Research Abstract for the California Leafy Greens Research Board April 2008 to March 2009

Project Title: Survival and biology of *E. coli* under field production environments

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Summary

Outbreaks of foodborne pathogens on leafy vegetables have highlighted the need for practical information on the dynamics of such organisms in the field. Applied field-oriented research is needed so that industry and regulators can make informed decisions on growing practices and food safety policies. This project is part of a continuing effort to develop information on the survival and ecology of *E. coli* strains under Salinas Valley farming conditions.

Our objectives were the following: (1) Establish and confirm the relationship between soil moisture and *E. coli* survival in a contaminated water context; (2) Examine generic *E. coli* survival in a Salinas Valley field managed under different irrigation systems; (3) Compare field survival between generic and attenuated *E. coli* strains; (4) Assemble an investigative local rapid response team to collect samples and analyze on-farm sites where possible contamination and high risk situations occur. Experiments used either generic *E. coli* strains that were selected for spontaneous, natural resistance to the antibiotic rifampicin (*E. coli*^{rif}) or a naturally selected attenuated O157:H7 *E. coli* strain (attenuated strains are *E. coli* O157:H7 bacteria that lack the genes that code for toxin production and are therefore of greatly reduced risk to humans). We used rifampicin-amended medium to selectively recover and obtain presumptive identification of the applied bacteria.

In a small plot field experiment, generic *E. coli* strains were sprayed onto beds, irrigated with overhead sprinklers, and the soil periodically tested to examine persistence of the applied strains. With catch-buckets, the irrigation rates in the plots were recorded. All of our *E. coli*^{rif} strains, inoculated to soil at high rates (10^6 to 10^8 CFU/ml), were recoverable from soil for only a short period. By 14 days after inoculation, recovery was at or near the practical detection limit for most plots. The soil *E. coli* strain, originally isolated from soil under romaine lettuce, and the cocktail of strains were both detected for up to 23 days post inoculation. Beds closer to the sprinkler line received greater amounts of irrigation water and were associated with greater *E. coli* survival. We did not detect *E. coli* in uninoculated plots, indicating that inter-plot contamination did not occur at a detectable level.

For the large plot field experiment, fertilizer (grower standard and supplemented rates) and romaine seed were first placed into the replicated plots. Generic or attenuated *E. coli*^{rif} was then sprayed onto beds at a high rate (10^8 CFU/ml). Half of the plots were irrigated with overhead sprinklers and half with surface drip tape. Lettuce was germinated and grown to harvestable size. Soil, irrigation runoff (sprinkler plots only), and romaine plants were periodically tested for the bacterium. *E. coli*^{rif} applied to soil declined rapidly for the first 14 days after inoculation. Both generic and attenuated strains were not detected after 30 days post inoculation. Irrigation method (sprinkler vs. drip) or fertilizer rates (standard vs. supplemental) did not appear to influence bacterial survival.
We did not recover *E. coli*^{rif} from lettuce plants, growing in inoculated beds, at any stage of growth. An enrichment detection method used on lettuce seedlings also did not recover applied strains. For runoff water from the sprinkler plots, we recovered *E. coli*^{rif} at low levels for up to 30 days post inoculation. By 40 days post inoculation all bacteria were at or below the practical detection level.

We had several opportunities to evaluate presumptive risk factors in relation to grower/handler identified fields. On-site reviews by team members were followed, in some cases, by sample collection and processing for indicator bacteria and pathogens. Examples of risk-based analysis included animal intrusion (5 locations) adjacent land use concerns (1 location) and presumptive positives in internal testing (2 locations).

Successful recovery of pathogen indicators was observed in only one field associated with adjacent land use activities. Evidence for gradients of indicator *E. coli* on lettuce around a presumptive risk-factor (i.e. animal scat in furrow or on beds) was not observed within multiple 5 foot radial samplings around flagged focal points.

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Introduction

Recent outbreaks of foodborne pathogens on leafy vegetables have highlighted the need for practical information on the dynamics of such organisms in the field. For example, studies are lacking that directly answer how *E. coli* O157:H7 enters agricultural systems, how it spreads in fields, its ability to survive and persist under field conditions, and other related aspects. Applied field-oriented research is needed so that industry and regulators can make informed decisions on growing practices and food safety policies. This project has initiated efforts to develop information on how nonpathogenic (generic) *E. coli* persists under Salinas Valley farming conditions. Environmental data developed with generic *E. coli* can be the basis for gaining grower, handler, agency, and public acceptance of future controlled field trials with reduced-risk strains of *E. coli* O157:H7. Reported here are results of the second year of our field oriented studies that are conducted in Salinas Valley locations.

