during storage of spot inoculated non-brushed cucumbers

		Log CFU/Cucumber ± SD * (Enrichments positive)		
Experiment	**Storage Temp**	**0 DPI**	**1 DPI**	**5 DPI**
1	15 °C	1.34 ± 0.4	0.83 ± 0.3 (1/2)	0.65 ± 0.0 (0/2)
	29 °C		0.68 ± 0.0 (1/2)	0.65 ± 0.0 (0/2)
2	15 °C	0.94 ± 0.3	0.65 ± 0.0 (0/2)	0.65 ± 0.0 (0/2)
	29 °C		0.65 ± 0.0 (0/2)	0.65 ± 0.0 (0/2)

*Limit of Detection = 0.65 log10

References

1. Ailes, E.C., Leon, J.S., Jaykus, L.A., Johnston, L.M., Clayton, H.A., Blanding, S., & Moe, C.L. (2008). Microbial concentrations on fresh produce are affected by postharvest processing, importation, and season. Journal of Food Protection, 71(12), 2389-2397.
2. Angelo, K.M., Chu, A., Anand, M., Nguyen, T.A., Bottichio, L., Wise, M., ... & Lance, S. (2015). Outbreak of Salmonella Newport infections linked to cucumbers—United States, 2014. MMWR. Morbidity and Mortality Weekly Report, 64(6), 144-147.
3. Bottichio, L., et al. (2016). Outbreak of Salmonella Oslo infections linked to persian cucumbers—United States, 2016. MMWR. Morbidity and Mortality Weekly Report, 65(5051), 1430-1433.
4. CDC. (2016). Multistate outbreak of Salmonella Poona infections linked to imported cucumbers. Final update. http://www.cdc.gov/salmonella/poona-09-15/index.html.
5. Côté, C., and Quessy, S. (2005). Persistence of Escherichia coli and Salmonella in surface soil following application of liquid hog manure for production of pickling cucumbers. Journal of Food Protection, 68(5), 900-905.
6. Furuta, M., Oda, T., Oumi, M., & Inamasu, T. (2005). Survival and growth of Escherichia coli O157 and Salmonella Enteritidis on uncut whole cucumbers [Cucumis sativus] and their sterilization experiments. Journal of Antibacterial and Antifungal Agents, Japan (Japan).
7. Gutiérrez-Rodríguez, E., Gundersen, A., Sbodio, A.O., & Suslow, T.V. (2012). Variable agronomic practices, cultivar, strain source and initial contamination dose differentially affect survival of Escherichia coli on spinach. Journal of Applied Microbiology, 112(1), 109-118.
8. Howard, L.R., and Gonzalez, A.R. (2001). Food safety and produce operations: What is the future? HortScience, 36(1), 33-39.
9. Jones, S. L., Parry, S. M., O'Brien, S. J., and Palmer, S. R. (2008). Operational practices associated with foodborne disease outbreaks in the catering industry in England and Wales. Journal of Food Protection, 71(8), 1659-1665.
10. Kusumaningrum, H.D., Riboldi, G., Hazeleger, W.C., and Beumer, R.R. (2003). Survival of foodborne pathogens on stainless steel surfaces and cross-contamination to foods. International Journal of Food Microbiology, 85(3), 227-236.
11. Likotrafiti, E., Smirniotis, P., Nastou, A., and Rhoades, J. (2013). Effect of relative humidity and storage temperature on the behavior of Listeria monocytogenes on fresh vegetables. Journal of Food Safety, 33(4), 545-551.
12. Lopez-Galvez, F., Allende, A., Pedrero-Salcedo, F., Alarcon, J. J., and Gil, M. I. (2014). Safety assessment of greenhouse hydroponic tomatoes irrigated with reclaimed and surface water. International Journal of Food Microbiology, 191, 97-102.
13. Lopez-Velasco, G., Tomas-Callejas, A., Sbodio, A.O., Pham, X., Wei, P., Diribsa, D., and Suslow, T.V. (2015). Factors affecting cell population density during enrichment and subsequent molecular detection of Salmonella enterica and Escherichia coli O157: H7 on lettuce contaminated during field production. Food Control, 54, 165-175.
14. Moraes, M.H., Chapin, T.K., Ginn, A., Wright, A.C., Parker, K., ... & Teplitski, M. (2016). Development of an avirulent Salmonella surrogate for modeling pathogen behavior in pre-and postharvest environments. Applied and Environmental Microbiology, 82(14), 4100-4111.

15. Orozco, L., Rico-Romero, L., and Escartin, E. F. (2008). Microbiological profile of greenhouses in a farm producing hydroponic tomatoes. Journal of Food Protection, 71(1), 60-65.
16. Orozco, L. R., Iturriaga, M. H., Tamplin, M. L., Fratamico, P. M., Call, J. E., Luchansky, J. B., and Escartin, E. F. (2008). Animal and environmental impact on the presence and distribution of Salmonella and Escherichia coli in hydroponic tomato greenhouses. Journal of Food Protection, 71(4), 676-683.
17. Powell, D. A., Bobadilla-Ruiz, M., Whitfield, A., Griffiths, M. W., and Luedtke, A. (2002). Development, implementation, and analysis of an on-farm food safety program for the production of greenhouse vegetables. Journal of Food Protection, 65(6), 918-923.
18. Reina, L. D., Fleming, H. P., and Breidt Jr, F. (2002). Bacterial contamination of cucumber fruit through adhesion. Journal of Food Protection, 65(12), 1881-1887.
19. Suslow, T. V., and Cantwell, M. (1997). Cucumber: Recommendations for maintaining postharvest quality. Postharvest Technology Center, University of California, Davis. http://postharvest.ucdavis.edu/Commodity_Resources/Fact_Sheets/Datastores/Vegetables_English/?uid=14&ds=799.
20. Toivonen, P. M., Brandenburg, J. S., and Luo, Y. (2009). Modified atmosphere packaging for fresh-cut produce. Modified and Controlled Atmospheres for the Storage, Transportation, and Packaging of Horticultural Commodities, 464-486.
21. U.S. FDA. (2017). Microbiological Surveillance Sampling: FY16-17 Cucumbers. https://www.fda.gov/food/sampling-protect-food-supply/microbiological-surveillance-sampling-fy16-17-cucumbers.
22. Yuk, H. G., Bartz, J. A., & Schneider, K. R. (2006). The effectiveness of sanitizer treatments in inactivation of Salmonella spp. from bell pepper, cucumber, and strawberry. Journal of Food Science, 71(3), 95-99.