CENTER for PRODUCE SAFETY

CPS 2015 RFP
FINAL PROJECT REPORT

Project Title

Establishing die-off rates of surrogate and virulent EHEC-STEC strains from strawberry and cilantro surfaces: time, inoculum dose and chemical intervention

Project Period

January 1, 2016 – December 31, 2016 (extended to January 31, 2017)

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Objectives

1. *Determine the die-off kinetics (using multiple predictive models) of virulent and surrogate strains of E. coli O157 and non-O157 STEC inoculated to the surface of fruit and leaves of strawberry and cilantro at two inoculum concentrations (log 3 and 6 CFU/ml) with and without the application of chlorine (100 ppm) or OxiDate 2.0 (PAA) (100 ppm).*
2. *Establish whether applying an interval of days between last irrigation and harvest, using a microbial die-off rate of 0.5 log per day, is a sound food safety practice for the selected crops.*

Funding for this project provided by the Center for Produce Safety through:
CPS Campaign for Research

FINAL REPORT

Abstract
The die-off kinetics of virulent strains of enterohemorrhagic *Escherichia coli* (EHEC) and Shiga toxin–producing *E. coli* (STEC) microorganisms have not been well characterized under different agricultural environments. Several studies have used currently available surrogates of these pathogens in an attempt to predict pathogen survival and persistence on the surface of plants, soil and irrigation water, with limited success. Under the FSMA Produce Safety Rule, agricultural water (AW) that will be used in direct contact with the edible portion of the crop must meet a rolling geometric mean of 126 CFU/100 ml and a Statistical Threshold Value of 410 CFU/100 ml of generic *E. coli.* If agricultural water does not meet this microbiological criterion, farmers are required to establish as soon as practical, and no later than the following year, corrective actions that will allow this AW to meet these standards. Farmers with AW outside these standards may also follow a set of alternative provisions, enabling them to use this water on their crops. One of these provisions allows growers to apply a time interval for in-field microbial die-off of a maximum of four consecutive days between the last irrigation and harvest, where a predicted die-off rate of 0.5 log per day is expected during this period. This concept proposal looked to (1) compare side by side the die-off kinetics of surrogate and pathogenic strains of *E. coli* O157:H7, non-O157 STEC including *E. coli* O45, *E. coli* O111 and *E. coli* O145, and generic *E. coli* in growing strawberry and cilantro plants, and whether they followed the proposed Produce Safety Rule die-off rates under BSL2/3 greenhouse conditions; and (2) establish and evaluate the effectiveness of a preharvest spray of chlorine or peroxyacetic acid (PAA) as a last intervention strategy to expedite in-field pathogen die-off when AW outside the microbial standards was used to irrigate cilantro or to frost-protect strawberry plants. Our results suggest that irrespective of pathogen fitness and virulence, die-off rates after 8 days post inoculation (DPI) in strawberry and cilantro did not adjust to a linear model as proposed by the FSMA Produce Safety Rule, and preharvest spray applications of chlorine or PAA at 100 ppm or 40 ppm, respectively, marginally impacted die-off rates of the inoculated strains.

Background
Agricultural water in contact with fresh produce at any point during the preharvest to postharvest continuum may pose a risk to the microbial safety of the crop[1-3]. This is especially true on fruits and herbs with no further commercially available disinfection steps and with multiple entry points along the cropping cycle capable of introducing pathogens along the supply chain[2]. The microbial quality of water used for irrigation or crop protection sprays is critical to reduce the risk of fruit or herb contamination close to harvest. Most gastrointestinal pathogens usually die off immediately after excretion[1, 2, 4], however multiple studies have shown variable survival and persistence in-field and on crop surfaces despite exposure to fluctuating temperature, UV radiation and rainfall[1, 2, 5]. Many of these studies have used surrogates of human pathogens to try to predict natural die-off rates of pathogenic microorganism in ag-environments. However, little information is available comparing die-off rates of these organisms inoculated side by side on the surface of crops in conditions that closely represent field environments.

The die-off kinetics of virulent strains of *E. coli* O157 and non-O157 STEC are not well characterized under different ag-environments[1]. Even though difference in survival rates of virulent vs. avirulent *E. coli* have been shown[6], multiple studies have used currently available surrogates of these pathogens in an attempt to predict pathogen survival and persistence on the surface of plants, in soil and in irrigation water, with limited success. Major obstacles in this effort are (1) a lack of open field environments or greenhouse facilities where researchers could make direct comparisons of the survival and persistence of these strains without compromising the health of research personnel; (2) the spread and persistence of these high risk pathogens into the environment following plant/soil inoculation studies; and (3) the potential to over- or underestimate their persistence in ag-environments. Despite these limitations the outcomes of multiple studies have been

used by FDA as part of their decision process to develop the FSMA Produce Safety Rule and to adopt the revised 2012 EPA recreational water microbial standards as the microbiological criterion to follow when using agricultural water.

Within the new Produce Safety Rule the standards associated with water quality are among the most contested by industry associations, state agencies and stakeholders. All of them have provided comments to FDA regarding their concerns, which led to a halt in the implementation process of these water standards. Current guidelines require AW that will be in direct contact with the crop, to meet specific microbiological thresholds (a rolling geometric mean of 126 CFU/100 ml, and a Statistical Threshold Value of 410 CFU/100 ml of generic *E. coli*) listed in the 2012 EPA standards. Alternative provisions to comply with these rules have also been provided by FDA when water does not meet these numerical values. These options take into consideration the microbial die-off, 0.5 log per day, which may occur naturally in the field between the last irrigation event and harvest and for no longer than four consecutive days. Despite these potentially useful alternatives, further science-based information to support these alternative provisions is needed, especially when multiple crop-specific and environmental factors significantly alter targeted die-off rates, and in crops like strawberry and cilantro for which no additional postharvest intervention strategies are available to potentially reduce this risk.

Multiple *E. coli* outbreaks have motivated the utilization of quantitative approaches to calculate the public health risk imposed by the consumption of fresh fruits and vegetables[7-10]. However, these efforts are hampered by the lack of models that can simulate pathogen behavior before harvest[1]. The systematic review of this body of work, however, revealed one key finding, which would challenge current industry guidelines: *E. coli* survival/die-off kinetics do not conform to first order kinetics[1, 11]. Other mathematical models—Weibull[12, 13], biphasic [1, 14]and log-linear[15]—have been put forward to more accurately describe *E. coli* survival/die-off. A simple analysis of previously published *E. coli* O157:H7 survival from the surface of spinach leaves [3] suggest variable die-off rates between 0.3 and 0.2 log per day, depending on the predictive model used to describe the rates. These values differ from those proposed by current FSMA provisions and bring into question the microbial safety of produce if following these guidelines. Systematic research on pathogen die-off dynamics after crop exposure to contaminated AW is imperative to create and parameterize accurate predictive models on preharvest risk factors of *E. coli* contamination.

Research Methods

Strawberry (cv. Chandler) and cilantro (cv. Santo) plants were grown following commercial practices for each crop. Strawberry mesocosms (25 x 25 cm) consisted of 2 strawberry plants per container (Figure 3 – in Appendix). Cilantro mesocosms (20 x 20 cm) contained ~50 plants (Figure 4). Each crop was inoculated with the following strains: (1) avirulent *E. coli* O157 (ATCC #43888), Rif resistant; (2) pathogenic *E. coli* O157 GFP + Kan resistant, isolate from lettuce-related outbreak; (3) pathogenic STEC non-O157: *E. coli* O45 (Rif and Kan resistant), *E. coli* O111, *E. coli* O145; and (4) generic *E. coli*: W778 (isolated from water and used on multiple field studies in CA and NC); or (5) Butterfield's phosphate buffer (BPB) as the control.

Bacterial growth

Strains were cultured following the procedures described by Gutierrez-Rodriguez et al. 2011[3], Li et al. 2013[16] and Rodriguez-Lazaro et al. 2014[17]. In brief, all selected microorganisms from stock solutions stored at −80°C were first streaked on ChromSTEC or ChromO157 and tryptic soy agar (TSA) amended with Rif, Kan, or no antibiotic. From the streaked TSA plates, individual colonies were selected and grown for 24 h on mEHEC amended with strain-specific antibiotics. After incubation the bacterial growth was cleaned through multiple wash steps with potassium phosphate, and the pellet was re-suspended using BPB, and then the optical density (OD) was adjusted to 0.8–0.9. The cleaned supernatant was then spread plated (100 µL) on TSA plates amended with the respective antibiotic, and incubated overnight to achieve a lawn bacterial growth. The bacterial lawn plates were used to prepare the targeted inoculum at concentrations of log 3 and 6 CFU/ml.

Bacteria inoculation and recovery

For strawberry, spot inoculation concentrations were log 6 or 3 CFU/ml and each leaf received a total of 10 10-µl droplets, for a final volume of 100 µl per leaf; a total of 288 leaves were inoculated per experiment (6 experiments in total). For cilantro, spray inoculations were performed at concentrations of log 6 and 3 CFU/400 cm^2; a total of 900 leaves were inoculated per experiment, and each mesocosm was inoculated with 10 ml of each solution. Once inoculated, bacteria were recovered for both crops at 0, 2, 3, 4, 7 and 8 days after inoculation, without (sterile water) or with chlorine or PAA applications. Both of these solutions were sprayed to the plants 4 days after inoculation, and the control treatment was sterile water. Bacterial recovery follow the procedure described by Gutierrez-Rodriguez et al. 2011[3], Li et al. 2013[16] and Rodriguez-Lazaro et al. 2014[17]. In brief, strawberry or cilantro replicates comprised 1 leaf or 4–6 leaves, respectively, per treatment. Each sample was harvested with sterile tweezers and placed inside a sterile 2-oz bag. The bags were placed inside a cooler with ice, and then transported from the greenhouse facility to the Gutierrez lab. Once at the lab, BPB amended with Tween 20 was added to each bag at a 1:2 ratio. Each sample was macerated with a mallet, and the supernatant was used to estimate the bacterial population of the inoculated strains. When populations were below the limit of detection, each sample was enriched following the procedures described by Gutierrez-Rodriguez et al. 2011[3], Li et al. 2013[16] and Rodriguez-Lazaro et al. 2014[17] to confirm the presence and persistence of the inoculated strains.