Objectives:

1. Establish and confirm the relationship between soil moisture and *E. coli* survival in a contaminated water context.

2. Examine generic *E. coli* survival in a Salinas Valley field managed under different irrigation systems.

3. Compare field survival between generic and attenuated *E. coli* strains.

4. Assemble an investigative local rapid response team to collect samples and analyze on-farm sites where positive pathogen-screening tests or relevant events (flooding, animal intrusion, adjacent land activities) indicate possible contamination situations.

Procedures:

1. Relationship between soil moisture and *E. coli* survival in a contaminated water context.

A replicated, small plot (plot dimensions: one 40-inch bed x 20 ft) field trial was established in the Salinas Valley. To simulate a one-time contaminated irrigation water event, three different strains of generic (nonpathogenic) *E. coli* were applied on April 29, 2008 in different concentrations and combinations after plot beds were formed. Control beds received no bacteria. There were four replications. Inoculum was applied with a CO_2 powered, handheld, backpack sprayer, two nozzle spray boom, and Teejet 8005 spray tips (selected to reduce spray drift); the sprayer was set at approx. 25-27 psi. Directed spray applications were made so that only the bedtops (approximately a 22 inch band) received the inoculum. All strains were selected from central coast sources (irrigation water, lettuce, and soil from a lettuce planting) for spontaneous, natural resistance to the antibiotic rifampicin (*E. coli*^{rif}) (Table 1). Because our strains are resistant to this antibiotic, for selective detection and enumeration of the strains we used media amended with rifampicin to facilitate recovery and identification of our strains. Each bacterial strain was shown to lack key genetic determinates of virulence associated with *E. coli* O157:H7. Each strain also has a distinct DNA-fingerprint, facilitating subspecies differentiation following recovery from field trial samples.

Tryptic soy agar (TSA) amended with 80 µg/liter rifampicin and 70 µg/liter MUG (a substrate reasonably diagnostic for *E. coli* due to production of a specific substrate degrading enzyme that results in fluorescence of the broth or agar medium) was used for bacteria recovery and identification. TSA was amended with 1.0% pentachloronitrobenzene (PCNB) fungicide to inhibit fungal growth that would interfere with counting bacterial colonies.

Table 1. Treatments for the small plot field experiment

Treatment	Source of strain	Concentrations*
E. coli^{rif} W353	water	10^6 and 10^8 CFU/ml
E. coli^{rif} P354	lettuce	10^6 and 10^8 CFU/ml
E. coli^{rif} S355	soil	10^6 and 10^8 CFU/ml
W353/P354/S355	"cocktail" mixture	$10^{7.5}$ CFU/ml
non-inoculated control	---	---

* CFU/ml = colony forming units per milliliter

After inoculation, plots were watered with overhead sprinklers to simulate the germination irrigations for direct seeded lettuce. Irrigations were subsequently applied every 2 to 3 days post-planting until 3 weeks after inoculation (4/29, 5/1, 5/5, 5/6, 5/8, 5/12, 5/20). Irrigations were made with a single sprinkler line located at the center of the trial so that less water was applied to plots located further from the water source. With catch-buckets, the water volumes were recorded throughout the plots for each irrigation. Soil was collected from each plot immediately after inoculation and at 1, 3, 6, 8, 14, and 23 days post-inoculation. Soil was collected using sanitized collection scoops; 8 soil subsamples were taken from the top 0.5 to 1.0 inch of

undisturbed bedtops, bulked into sterile bags, stored on ice, and taken to the lab for analysis. For each sample, a 100 gram subsample was tested by adding 200 ml 0.01 M sodium phosphate buffer + 0.05% Tween 20, agitating the mixture vigorously for 1 minute, and allowing the mixture to settle for 1 hour at room temperature. 25 ml of the supernatant were removed and placed in sterile Falcon tubes (50 ml). This liquid was vortexed for 30 seconds, then a 200 µl aliquot was plated and spread onto each of two TSA + rif + MUG + PCNB plates. Plates were incubated at room temperature for 2 hours then placed in an incubator for 48 hours at 42 °C. After 48 hours, the number of colonies was determined for all plates.