Recovery of background indicator organisms

For all experiments the presence of naturally occurring coliforms, generic *E. coli* and *Enterococci* was quantified from the surface of strawberry and cilantro leaves with and without the presence of the inoculated strains and the application of sterile water, chlorine or PAA at 4 days after inoculation. Recovery of these indicator microorganisms was achieved by plating the supernatant from each sample on ChromECC and M-Enterococcus selective and differential media. When necessary, the presence of *Enterococci* was further confirmed RT-PCR following the procedure described by Haugland et al. 2005[18].

Chlorine and PAA concentrations

At 4 days post inoculation a subset of previously inoculated strawberry and cilantro leaves were treated with a chlorine or PAA solution at a concentration of 100 and 40 ppm, respectively (both adjusted to pH 6.5), which represents the average concentrations used in the industry to treat water or disinfect leaves/fruit without causing any damage to the crop. Both crops were sprayed with 4 ml of either solution, while the control treatment received 4 ml of sterile water.

Environmental data

Environmental data was collected during the length of each experiment and used to evaluate the die-off kinetics of the selected strains using the following predictive models: (i) classical log-linear, (ii) sigmoidal-like, (iii) concave and convex, and (iv) biphasic[5, 6]. Recorded parameters included relative humidity, ambient temperature, and photosynthetic active radiation and UV light.

Research Results

Strawberry

The die-off kinetics of 6 different *E. coli* strains were determined from the surface of strawberry leaves inoculated at log 3 and 6 CFU/ml. All inoculations were performed at dusk to provide conditions conducive to bacteria establishment; the overall temperature of the greenhouse fluctuated between 10 and 30°C and an average relative humidity (RH) of 35%. Each experiment consisted of 2 to 4 strains inoculated at the same time on different plants. The goal was to compare the die-off of surrogate, pathogenic and attenuated strains of *E. coli* under the same growing conditions. Irrespective of experiment and marginal variations in temperature and RH, all inoculated strains followed similar die-off patterns between independent experiments (Tables 1–4). Background populations of coliforms and generic *E. coli* were similar between experiments (Tables 5–8). In general, coliforms were present at higher concentrations than generic *E. coli*, and the presence of *Enterococci* was not detected in any experiment. Coliform populations varied between 0 and 4 log CFU/g, while generic *E. coli* populations varied between 0 and 1.2 log CFU/g. There was no correlation between the presence of any of these indicator organisms and the persistence of any of the inoculated strains.

The die-off kinetics for generic *E. coli*, pathogenic O157, attenuated O157, and *E. coli* O145 followed a biphasic model irrespective of inoculum concentrations. Log linear, linear, sigmoidal and convex models were not able to accurately describe die-off of these strains. Die-off for this group of four strains followed a polynomial approach and was consistent across all experiments (Figure 1). At 7 or 8 days after inoculation most of the inoculated strains remained below the limit of detection for all treatments (sterile water, chlorine and PAA). A small portion of these samples continued to be positive after enrichment and no clear differences between the control (sterile water) and the spray treatments (chlorine and PAA) were observed since recovery efficacy fluctuated between 0 and 31%. For instance in experiment 1, recovery at 8 days after enrichment for all 3 treatments was 0%, while for experiments 2–4 recovery varied by strain and it was irrespective of inoculum dose and treatment. For this group of experiments, recovery after 8 days was 0% for generic *E. coli*, 13% for *E. coli* O145, and 17 or 19% for pathogenic or attenuated *E. coli* O157, respectively.

The die-off kinetics for *E. coli* O45 and *E. coli* O111 were different than for the other strains used in this project, irrespective of inoculum concentrations (Figure 1). Log linear, linear, sigmoidal and convex models were also not able to accurately describe the die-off of these strains. Persistence of *E. coli* O45 and *E. coli* O111 was also different than for the other inoculated strains. *E. coli* O111 presented the highest die-off rates of all the inoculated strains (Figure 1, Table 3) since it was not detected after 2 and 4 days after inoculation at log 3 and 6 CFU/ml, respectively. For *E. coli* O45 and *E. coli* O111 strains there was no clear differences in their persistence after the application of chlorine, PAA and sterile water, since 7 days after inoculation all samples were negative after enrichment (Table 3).

Die-off rates for the 6 inoculated strains were higher when inoculated at log 6 CFU/ml than when inoculated at log 3 CFU/ml. If we assume a linear die-off, all 6 strains at the low inoculum dose presented an average die-off of 0.4 CFU/day, while at the higher inoculum dose the average die-off rate was 0.82 CFU/day. However, in reality this linear die-off was not observed with any of the treatments. No clear reduction in the population of the inoculated strains was observed when plants were sprayed with chlorine (100 ppm) or PAA (40 ppm).

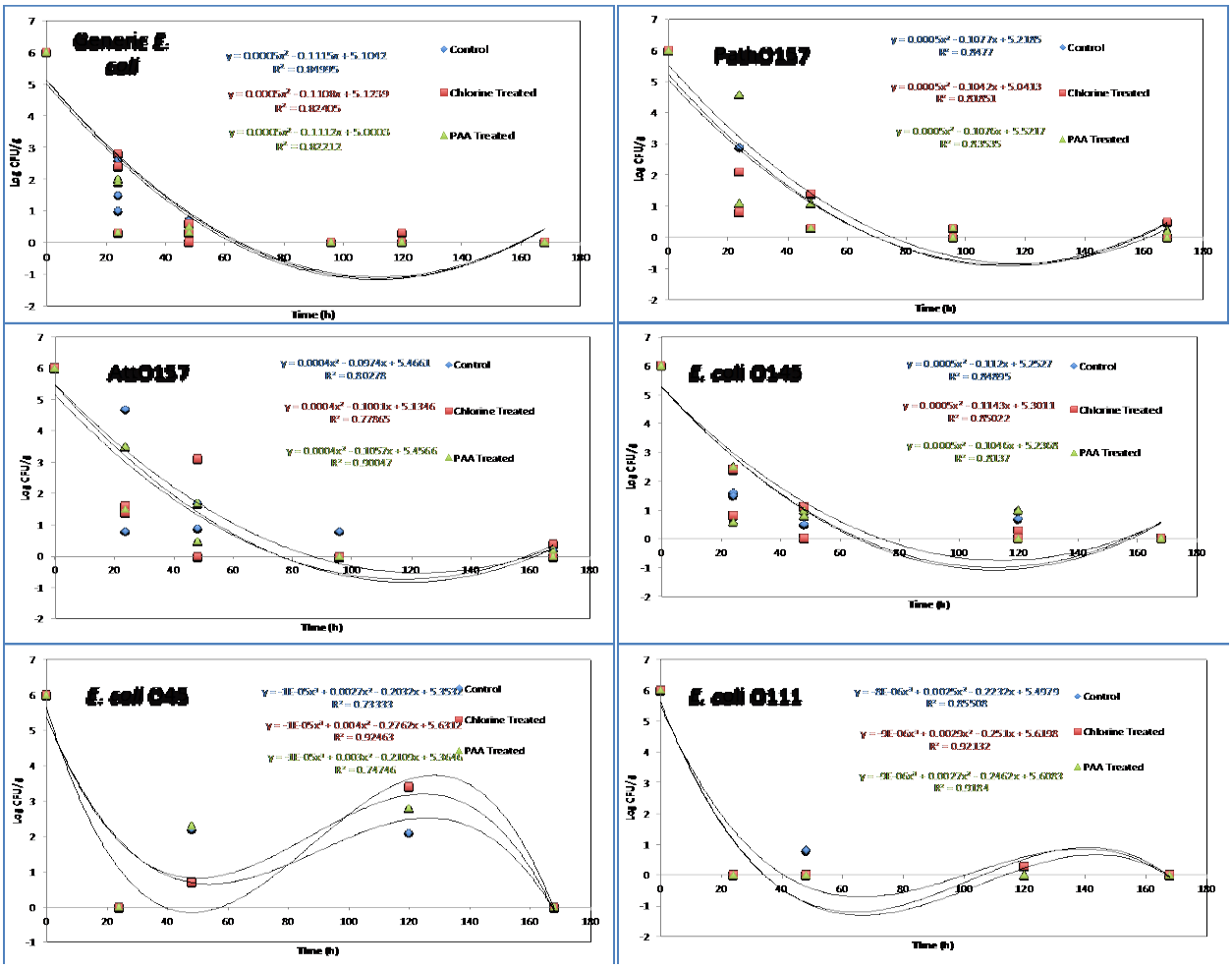

Figure 1. Die-off kinetics of generic *E. coli*, pathogenic and attenuated *E. coli* O157:H7, *E. coli* O145, *E. coli* O45 and *E. coli* O111 from the surface of strawberry leaves. Data combines results from two independent experiments (Tables 1–4) and uses a polynomial best-fit approach to describe die-off of these pathogens inoculated to the surface of strawberry leaves at log 6 CFU/ml. Similar die-off kinetics were observed when these strains were inoculated at log 3 CFU/ml.