The effects of time, initial concentration of inoculum, and amount of irrigation water on survival rates of *E. coli* in the soil were evaluated using a step-wise multivariate linear regression procedure in SAS (statistical analysis software) for each inoculum source (plant, water, soil, combined sources).

2. Generic *E. coli* survival in a Salinas Valley field managed under different irrigation systems.
3. Field survival between generic and attenuated *E. coli* strains.

A replicated (3 blocks), large plot, open field trial was established in the Salinas Valley and used a randomized complete block design. Irrigation treatments (sprinkler or drip) were set up for the main plots of nine beds (40-inch) wide x 145 feet long, and fertilizer treatments (grower standard or supplemental nitrogen and phosphorus) were incorporated prior to bed shaping into sub-plots of three beds (40-inch) wide x 145 feet long. Beds were seeded with romaine lettuce followed by inoculation on July 22, 2008 with either a three strain combination (water, plant, and soil) of generic *E. coli*^{rif} or a naturally selected attenuated O157:H7 *E. coli* strain (attenuated strains are *E. coli* O157:H7 bacteria that lack the genes that code for toxin production and are therefore of greatly reduced risk to humans) (Table 2). All bacteria were applied at a high rate (10^8 CFU/ml). Control plots received no bacterial inoculum. This start date was selected so that the romaine would reach harvestable stage by mid-September. The strains were applied using the same equipment and methods as used for the small plot study. Directed spray applications were made so that only the bedtops (approximately a 22 inch band) received the inoculum.

Table 2. Treatments for the large plot field experiment

Inoculation	Irrigation method	Fertilizer level*
generic *E. coli*^{rif}	sprinkler	standard rate
generic *E. coli*^{rif}	drip	standard rate
generic *E. coli*^{rif}	sprinkler	supplemented rate
generic *E. coli*^{rif}	drip	supplemented rate
attenuated *E. coli*^{rif}	sprinkler	standard rate
attenuated *E. coli*^{rif}	drip	standard rate
non-inoculated	sprinkler	standard rate
non-inoculated	drip	standard rate
non-inoculated	sprinkler	supplemented rate
non-inoculated	drip	supplemented rate

* Standard fertilizer rate = 300 lb per acre of 7-7-7; supplemented fertilizer rate = 300 lb per acre of 7-7-7 plus 350 lb N and 250 lb P per acre.

After inoculation, all plots were watered once with overhead sprinklers to initiate the germination of the lettuce. Subsequently, plots were irrigated with either surface drip or overhead sprinklers. To germinate and establish the lettuce, irrigations were applied 2, 4, 7, 11, and 14 days after the first watering. Soil moisture tension was monitored in the irrigation plots at 8-inch and 18-inch depths using watermark blocks from the 3rd irrigation until harvest except for a 7-day period when the crop was thinned and cultivated. In one of the sprinkler irrigated plots, relative humidity and air temperature were monitored hourly at a height of 8 inches above the bed top using an HMP35C relative humidity/air temperature sensor and a Campbell Scientific CR1000 datalogger. The lettuce was irrigated, grown, thinned, and managed according to standard commercial practices. The experiment was terminated when the lettuce reached harvest size and a final plant sample taken at that time.

All soil and plant samples were collected from undisturbed areas on the center bed of the three-bed plots. Soil was collected from each plot immediately after inoculation and at 1, 3, 8, 15, 22, and 30 days post-inoculation. Soil was collected, stored, and tested for *E. coli*^{rif} according to the same procedures as described for the small plot study. However, to increase the chances of recovering *E. coli* late in the experiment, the soil samples collected on days 15, 22, and 30 were processed in 100 ml of the sodium buffer and not 200 ml (as used with day 1, 3, and 8 samples).