Cilantro

The die-off kinetics of the same 6 *E. coli* strains used with strawberry also were determined from the surface of cilantro leaves spray inoculated at log 3 and 6 CFU/ml. As described earlier the only difference between inoculation approaches for strawberry and cilantro was the use of a small spray system instead of spot inoculation. This spray system allowed even distribution of small droplets on the surface of the leaves and prevented excessive plant bending and contamination with soil debris. All inoculations were also performed at dusk, and the overall temperature of the greenhouse fluctuated between 5 and 23°C and an average RH of 34%. Each experiment consisted of 3 strains inoculated at the same time on different plants. Irrespective of experiment and marginal variations in temperature and RH, all inoculated strains followed similar die-off patterns between experiments (Table 9 and 10, Figure 2). Background populations of coliforms and generic *E. coli* were similar between experiments (Table 11 and 12). In general, coliforms were present at higher concentrations than generic *E. coli*, and the presence of *Enterococci* was detected in only one sample. Coliform populations varied between 0 and 4.9 log CFU/g, while generic *E. coli* populations varied between 0 and 3.6 log CFU/g. As expected the number of leaves with coliforms and *E. coli* was greater for cilantro

than for strawberry. This result was expected since cilantro-growing conditions utilize sprinkle irrigation, no plastic cover, and plants are in close proximity to the soil. Similarly to strawberry plants, there was no correlation between the presence of any of these indicator organisms and the persistence of any of the inoculated strains. The application of chlorine (100 ppm) or PAA (40 ppm) also had marginal effects on the population of these background microorganisms (Table 11 and 12).

As with strawberry, the die-off kinetics for generic *E. coli* and pathogenic and attenuated *E. coli* O157:H7 also followed a biphasic model, irrespective of inoculum concentrations. Log linear, linear, sigmoidal and convex models were not able to accurately describe die-off of these strains. Die-off for this group of strains followed a polynomial approach and was consistent across all experiments (Figure 2).

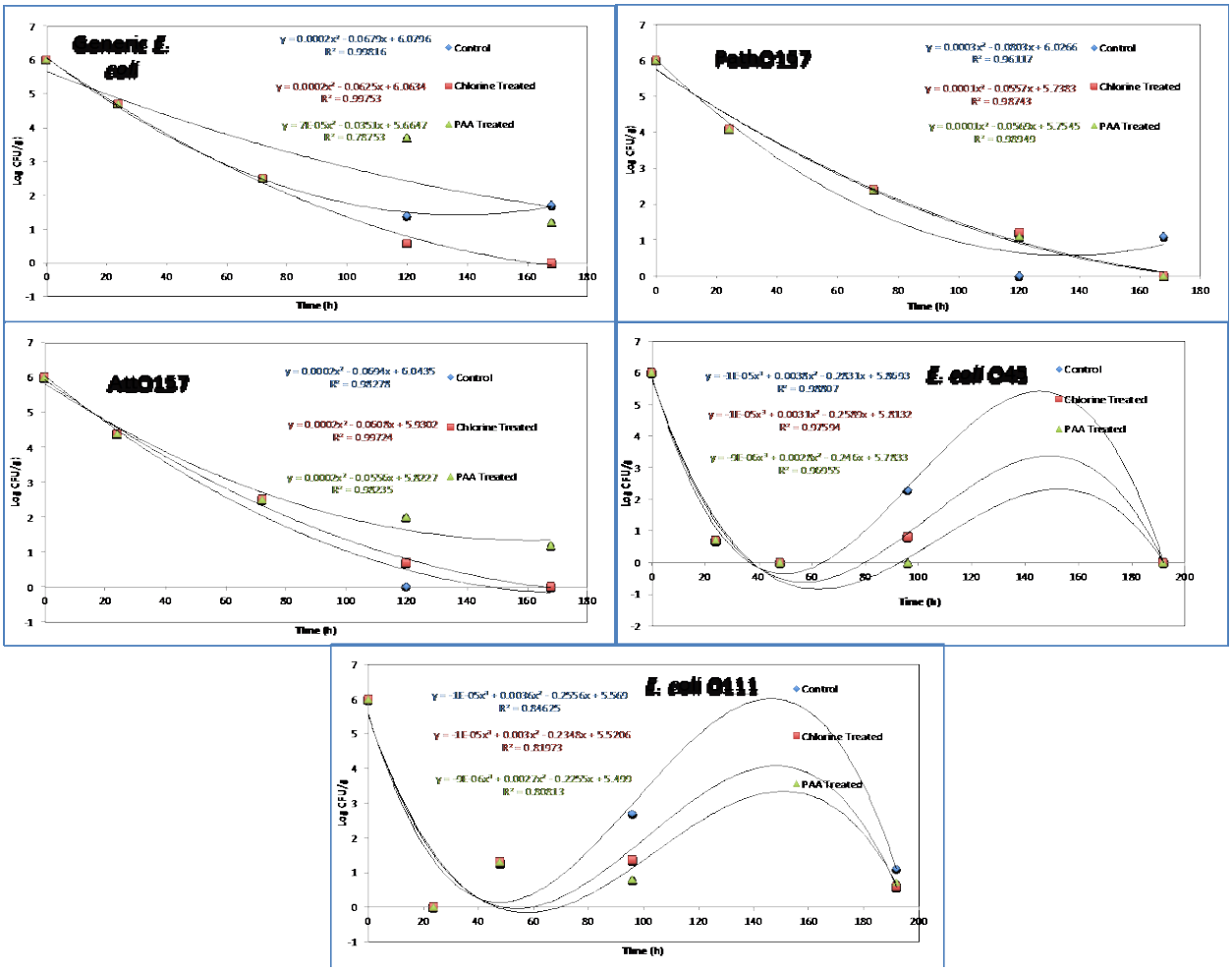

Figure 2. Die-off kinetics of generic *E. coli*, pathogenic and attenuated *E. coli* O157:H7, *E. coli* O45 and *E. coli* O111 from the surface of cilantro leaves. Data combines results from two independent experiments (Table 9 and 10) and uses a polynomial best-fit approach to describe die-off of these pathogens inoculated to the surface of cilantro leaves at log 6 CFU/ml. Similar die-off kinetics were observed when these strains were inoculated at log 3 CFU/ml.

At 7 or 8 days after inoculation most of the inoculated strains remained below the limit of detection for all treatments (sterile water, chlorine and PAA); however, a larger number of positive samples after enrichment were detected for cilantro than for strawberry. This result was expected, since cilantro leaves were well covered with the inoculum when compared with inoculated strawberry leaves. No clear difference in the survival and persistence of the inoculated strains was observed between the control (sterile water) and the

spray treatments (chlorine and PAA) (Table 9 and 10). Similarly to strawberry, the die-off rates for all strains were higher when inoculated at log 6 CFU/ml than when inoculated at log 3 CFU/ml. Assuming a linear die-off for all strains, the low inoculum dose presented an average die-off of 0.36 CFU/day, while for the higher inoculum dose the average die-off was 0.75 CFU/day. Both of these values are slightly lower than for strawberry but are not significantly different. However, in reality this linear die-off was not observed with any of the treatments.

Despite the difference in inoculation method, the die-off kinetics for *E. coli* O45 and *E. coli* O111 on cilantro were similar to those observed on strawberry, and significantly different than the other strains used in this project, irrespective of inoculum concentrations (Figure 1 and 2). Log linear, linear, sigmoidal and convex models were also not able to accurately describe die-off of these strains. On cilantro, persistence of *E. coli* O45 and *E. coli* O111 was similar to the rest of the inoculated strains (Table 9 and 10). However, both strains had the slowest die-off rates compared with the other strains when inoculated at log 3 CFU/ml. For these two strains there also was no clear difference in their persistence after the application of chlorine (100 ppm), PAA (40 ppm) or sterile water (Table 9 and 10).

Overall, irrespective of inoculum dose and plant type, the die-off kinetics for generic *E. coli*, pathogenic and attenuated *E. coli* O157:H7 and *E. coli* O145 were identical along all experiments. *E. coli* O45 and *E. coli* O111 behaved differently than the other strains but similarly when inoculated on both plant systems. The proposed preharvest spray applications of chlorine (100 ppm) and PAA (40 ppm) had marginal and no conclusive positive impact in reducing the survival and persistence of any of the inoculated strains. The proposed surrogate generic *E. coli* W778 was able to accurately represent die-off rates of 4 out the 6 strains used in this study. Although the kinetics for *E. coli* O45 and *E. coli* O111 were different than the proposed surrogate, the overall persistence and survival of *E. coli* W778 was similar to these other two pathogens, suggesting that this surrogate could be used in open field environments to further test die-off kinetics under standard growing practices.

Outcomes and Accomplishments

1. Die-off rates for EHEC, STEC and generic *E. coli* were strain and concentration dependent.
2. Die-off rates for the inoculated pathogens varied between 0.09 and 1.5 CFU per day.
3. Irrespective of pathogen fitness and virulence, die-off rates after 8 days postinoculation (DPI) did not adjust to a linear model as proposed by the FSMA Produce Safety Rule.
4. Marginal efficacy in reducing the survival and persistence of artificially inoculated EHEC, STEC and generic *E. coli* strains was observed when chlorine and PAA were applied to the surface of strawberry and cilantro leaves over a period of 4 days.
5. No in-field phytotoxicity was observed in either crop when chlorine or PAA were applied at 100 and 40 ppm, respectively, over a period of 4 days.
6. *E. coli* W778 (surrogate) die-off rate accurately mimicked the die-off rates of 4 out of the 6 strains used in this study.
7. *E. coli* W778 (surrogate) had similar survival and persistence when compared with the other strains used in this study.

Summary of Findings and Recommendations

Strawberry

Die-off rates for EHEC, STEC and generic *E. coli* were strain and concentration dependent. However, all strains displayed bimodal die-off dynamics, with higher rates between 0 and 2-DPI and lower rates thereafter. All inoculated strains at log 6.0 CFU/ml displayed linear die-off rates >0.5 log CFU/day throughout the duration of the experiments. At log 3.0 CFU/ml, all inoculated strains displayed linear die-off rates <0.5 log CFU/day. However, in reality this linear die-off was not observed with any of the treatments. Similar die-off kinetics were observed for generic *E. coli*, pathogenic and attenuated *E. coli* O157:H7, and *E. coli* O145. Chlorine and PAA treatments had marginal to no conclusive effects on reducing the population of the inoculated pathogens 8 days after inoculation.