At 22 and 30 days post-planting, a bulked romaine seedling sample was collected from each plot and tested for *E. coli*^{rif}. For each plot, 50 random plants were unearthed and the roots and adhering soil placed in one collection bag. The foliage (crowns and leaves) was placed in a second bag. Roots and rhizosphere soil were tested by adding 100 ml 0.01 M sodium phosphate buffer + 0.05% Tween 20, macerating the plant material to expose vascular tissue (xylem), shaking and agitating the mixture vigorously for 1 minute, allowing the mixture to settle for 10 minutes, then plating 250 µl aliquots onto each of 2 TSA + rif + MUG + PCNB plates. Plates were incubated and evaluated according to the same procedures as described for the small plot study. Lettuce foliage was tested in a similar manner except that 100 ml of buffered peptone water (Difco Laboratories) was added to this material. To further test lettuce seedlings for internalized *E. coli*^{rif}, an additional set of individual subsamples (5 plants per sample per plot) was collected and bagged in groups of five in separate bags. Each set was tested for *E. coli*^{rif} in a similar way.

As the romaine crop developed, later plant collections consisted only of the above ground portion of the lettuce. Ten plants per plot were cut at the soil level (leaving behind the oldest, lower 3 to 4 leaves), placed in one large plastic bag, stored on ice, and transported to the lab. Plants were analyzed by chopping all leaves and crowns into approximately 1 inch square pieces, mixing all chopped pieces in a tray, and removing 150 g of the material for analysis. 100 ml of buffered peptone water (BPW) was added to the 150 g and the mixture was agitated vigorously for 1 minute. A 250 µl aliquot was removed and plated onto each of two TSA + rif + MUG + PCNB plates (hence 2 replicates per sample). Plates were incubated and evaluated as previously

described. The lettuce tissue + BPW mixture was incubated further for another 24 hours at 37 C; additional 250 µl aliquots were removed and plated onto TSA + rif + MUG + PCNB plates.

These procedures were followed for all subsequent lettuce tissue samples with the exception of increasing the amount of BPW liquid used to process the 150 g lettuce samples. The last two lettuce tissue samples were also processed with an enrichment incubation step (24 hours at 37 C).

For sprinkler plots only, irrigation runoff water was collected and tested for *E. coli*^{rif}. Samples were taken 13, 27, 38, and 49 days after planting. Using the QuantiTray 2000 Colilert system (Idexx Corporation), 100 ml of collected water were mixed with Colilert reagents, dispensed into the assay trays, sealed, and incubated at 37 °C for 48 hours according to manufacturer recommendations. A set of 10^{-1} dilutions was also prepared and tested with the Colilert system.

Bacterial count data determined from soil, plant, and run-off samples were subjected to general linear means procedures using SAS to determine if irrigation, nutrient, or *E. coli* source treatments were statistically different.

4. Local rapid response team to collect samples and analyze on-farm sites.

Our team worked with local growers and shippers to mobilize a rapid sampling response for fields having potential food safety issues. Four fields were investigated during the summer of 2008 (Table 3). Candidate fields were selected if product testing resulted in a presumptive positive for microbial contamination, or if the field had identified risk factors that could indicate microbial contamination.

Table 3. Rapid response sites in 2008

Date sampled	Location	Potential risk factor
May 21	Gonzales	animal intrusion
June 11	Chualar	undetermined
October 8	south Salinas	aerosols
October 10	south Salinas	animal fecal material

Soil and crop tissue samples were collected in a systematic grid pattern. Each site was also examined and evaluated for evidence of wildlife habitat and activity.

Results:

1. Relationship between soil moisture and *E. coli* survival in a contaminated water context.

The water, plant, and soil strains of generic *E. coli*^{rif}, inoculated to soil at two high rates (10^6 or 10^8 CFU/ml), were recoverable from soil for relatively short periods of time. One day after inoculation, all inoculated soils yielded bacterial counts that were significantly lower than the original inoculum concentrations (Fig. 1). Bacterial counts for all inoculated treatments

continued to decline during the first week, but at a rate slower than initially. By 14 days after inoculation, recovery was at or below the detection limit for the plant and water *E. coli* strains sprayed at 10^6 CFU/ml. *E. coli* bacteria were recovered from all other inoculated plots by day 14 (plant and water strains at 10^8 CFU/ml, soil strain at 10^6 and 10^8 CFU/ml, and cocktail combination of all strains at $10^{7.5}$ CFU/ml). By day 14, the highest levels of *E. coli* bacteria were recovered from plots sprayed with the highest concentrations of inocula (10^8 CFU/ml).