Cilantro

As with strawberry, die-off rates for EHEC, STEC and generic *E. coli* also were strain and concentration dependent and similarly showed bimodal die-off dynamics, with higher rates between 0 and 2-DPI and lower rates thereafter. As with strawberry, all inoculated strains at log 6.0 CFU/ml displayed linear die-off rates >0.5 log CFU/day throughout the duration of the experiments, while at log 3.0 CFU/ml, all inoculated strains displayed linear die-off rates <0.5 log CFU/day. However, in reality this linear die-off was not observed with any of the treatments. Higher numbers of positive samples after enrichment were detected on cilantro than on strawberry leaves. For cilantro, there was no discernible difference between applications of chlorine and PAA. *E. coli* O45 and *E. coli* O111 behaved differently than the other strains but were similar to each other when inoculated on both plant systems

Strawberry and cilantro combined

Irrespective of pathogen fitness and virulence, die-off rates after 8-DPI did not adjust to a linear model as proposed by the FSMA Produce Safety Rule, and were strain and concentration dependent. No in-field phytotoxicity was observed in either crop when chlorine or PAA were applied at 100 and 40 ppm, respectively, over a period of 4 days. Chlorine and PAA treatments had marginal to no conclusive effects on reducing the population of the inoculated pathogens 8 days after inoculation.

Overall recommendation

Based on our project findings the current alternative provision of applying an in-field die-off rate of 0.5 log per day within 4 consecutive days between the last irrigation event and harvest (potential cumulative die-off of 2.0 log) needs further revision and may not provide the necessary level of protection against postharvest pathogen contamination in cilantro or strawberry plants.

Pitfalls of the project:

Phytotron access. It was not possible to perform our experiments at this location because of unfulfilled commitments to allow entry of strawberry and cilantro plants grown under field conditions as was originally accepted and listed in the grant. A new BSL2 greenhouse was built in collaboration with the other Co-PIs of this grant, where all experiments were performed until the end of the project.

Strawberry experiments. Data gathered in 5 out of 9 experiments was discarded and not included in this final report. Several unforeseen problems were encountered with lab personnel, who are no longer part of the Gutierrez Lab, and with greenhouse growing conditions, which hindered our ability to use this information. All issues were resolved by September–October 2016 and most experiments were repeated during the duration of the grant. Fruit was not tested, but will be completed before the 2017 CPS Research Symposium.

Cilantro inoculations. There were no issues with these set of experiments, however because of delays with strawberry evaluations at the moment this report was submitted we were only able to complete 50% of all planned activities.

Next steps in the project:

During the months of April and May 2017 we will complete the rest of activities specific to cilantro (50%) and we will also incorporate strawberry fruit evaluations. If allowed, an addendum to this report will be submitted June 1st 2017.

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APPENDICES

Publications and Presentations

IAFP 2017 Symposium – Tampa, Florida (July 2017)
Title of abstract/presentation: Die-off Rates of Surrogate and Virulent EHEC-STEC Strains from the Surface of Strawberry vary with Time, Inoculum Dose and Chemical Interventions.
Authors: Albarracin, M.A., Gunter, C., Thakur, S., and Gutierrez-Rodriguez, E.

Budget Summary

The majority of funds allocated to this project (~$46,400) have been spent; the remaining funds will be used to travel to the CPS Research Symposium in Denver and present final research results.

Description	**Current Budget**	**Current Month Expenses**	**Project to Date Expenses**	**Budget Balance Available**
Salaries	16,100.00	-3,000	17,479.97	-1,379.97
Fringe Benefits	3,150.00	-229.50	1530.01	1,619.99
Contracted Services	0	0	0	0
Supplies and Materials	24,440.00	0	24,440.00	0
Domestic Travel	4,500.00	0	1,639.80	2,860.20
Foreign Travel	0	0	0	0
Other Travel	0	0	0	0
Current Services	300	0	193.55	106.45
Fixed Charges	0	0	0	0
Equipment	0	0	0	0
Student Aid	0	0	0	0
Subcontracts	0	0	0	0
Other Direct Charges	0	0	0	0
Budget Pool	145	0	0	145
Total Direct Costs	48,635.00	-282.20	45,283.33	3,351.67
Total Indirect Costs	963	0	1,112.02	-149.02
TOTAL COSTS	49,598.00	-282.20	46,395.35	3,202.65

Tables 1–12 and Figures 3–4

Strawberry Data

Table 1. Microbial log reduction and die-off rates of pathogenic and attenuated *E. coli* O157:H7 from the surface of strawberry leaves without and with the application of chlorine (100 ppm) and PAA (40 ppm) at 4 days after inoculation and over a period of 7 days post inoculation.

Strain	Inoculum Log CFU/ml	Day 0.5			Day 2			Day 7			Die-off (CFU) /day
		Log CFU/g +/- STE*									
Control (Sterile Water)											
Path O157	6	2.9	+/-	1.0	1.4	+/-	1.0	0.5	+/-	0.5	0.78
	3	3.5	+/-	1.3	1.2	+/-	0.7	0.0	+/-	0.0	0.4
Att O157	6	4.7	+/-	0.1	1.7	+/-	0.6	0.2	+/-	0.2	0.83
	3	3.9	+/-	1.3	2.6	+/-	0.3	0.2	+/-	0.2	0.4
Chlorine application (100 ppm)											
Path O157	6	2.1	+/-	1.2	0.3	+/-	0.3	0.5	+/-	0.2	0.78
	3	3.6	+/-	1.2	0.3	+/-	0.3	0.3	+/-	0.2	0.38
Att O157	6	1.6	+/-	1.6	3.1	+/-	0.3	0.4	+/-	0.4	0.8
	3	3.0	+/-	0.4	2.4	+/-	0.3	0.2	+/-	0.2	0.4
PAA application (40 ppm)											
Path O157	6	4.6	+/-	1.2	1.1	+/-	0.6	0.0	+/-	0.0	0.86
	3	3.4	+/-	0.1	1.1	+/-	0.4	0.3	+/-	0.2	0.38
Att O157	6	3.5	+/-	1.2	1.7	+/-	0.2	0.2	+/-	0.2	0.83
	3	2.8	+/-	1.6	3.3	+/-	0.3	0.2	+/-	0.2	0.4

PathO157= pathogenic *E. coli* O157:H7 and AttO157=attenuated *E. coli* O157:H7. Data represents average values for 4 replicates per treatment and day of evaluation. Die-off per day was calculated by subtracting the initial bacterial concentration applied to the leaves and the final concentration of the same bacteria after the sampling event where no additional bacteria were recovered by direct plating and enrichment, and dividing this value by the number of sampling days when it was no longer recovered (assuming linear die-off).
* STE = standard error

Table 2. Microbial log reduction and die-off rates of two different pathogenic *E. coli* O145 strains and generic *E. coli* from the surface of strawberry leaves without and with the application of chlorine (100 ppm) and PAA (40 ppm) at 4 days after inoculation and over a period of 7 days post inoculation.

Run	Inoculum Log CFU/ml	Day 0.5	Day 2	Day 5	Day 8	Die-off (CFU) /day
		Log CFU/g +/- STE*				
Control (Sterile Water)						
E. coli O145 (A)	6	1.6 +/- 0.68	0.5 +/- 0.29	0.7 +/- 0.74	0.0 +/- 0.00	0.75
	3	0.0 +/- 0.00	0.0 +/- 0.00	0.0 +/- 0.00	0.4 +/- 0.42	0.33
E. coli O145 (B)	6	1.5 +/- 0.29	0.5 +/- 0.29	0.0 +/- 0.00	0.0 +/- 0.00	1.2
	3	1.1 +/- 0.65	1.1 +/- 0.76	0.0 +/- 0.00	0.0 +/- 0.00	0.6
Generic *E. coli*	6	2.6 +/- 0.35	0.0 +/- 0.00	0.0 +/- 0.00	0.0 +/- 0.00	0.75
	3	0.6 +/- 0.62	0.0 +/- 0.00	0.0 +/- 0.00	0.0 +/- 0.00	0.38
Chlorine application (100 ppm)						
E. coli O145 (A)	6	0.8 +/- 0.25	0.0 +/- 0.00	0.3 +/- 0.25	0.0 +/- 0.00	0.75
	3	1.3 +/- 0.80	0.0 +/- 0.00	0.5 +/- 0.50	0.3 +/- 0.25	0.34
E. coli O145 (B)	6	2.4 +/- 0.26	1.1 +/- 1.06	0.0 +/- 0.00	0.0 +/- 0.00	1.2
	3	1.7 +/- 0.71	0.8 +/- 0.84	0.0 +/- 0.00	0.0 +/- 0.00	0.6
Generic *E. coli*	6	2.8 +/- 0.20	0.0 +/- 0.00	0.0 +/- 0.00	0.0 +/- 0.00	0.75
	3	1.4 +/- 0.81	0.0 +/- 0.00	0.0 +/- 0.00	0.0 +/- 0.00	0.38
PAA application (40 ppm)						
E. coli O145 (A)	6	0.6 +/- 0.59	0.8 +/- 0.80	1.0 +/- 0.97	0.0 +/- 0.00	0.75
	3	0.0 +/- 0.00	0.5 +/- 0.29	0.0 +/- 0.00	0.3 +/- 0.25	0.34
E. coli O145 (B)	6	2.5 +/- 0.16	1.0 +/- 0.58	0.0 +/- 0.00	0.0 +/- 0.00	0.75
	3	0.9 +/- 0.63	0.0 +/- 0.00	0.3 +/- 0.25	0.0 +/- 0.00	0.38
Generic *E. coli*	6	1.9 +/- 0.67	0.5 +/- 0.50	0.0 +/- 0.00	0.0 +/- 0.00	0.75
	3	0.0 +/- 0.00	0.0 +/- 0.00	0.0 +/- 0.00	0.0 +/- 0.00	0.38

Data represents average values for 4 replicates per treatment and day of evaluation. Die-off per day was calculated by subtracting the initial bacterial concentration applied to the leaves and the final concentration of the same bacteria after the sampling event where no additional bacteria were recovered by direct plating and enrichment, and dividing this value by the number of sampling days when it was no longer recovered (assuming linear die-off).
* STE = standard error

Table 3. Microbial log reduction and die-off rates of pathogenic *E. coli* O45 and *E. coli* O111 and generic *E. coli* from the surface of strawberry leaves without and with the application of chlorine (100 ppm) and PAA (40 ppm) at 4 days after inoculation and over a period of 7 days post inoculation.

Run	Inoculum Log CFU/ml	Day 0.5	Day 2	Day 5	Day 7	Die-off (CFU) /day
		Log CFU/g +/- STE*				
Control (Sterile Water)						
E. coli O45	6	0.0 +/- 0.00	2.2 +/- 0.33	2.1 +/- 0.11	0.0 +/- 0.00	0.86
	3	0.0 +/- 0.00	2.2 +/- 0.24	2.6 +/- 0.88	0.0 +/- 0.00	0.43
E. coli O111	6	0.0 +/- 0.00	0.8 +/- 0.76	0.3 +/- 0.33	0.0 /- 0.00	0.86
	3	0.0 +/- 0.00	0.4 +/- 0.40	0.0 +/- 0.00	0.0 +/- 0.00	0.6
Generic *E. coli*	6	1.0 +/- 0.33	0.7 +/- 0.38	0.0 +/- 0.00	0.0 +/- 0.00	0.86
	3	0.3 +/- 0.33	0.0 +/- 0.00	0.0 +/- 0.00	0.0 +/- 0.00	0.43
Chlorine application (100 ppm)						
E. coli O45	6	0.0 +/- 0.00	0.7 +/- 0.67	3.4 +/- 0.22	0.0 +/- 0.00	0.86
	3	0.0 +/- 0.00	1.2 +/- 0.40	2.3 +/- 0.61	0.0 +/- 0.00	0.43
E. coli O111	6	0.0 +/- 0.00	0.0 +/- 0.00	0.3 +/- 0.33	0.0 +/- 0.00	0.86
	3	0.0 +/- 0.00	0.0 +/- 0.00	0.0 +/- 0.00	0.0 +/- 0.00	0.6
Generic *E. coli*	6	0.3 +/- 0.33	0.3 +/- 0.33	0.0 +/- 0.00	0.0 +/- 0.00	0.86
	3	0.0 +/- 0.00	0.0 +/- 0.00	0.0 +/- 0.00	0.0 +/- 0.00	N/A
PAA application (40 ppm)						
E. coli O45	6	0.0 +/- 0.00	2.3 +/- 0.30	2.8 +/- 0.40	0.0 +/- 0.00	0.86
	3	0.0 +/- 0.00	0.4 +/- 0.44	1.9 +/- 0.71	0.0 +/- 0.00	0.43
E. coli O111	6	0.0 +/- 0.00	0.0 +/- 0.00	0.0 +/- 0.00	0.0 +/- 0.00	1.2
	3	0.0 +/- 0.00	0.0 +/- 0.00	0.0 +/- 0.00	0.0 +/- 0.00	1.5
Generic *E. coli*	6	0.3 +/- 0.33	0.0 +/- 0.00	0.0 +/- 0.00	0.0 +/- 0.00	1.2
	3	0.0 +/- 0.00	0.0 +/- 0.00	0.0 +/- 0.00	0.0 +/- 0.00	0.6

Data represents average values for 4 replicates per treatment and day of evaluation. Die-off per day was calculated by subtracting the initial bacterial concentration applied to the leaves and the final concentration of the same bacteria after the sampling event where no additional bacteria were recovered by direct plating and enrichment, and dividing this value by the number of sampling days when it was no longer recovered (assuming linear die-off).
* STE = standard error

Table 4. Microbial log reduction and die-off rates of pathogenic and attenuated *E. coli* O157:H7 and generic *E. coli* from the surface of strawberry leaves without and with the application of chlorine (100 ppm) and PAA (40 ppm) at 4 days after inoculation and over a period of 7 days post inoculation.

Run	Inoculum Log CFU/ml	Day 0.5	Day 2	Day 4	Day 7	Die-off (CFU) /day
		Log CFU/g +/- STE*				
Control (Sterile Water)						
Path O157	6	0.8 +/- 0.25	0.3 +/- 0.29	0.0 +/- 0.00	0.0 +/- 0.00	0.86
	3	0.6 +/- 0.36	0.7 +/- 0.44	0.0 +/- 0.00	0.0 +/- 0.00	0.43
Att O157	6	0.8 +/- 0.25	0.9 +/- 0.50	0.8 +/- 0.45	0.7 +/- 0.41	0.76
	3	0.0 +/- 0.00	0.0 +/- 0.00	0.0 +/- 0.00	0.0 +/- 0.00	0.75
E. coli	6	1.5 +/- 0.88	0.4 +/- 0.37	0.0 +/- 0.00	0.0 +/- 0.00	0.86
	3	0.9 +/- 0.32	0.0 +/- 0.00	0.4 +/- 0.39	0.0 +/- 0.00	0.43
Chlorine application (100 ppm)						
Path O157	6	0.8 +/- 0.25	1.4 +/- 0.13	0.3 +/- 0.25	0.0 +/- 0.00	0.86
	3	0.0 +/- 0.00	0.3 +/- 0.25	0.0 +/- 0.00	0.0 +/- 0.00	0.43
Att O157	6	1.4 +/- 0.59	0.0 +/- 0.00	0.0 +/- 0.00	0.0 +/- 0.00	1.5
	3	0.3 +/- 0.25	0.0 +/- 0.00	0.0 +/- 0.00	0.0 +/- 0.00	1.5
E. coli	6	2.4 +/- 0.79	0.6 +/- 0.34	0.0 +/- 0.00	0.0 +/- 0.00	0.86
	3	0.4 +/- 0.37	0.0 +/- 0.00	0.4 +/- 0.37	0.0 +/- 0.00	0.43
PAA application (40 ppm)						
Path O157	6	1.1 +/- 0.75	0.3 +/- 0.25	0.3 +/- 0.25	0.2 +/- 0.17	0.83
	3	1.1 +/- 0.18	0.0 +/- 0.00	0.0 +/- 0.00	0.0 +/- 0.00	0.75
Att O157	6	1.5 +/- 0.72	0.5 +/- 0.29	0.0 +/- 0.00	0.0 +/- 0.00	0.86
	3	0.0 +/- 0.00	0.3 +/- 0.25	0.0 +/- 0.00	0.0 +/- 0.00	0.75
E. coli	6	2.0 +/- 0.71	0.3 +/- 0.25	0.0 +/- 0.00	0.0 +/- 0.00	0.86
	3	0.3 +/- 0.25	0.0 +/- 0.00	0.0 +/- 0.00	0.0 +/- 0.00	0.75

PathO157= pathogenic *E. coli* O157:H7 and AttO157=attenuated *E. coli* O157:H7. Data represents average values for 4 replicates per treatment and day of evaluation. Die-off per day was calculated by subtracting the initial bacterial concentration applied to the leaves and the final concentration of the same bacteria after the sampling event where no additional bacteria were recovered by direct plating and enrichment, and dividing this value by the number of sampling days when it was no longer recovered (assuming linear die-off).

* STE = standard error

Table 5. Background population of coliforms and generic *E. coli* present on strawberry leaves inoculated with pathogenic and attenuated strains of *E. coli* O157:H7 at log 3 and 6 CFU/ml without and with the application of chlorine and PAA at concentrations of 100 and 40 ppm, respectively.

Day	Strain	Inoculum dose Log CFU/ml	Treatment	Coliforms Log CFU/g	STE	*E. coli* Log CFU/g	STE
0.5	Path O157	6	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	4.7	0.1	0.0	0.0
			No-PAA	2.2	1.3	0.0	0.0
		3	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	1.1	1.1	0.0	0.0
			No-PAA	1.1	1.1	0.0	0.0
	Att O157	6	No-Water	4.7	0.1	0.0	0.0
			No-Chlorine	4.5	0.1	0.0	0.0
			No-PAA	4.2	0.2	0.0	0.0
		3	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
2	Path O157	6	No-Water	1.1	1.1	0.0	0.0
			No-Chlorine	2.0	0.7	0.0	0.0
			No-PAA	0.7	0.7	0.0	0.0
		3	No-Water	1.5	1.0	0.0	0.0
			No-Chlorine	0.9	0.9	0.0	0.0
			No-PAA	1.3	0.8	0.0	0.0
	Att O157	6	No-Water	0.6	0.6	0.0	0.0
			No-Chlorine	2.3	0.8	0.0	0.0
			No-PAA	1.0	0.6	0.0	0.0
		3	No-Water	0.9	0.9	0.0	0.0
			No-Chlorine	1.3	0.8	0.0	0.0
			No-PAA	1.3	0.8	0.0	0.0
7	Path O157	6	Water	1.4	0.8	0.0	0.0
		3		2.4	0.9	0.0	0.0
		6	Chlorine	0.8	0.8	0.0	0.0
		3		2.6	0.9	0.0	0.0
		6	PAA	3.5	0.6	0.0	0.0
		3		1.2	0.7	0.0	0.0
	Att O157	6	Water	0.6	0.6	0.0	0.0
		3		1.7	0.6	0.0	0.0
		6	Chlorine	1.9	0.7	0.0	0.0
		3		2.7	0.5	0.0	0.0
		6	PAA	2.4	0.9	0.0	0.0
		3		0.5	0.5	0.0	0.0

Data represents averages of four replicates per sample and STE (standard error). Chlorine and PAA were applied at day 4 post inoculation. PathO157= pathogenic *E. coli* O157:H7. AttO157= attenuated *E. coli* O157:H7.

Table 6. Background population of coliforms and generic *E. coli* present on strawberry leaves inoculated with two different pathogenic *E. coli* O145 strains and generic *E. coli* at log 3 and 6 CFU/ml, without and with the application of chlorine and PAA at concentrations of 100 and 40 ppm, respectively.

Day	Strain	Inoculum dose Log CFU/ml	Treatment	Coliforms Log CFU/g	STE	*E. coli* Log CFU/g	STE
0.5	*E. coli* O145 (A)	6	No-Water	0.5	0.5	0.0	0.0
			No-Chlorine	1.0	0.7	0.0	0.0
			No-PAA	1.2	0.7	0.0	0.0
		3	No-Water	0.7	0.7	0.0	0.0
			No-Chlorine	0.7	0.7	0.0	0.0
			No-PAA	0.6	0.6	0.0	0.0
	E. coli O145 (B)	6	No-Water	1.4	0.8	0.0	0.0
			No-Chlorine	0.3	0.3	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
		3	No-Water	0.8	0.8	0.0	0.0
			No-Chlorine	1.5	0.9	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
	Generic *E. coli*	6	No-Water	1.0	1.0	1.2	0.7
			No-Chlorine	0.7	0.7	1.3	0.8
			No-PAA	1.7	1.0	1.4	0.8
		3	No-Water	0.6	0.6	0.0	0.0
			No-Chlorine	0.7	0.7	0.0	0.0
			No-PAA	1.4	1.0	0.3	0.3
2	*E. coli* O145 (A)	6	No-Water	0.6	0.6	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
		3	No-Water	0.5	0.5	0.0	0.0
			No-Chlorine	0.9	0.6	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
	E. coli O145 (B)	6	No-Water	0.8	0.8	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
		3	No-Water	0.8	0.8	0.0	0.0
			No-Chlorine	1.7	1.0	0.0	0.0
			No-PAA	0.8	0.8	0.0	0.0
	Generic *E. coli*	6	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
		3	No-Water	0.8	0.8	0.0	0.0
			No-Chlorine	0.3	0.3	0.0	0.0
			No-PAA	1.2	0.8	0.0	0.0

5	*E. coli* O145 (A)	6	No-Water	1.1	1.1	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
		3	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
	E. coli O145 (B)	6	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
		3	No-Water	0.5	0.5	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
	Generic *E. coli*	6	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.4	0.4	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
		3	No-Water	0.7	0.7	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
8	*E. coli* O145 (A)	6	Water	1.3	0.9	0.0	0.0
		3		0.0	0.0	0.0	0.0
		6	Chlorine	1.0	1.0	0.0	0.0
		3		0.4	0.4	0.0	0.0
		6	PAA	0.4	0.4	0.0	0.0
		3		0.0	0.0	0.0	0.0
	E. coli O145 (B)	6	Water	0.0	0.0	0.0	0.0
		3		0.5	0.5	0.0	0.0
		6	Chlorine	0.0	0.0	0.0	0.0
		3		0.6	0.6	0.0	0.0
		6	PAA	0.0	0.0	0.0	0.0
		3		0.4	0.4	0.0	0.0
	Generic *E. coli*	6	Water	1.6	1.0	0.0	0.0
		3		0.9	0.9	0.0	0.0
		6	Chlorine	1.5	1.0	0.0	0.0
		3		0.7	0.7	0.0	0.0
		6	PAA	0.8	0.8	0.0	0.0
		3		0.3	0.3	0.0	0.0

Data represents averages of four replicates per sample and standard error (STE). Chlorine and PAA were applied at day 4 post inoculation.

Table 7. Background populations of coliforms and generic *E. coli* present on strawberry leaves inoculated with pathogenic *E. coli* O45 and *E. coli* O111 and generic *E. coli* at log 3 and 6 CFU/ml, without and with the application of chlorine and PAA at concentrations of 100 and 40 ppm, respectively.

Day	Strain	Inoculum dose Log CFU/ml	Treatment	Coliforms Log CFU/g	STE	*E. coli* Log CFU/g	STE
0.5	*E. coli* O111	6	No-Water	0.6	0.6	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
		3	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.8	0.8	0.0	0.0
			No-PAA	0.8	0.8	0.0	0.0
	E. coli O45	6	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.6	0.6	0.0	0.0
			No-PAA	0.4	0.4	0.0	0.0
		3	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.7	0.7	0.0	0.0
	Generic *E. coli*	6	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
		3	No-Water	0.5	0.5	0.0	0.0
			No-Chlorine	0.6	0.6	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
2	*E. coli* O111	6	No-Water	0.3	0.3	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.4	0.4	0.0	0.0
		3	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	1.1	1.1	0.0	0.0
			No-PAA	0.7	0.7	0.0	0.0
	E. coli O45	6	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.6	0.6	0.0	0.0
			No-PAA	1.0	0.6	0.0	0.0
		3	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.6	0.6	0.0	0.0
			No-PAA	1.0	1.0	0.0	0.0
	Generic *E. coli*	6	No-Water	0.5	0.5	0.0	0.0
			No-Chlorine	0.3	0.3	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
		3	No-Water	1.0	1.0	0.0	0.0
			No-Chlorine	0.6	0.6	0.0	0.0
			No-PAA	0.4	0.4	0.0	0.0

5	*E. coli* O111	6	No-Water	1.1	1.1	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
		3	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
	E. coli O45	6	No-Water	0.8	0.8	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.8	0.8	0.0	0.0
		3	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.7	0.7	0.0	0.0
	Generic *E. coli*	6	No-Water	1.1	0.6	0.0	0.0
			No-Chlorine	1.1	0.7	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
		3	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	1.0	0.6	0.0	0.0
			No-PAA	0.6	0.6	0.0	0.0
7	*E. coli* O111	6	Water	0.0	0.0	0.0	0.0
		3		0.0	0.0	0.0	0.0
		6	Chlorine	0.0	0.0	0.0	0.0
		3		0.0	0.0	0.0	0.0
		6	PAA	0.0	0.0	0.0	0.0
		3		0.0	0.0	0.0	0.0
	E. coli O45	6	Water	0.0	0.0	0.0	0.0
		3		0.0	0.0	0.0	0.0
		6	Chlorine	0.0	0.0	0.0	0.0
		3		0.0	0.0	0.0	0.0
		6	PAA	0.0	0.0	0.0	0.0
		3		0.0	0.0	0.0	0.0
	Generic *E. coli*	6	Water	0.0	0.0	0.0	0.0
		3		0.0	0.0	0.0	0.0
		6	Chlorine	0.0	0.0	0.0	0.0
		3		0.0	0.0	0.0	0.0
		6	PAA	0.0	0.0	0.0	0.0
		3		0.0	0.0	0.0	0.0

Data represents averages of four replicates per sample and standard error (STE). Chlorine and PAA were applied at day 4 post inoculation.

Table 8. Background populations of coliforms and generic *E.coli* present on strawberry leaves inoculated with pathogenic and attenuated strains of *E. coli* O157:H7 and generic *E. coli* at log 3 and 6 CFU/ml, without and with the application of chlorine and PAA at concentrations of 100 and 40 ppm, respectively.

Day	Strain	Inoculum dose Log CFU/ml	Treatment	Coliforms Log CFU/g	STE	*E. coli* Log CFU/g	STE
0.5	Path O157	6	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
		3	No-Water	0.8	0.8	0.0	0.0
			No-Chlorine	0.9	0.9	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
	Att O157	6	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.6	0.6	0.0	0.0
		3	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.6	0.6	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
	E. coli	6	No-Water	0.6	0.6	0.0	0.0
			No-Chlorine	0.0	0.0	0.5	0.5
			No-PAA	1.2	0.4	2.0	0.7
		3	No-Water	0.3	0.3	0.4	0.4
			No-Chlorine	0.0	0.0	0.3	0.3
			No-PAA	0.0	0.0	0.0	0.0
2	Path O157	6	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
		3	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
	Att O157	6	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
		3	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
	E. coli	6	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
		3	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0

4	Path O157	6	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
		3	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
	Att O157	6	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
		3	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
	E. coli	6	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
		3	No-Water	0.0	0.0	0.0	0.0
			No-Chlorine	0.0	0.0	0.0	0.0
			No-PAA	0.0	0.0	0.0	0.0
7	Path O157	6	Water	0.0	0.0	0.0	0.0
		3		0.0	0.0	0.0	0.0
		6	Chlorine	0.0	0.0	0.0	0.0
		3		0.8	0.8	0.0	0.0
		6	PAA	0.0	0.0	0.0	0.0
		3		0.0	0.0	0.0	0.0
	Att O157	6	Water	0.0	0.0	0.0	0.0
		3		0.0	0.0	0.0	0.0
		6	Chlorine	0.0	0.0	0.0	0.0
		3		0.0	0.0	0.0	0.0
		6	PAA	0.0	0.0	0.0	0.0
		3		0.0	0.0	0.0	0.0
	E. coli	6	Water	0.0	0.0	0.0	0.0
		3		0.0	0.0	0.0	0.0
		6	Chlorine	0.0	0.0	0.0	0.0
		3		0.0	0.0	0.0	0.0
		6	PAA	0.0	0.0	0.0	0.0
		3		0.0	0.0	0.0	0.0

Data represents averages of four replicates per sample and standard error (STE). Chlorine and PAA were applied at day 4 post inoculation. PathO157= pathogenic *E. coli* O157:H7. AttO157= attenuated *E. coli* O157:H7.

Cilantro Data

Table 9. Microbial log reduction and die-off rates of pathogenic and attenuated *E. coli* O157:H7 and generic *E. coli* on the surface of cilantro leaves without and with the application of chlorine (100 ppm) and PAA (40 ppm) at 4 days after inoculation and over a period of 7 days post inoculation.

Strain	Inoculum Log CFU/ml	Day 0.5	Day 3	Day 5	Day 7	Die-off (CFU) /day
		Log CFU/g ± STE*				
Control (Sterile Water)						
PathO157	6.0	4.1 ± 0.2	2.4 ± 1.4	0.0 ± 0.0	1.1 ± 1.1	0.7
	3.0	3.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4
AttO157	6.0	3.6 ± 0.7	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.9
	3.0	0.0 ± 0.0	0.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.4
Generic *E. coli*	6.0	4.7 ± 0.1	2.5 ± 1.0	1.4 ± 0.8	1.7 ± 1.0	0.6
	3.0	3.9 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4
Chlorine application (100 ppm)						
PathO157	6.0	4.4 ± 0.1	1.3 ± 0.4	1.2 ± 0.7	0.0 ± 0.0	0.9
	3.0	3.8 ± 0.3	0.0 ± 0.0	1.2 ± 0.4	0.0 ± 0.0	0.4
AttO157	6.0	4.7 ± 0.1	0.0 ± 0.0	0.7 ± 0.7	0.0 ± 0.0	0.9
	3.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.4
Generic *E. coli*	6.0	4.7 ± 0.1	0.0 ± 0.0	0.6 ± 0.6	0.0 ± 0.0	0.9
	3.0	2.7 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.6	0.3
PAA application (40 ppm)						
PathO157	6.0	4.4 ± 0.1	0.0 ± 0.0	1.1 ± 0.6	0.0 ± 0.0	0.9
	3.0	3.2 ± 0.2	0.0 ± 0.0	0.4 ± 0.4	0.0 ± 0.0	0.4
AttO157	6.0	4.4 ± 0.1	2.5 ± 0.9	2.0 ± 0.7	1.2 ± 0.7	0.7
	3.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ±0.0	0.4
Generic *E. coli*	6.0	4.6 ± 0.1	0.5 ± 0.5	3.7 ± 1.6	1.2 ± 0.7	0.7
	3.0	2.4 ± 0.1	0.9 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.4

PathO157= pathogenic *E. coli* O157:H7 and AttO157=attenuated *E. coli* O157:H7. Data represents average values for 4 replicates per treatment and day of evaluation. Die-off per day was calculated by subtracting the initial bacterial concentration applied to the leaves and the final concentration of the same bacteria after the sampling event where no additional bacteria were recovered by direct plating and enrichment, and dividing this value by the number of sampling days when it was no longer recovered (assuming linear die-off).

* STE = standard error

Table 10. Microbial log reduction and die-off rates of *E. coli* O45, *E. coli* O111 and generic *E. coli* on the surface of cilantro leaves without and with the application of chlorine (100 ppm) and PAA (40 ppm) at 4 days after inoculation and over a period of 8 days post inoculation.

Strain	Inoculum Log CFU/ml	Day 0.5†	Day 2†	Day 4	Day 8	Die-off (CFU) /day
		Log CFU/g ± STE*				
Control (Sterile Water)						
E. coli O45	6.0	0.7 ± 0.4	0.0 ± 0.0	2.3 ± 0.8	0.0 ± 0.0	0.75
	3.0	0.4 ± 0.3	0.0 ± 0.0	0.8 ± 0.8	0.0 ± 0.0	0.4
E. coli O111	6.0	0.0 ± 0.0	1.3 ± 0.8	2.7 ± 0.2	1.1 ± 0.7	0.6
	3.0	0.0 ± 0.0	1.8 ± 0.6	1.1 ± 0.6	2.3 ± 0.9	0.09
Generic *E. coli*	6.0	4.1 ± 0.2	2.6 ± 1.0	2.1 ± 0.7	0.0 ± 0.0	0.75
	3.0	3.9 ± 0.2	1.7 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.4
Chlorine application (100 ppm)						
E. coli O45	6.0	0.7 ± 0.4	0.0 ± 0.0	0.8 ± 0.5	0.0 ± 0.0	0.75
	3.0	0.4 ± 0.3	0.0 ± 0.0	0.9 ± 0.9	0.0 ± 0.0	0.4
E. coli O111	6.0	0.0 ± 0.0	1.3 ± 0.8	1.4 ± 0.8	0.6 ± 0.6	0.7
	3.0	0.0 ± 0.0	1.8 ± 0.6	0.8 ± 0.7	1.5 ± 0.9	0.2
Generic *E. coli*	6.0	4.1 ± 0.2	2.6 ± 1.0	1.6 ± 0.6	0.4 ± 0.4	0.7
	3.0	3.9 ± 0.2	1.7 ± 0.6	0.8 ± 0.8	0.0 ± 0.0	0.4
PAA application (40ppm)						
E. coli O45	6.0	0.7 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.75
	3.0	0.4 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.75
E. coli O111	6.0	0.0 ± 0.0	1.3 ± 0.8	0.8 ± 0.8	0.7 ± 0.4	0.66
	3.0	0.0 ± 0.0	1.8 ± 0.6	0.4 ± 0.4	1.0 ± 0.6	0.25
Generic *E. coli*	6.0	4.1 ± 0.2	2.6 ± 1.0	0.5 ± 0.5	0.4 ± 0.4	0.66
	3.0	3.9 ± 0.2	1.7 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.4

Data represents average values for 4 replicates per treatment and day of evaluation. †For days 0.5 and 2, data from each sampling date was combined between treatments since no chlorine or PAA was applied until day 4. Die-off per day was calculated by subtracting the initial bacterial concentration applied to the leaves and the final concentration of the same bacteria after the sampling event where no additional bacteria were recovered by direct plating and enrichment, and dividing this value by the number of sampling days when it was no longer recovered (assuming linear die-off).
* STE = standard error

Table 11. Background population of naturally occurring coliforms and generic *E. coli* present on cilantro inoculated with pathogenic and attenuated strains of *E. coli* O157:H7 and generic *E. coli* at log 3 and 6 CFU/ml, treated without and with chlorine and PAA at 4 days after inoculation (experiment 1).

Day	Strain	Treatment	Inoculum dose Log CFU/ml	Coliforms Log CFU/g	STE	*E. coli* Log CFU/g	STE
Day 0.5	PathO157	No - Water	6	0.7	0.5	0.7	0.5
		No-Chlorine		1.9	0.3	2.1	0.1
		No-PAA		0.0	0.0	1.1	0.8
		No - Water	3	1.3	0.9	0.0	0.0
		No-Chlorine		0.0	0.0	1.0	0.7
		No-PAA		0.0	0.0	1.6	1.2
	AttO157	No - Water	6	0.0	0.0	0.0	0.0
		No-Chlorine		1.2	0.8	2.8	1.0
		No-PAA		1.8	1.3	0.0	0.0
		No - Water	3	0.0	0.0	0.0	0.0
		No-Chlorine		0.0	0.0	0.8	0.5
		No-PAA		0.0	0.0	2.4	1.7
	E. coli	No - Water	6	0.0	0.0	4.0	0.2
		No-Chlorine		0.0	0.0	3.3	0.7
		No-PAA		0.0	0.0	2.7	0.7
		No - Water	3	0.0	0.0	3.6	0.3
		No-Chlorine		0.8	0.6	3.3	0.7
		No-PAA		0.0	0.0	1.0	0.7
Day 3	PathO157	No - Water	6	2.2	1.5	0.0	0.0
		No-Chlorine		0.0	0.0	0.0	0.0
		No-PAA		2.2	0.2	0.0	0.0
		No - Water	3	1.1	0.8	0.0	0.0
		No-Chlorine		0.0	0.0	0.0	0.0
		No-PAA		1.9	1.3	0.0	0.0
	AttO157	No - Water	6	0.8	0.5	0.0	0.0
		No-Chlorine		0.0	0.0	0.0	0.0
		No-PAA		2.4	1.7	0.0	0.0
		No - Water	3	0.6	0.5	0.0	0.0
		No-Chlorine		0.8	0.6	0.0	0.0
		No-PAA		2.6	1.8	1.4	1.0
	E. coli	No - Water	6	2.0	1.4	0.0	0.0
		No-Chlorine		0.0	0.0	0.0	0.0
		No-PAA		0.0	0.0	0.0	0.0
		No - Water	3	1.9	1.3	0.0	0.0
		No-Chlorine		2.7	1.9	0.0	0.0
		No-PAA		3.8	1.2	0.0	0.0

Day 5	PathO157	Water	6	0.0	0.0	0.0	0.0
			3	2.0	1.4	0.0	0.0
		Chlorine	6	2.2	1.5	0.0	0.0
			3	0.0	0.0	0.0	0.0
		PAA	6	3.2	0.1	0.0	0.0
			3	2.4	1.7	0.0	0.0
	AttO157	Water	6	1.1	0.8	0.0	0.0
			3	1.2	0.9	0.0	0.0
		Chlorine	6	1.4	1.0	0.0	0.0
			3	2.4	1.7	0.0	0.0
		PAA	6	0.0	0.0	0.0	0.0
			3	0.9	0.6	0.0	0.0
	E. coli	Water	6	2.5	1.7	1.3	0.9
			3	0.0	0.0	0.0	0.0
		Chlorine	6	2.4	1.7	0.0	0.0
			3	1.1	0.8	0.0	0.0
		PAA	6	2.2	1.6	0.0	0.0
			3	2.0	1.4	0.0	0.0
Day 7	PathO157	Water	6	2.5	1.8	0.0	0.0
			3	3.0	0.9	0.0	0.0
		Chlorine	6	0.8	0.6	0.0	0.0
			3	3.0	1.3	0.0	0.0
		PAA	6	1.0	0.7	0.0	0.0
			3	3.6	0.8	0.0	0.0
	AttO157	Water	6	2.6	1.8	0.8	0.6
			3	1.6	1.1	0.0	0.0
		Chlorine	6	2.9	0.1	0.0	0.0
			3	4.3	0.2	0.0	0.0
		PAA	6	2.7	1.9	0.0	0.0
			3	0.8	0.6	0.7	0.5
	E. coli	Water	6	4.0	0.5	1.2	0.9
			3	2.6	1.8	0.0	0.0
		Chlorine	6	0.9	0.6	0.0	0.0
			3	2.7	1.9	0.9	0.6
		PAA	6	1.3	0.9	1.2	0.8
			3	1.9	1.4	1.2	0.8

Data represents averages of 4 replicates per bacteria-treatment-inoculum combination recovered over a period of 7 days. No *Enterococci* were recovered from any samples. STE=standard error of the means, Chlorine= chlorine at 100 ppm, PAA=Peroxyacetic acid at 40 ppm. PathO157= pathogenic *E. coli* O157:H7 and AttO157=attenuated *E. coli* O157:H7.

Table 12. Background population of naturally occurring coliforms and generic *E. coli* present on cilantro inoculated with pathogenic *E. coli* O45 and *E. coli* O111 and generic *E. coli* at log 3 and 6 CFU/ml, treated without and with chlorine and PAA at 4 days after inoculation (experiment 2).

Day	Strain	Treatment	Inoculum dose Log CFU/ml	Coliforms Log CFU/g	STE	*E. coli* Log CFU/g	STE
Day 1	*E. coli* O45	No-Water	6	3.51	0.78	0.00	0.00
		No-Chlorine		2.98	0.74	1.53	0.08
		No-PAA		3.76	0.18	0.00	0.00
		No-Water	3	4.40	0.16	1.54	0.09
		No-Chlorine		0.00	0.00	0.00	0.00
		No-PAA		0.00	0.00	0.00	0.00
	E. coli O111	No-Water	6	1.28	0.91	0.00	0.00
		No-Chlorine		3.99	0.49	0.00	0.00
		No-PAA		3.77	0.24	0.00	0.00
		No-Water	3	4.68	0.13	0.00	0.00
		No-Chlorine		4.79	0.23	0.00	0.00
		No-PAA		2.80	0.71	0.00	0.00
	E. coli	No-Water	6	3.25	1.06	4.00	0.05
		No-Chlorine		1.85	1.31	2.20	1.56
		No-PAA		3.70	0.08	4.50	0.03
		No-Water	3	1.20	0.85	0.00	0.00
		No-Chlorine		3.40	0.89	3.13	0.08
		No-PAA		1.06	0.75	1.71	1.21
Day 2	*E. coli* O45	No-Water	6	2.52	1.78	0.00	0.00
		No-Chlorine		4.19	0.03	0.00	0.00
		No-PAA		3.92	0.42	0.00	0.00
		No-Water	3	1.67	1.18	0.00	0.00
		No-Chlorine		0.90	0.64	0.00	0.00
		No-PAA		0.00	0.00	0.00	0.00
	E.coli O111	No-Water	6	2.11	1.49	0.00	0.00
		No-Chlorine		1.71	1.21	0.00	0.00
		No-PAA		4.43	0.09	0.00	0.00
		No-Water	3	4.81	0.02	0.00	0.00
		No-Chlorine		4.23	0.10	0.00	0.00
		No-PAA		4.85	0.17	0.00	0.00
	E. coli	No-Water	6	0.00	0.00	0.00	0.00
		No-Chlorine		0.00	0.00	0.00	0.00
		No-PAA		0.00	0.00	0.00	0.00
		No-Water	3	0.00	0.00	0.00	0.00
		No-Chlorine		0.00	0.00	0.00	0.00
		No-PAA		0.00	0.00	0.00	0.00

Day 4	*E. coli* O45	Water	6	4.63	0.09	0.00	0.00
			6	3.50	0.09	0.00	0.00
		Chlorine	6	4.78	0.06	0.00	0.00
			3	2.53	0.09	0.00	0.00
		PAA	3	4.12	0.24	0.00	0.00
			3	2.42	0.26	0.00	0.00
	E.coli O111	Water	6	2.38	0.43	0.00	0.00
			6	4.56	0.14	0.56	0.40
		Chlorine	6	4.50	0.01	0.00	0.00
			3	4.49	0.08	0.00	0.00
		PAA	3	3.80	0.84	0.00	0.00
			3	2.54	0.17	0.00	0.00
	E. coli	Water	6	1.92	0.08	0.00	0.00
			6	3.50	0.23	1.58	1.12
		Chlorine	6	4.08	0.46	2.56	0.15
			3	2.53	0.51	0.00	0.00
		PAA	3	4.46	0.46	0.65	0.46
			3	3.18	0.98	0.68	0.48
Day 8	*E. coli* O45	Water	6	5.0	0.0	0.00	0.00
			3	3.5	1.3	0.00	0.00
		Chlorine	6	4.7	0.2	0.00	0.00
			3	3.8	0.7	0.00	0.00
		PAA	6	4.3	0.2	0.00	0.00
			3	2.6	0.1	0.00	0.00
	E. coli O111	Water	6	2.1	0.6	0.00	0.00
			3	4.3	0.8	0.00	0.00
		Chlorine	6	3.5	0.2	0.00	0.00
			3	4.6	0.4	0.00	0.00
		PAA	6	3.5	0.3	0.00	0.00
			3	2.4	1.1	0.00	0.00
	E. coli	Water	6	4.3	0.1	0.00	0.00
			3	4.4	0.0	0.00	0.00
		Chlorine	6	4.4	0.0	0.00	0.00
			3	4.6	0.1	0.00	0.00
		PAA	6	3.6	0.1	0.00	0.00
			3	3.10	1.20	0.00	0.00

Data represents averages of 4 replicates per bacteria-treatment-inoculum combination recovered over a period of 7 days. No *Enterococci* were recovered from any samples. STE=standard error of the means, Chlorine= chlorine at 100 ppm, PAA=Peroxyacetic acid at 40 ppm.

Figure 3. Greenhouse layout configuration (A), spot inoculation of new strawberry plant (B), and inoculated plants with generic *E. coli* (C), and pathogenic *E. coli* O157:H7 (D).

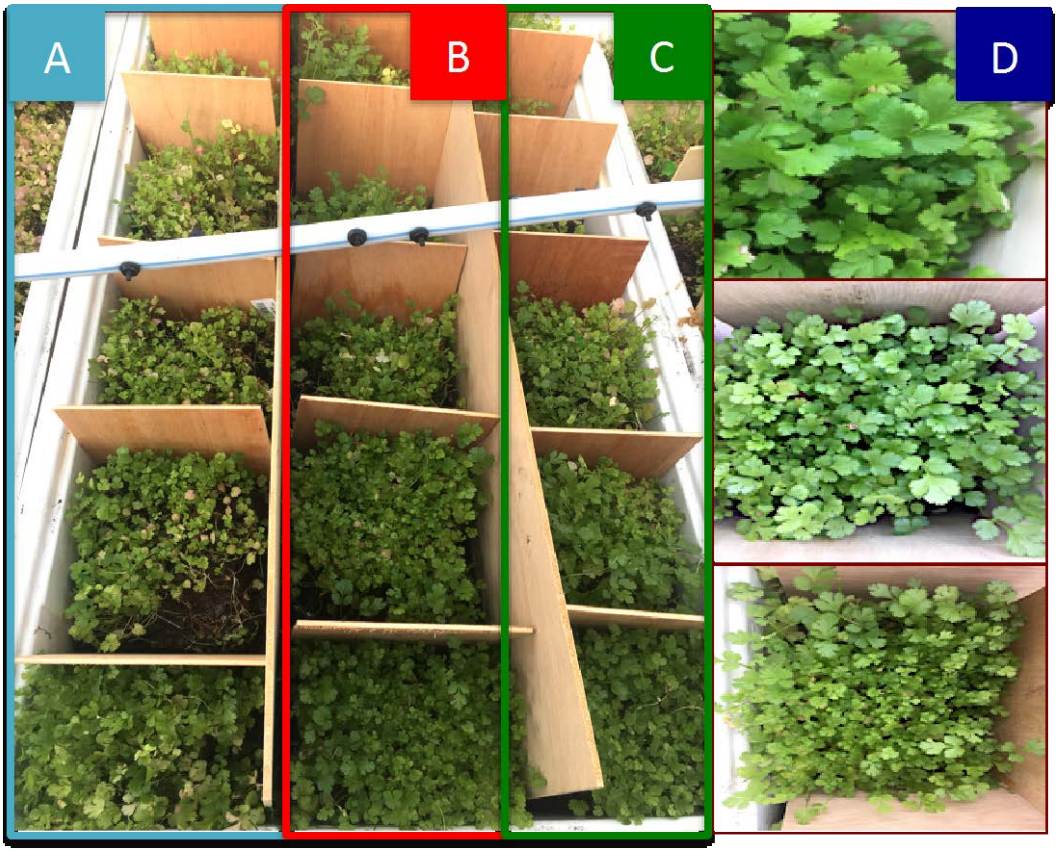

Figure 4. Greenhouse layout configuration of cilantro plantings: (A) control plants, (B) PAA-treated plants, and (C) chlorine-treated plants, each spray inoculated with generic *E. coli*, pathogenic and attenuated *E. coli* O157:H7, and *E. coli* O145, O111. D represents the general condition of cilantro plants within each box. This same layout was followed for all cilantro inoculation events.