The soil strain and cocktail combination strains were detected in soil for up to 23 days after inoculation (Fig. 2). Soils samples as deep as 10 cm (4 inches) had detectable levels at 23 days after spraying. We did not detect *E. coli*^{rif} in uninoculated plots, indicating that inter-plot contamination did not occur at a detectable level.

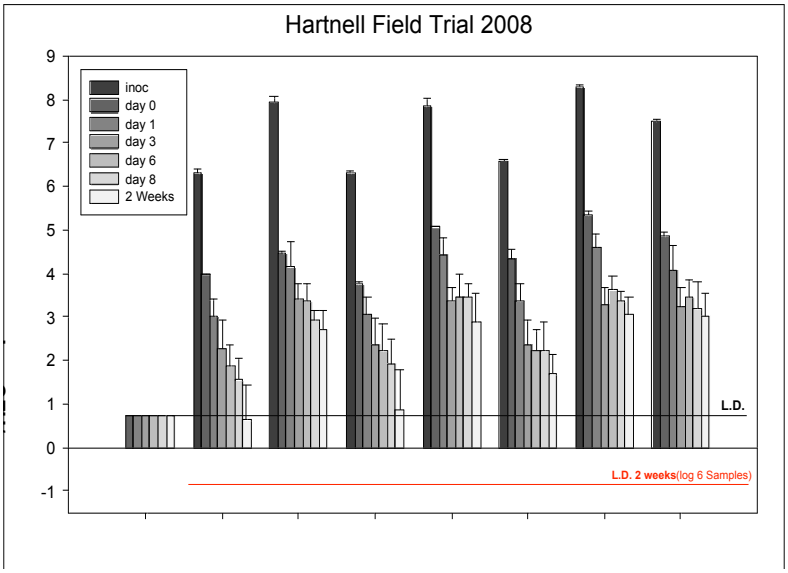

Figure 1. Recovery, up to 14 days post inoculation, of generic *E. coli*^{rif} strains that were inoculated to soil in a small plot field study. Inocula were sprayed in the following order: plant strain, soil strain, water strain. L. D. = limit of detection.

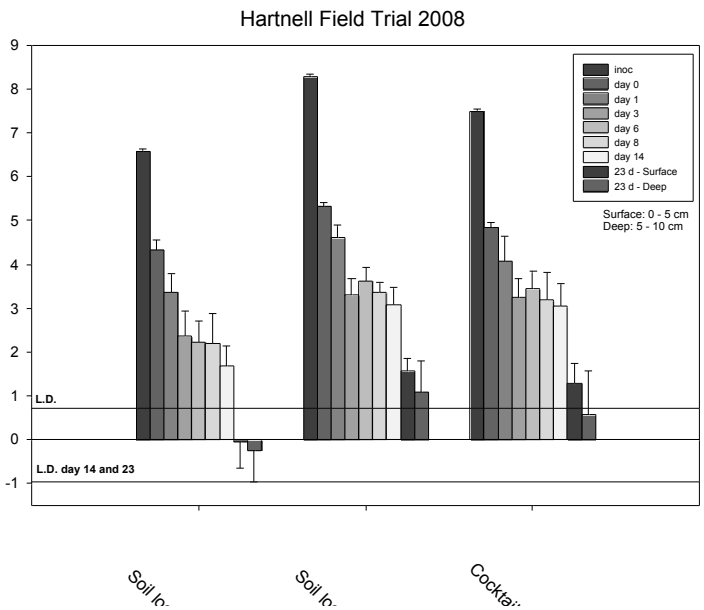

Figure 2. Recovery, up to 23 days post inoculation, of generic *E. coli*^{rif} soil and cocktail strains that were inoculated to soil in a small plot field study. L. D. = limit of detection.

The cumulative amount of water applied to the plots using the single sprinkler line varied between 2 inches and 3.6 inches during the first 2 weeks of the trial. Catch-bucket data demonstrated that the cumulative amount of water applied to the plots declined with distance from the sprinkler line (Fig. 3). The downwind, east side of the plots received significantly more water; the upwind, west plots had less applied water (Fig. 3). Multivariate regression analysis of the data demonstrated that the amount of water applied by overhead sprinklers, the concentration of inoculum sprayed on the plots, and time were statistically significant predictors of concentration of E. coli bacteria recovered from surface soil samples (Table 4).

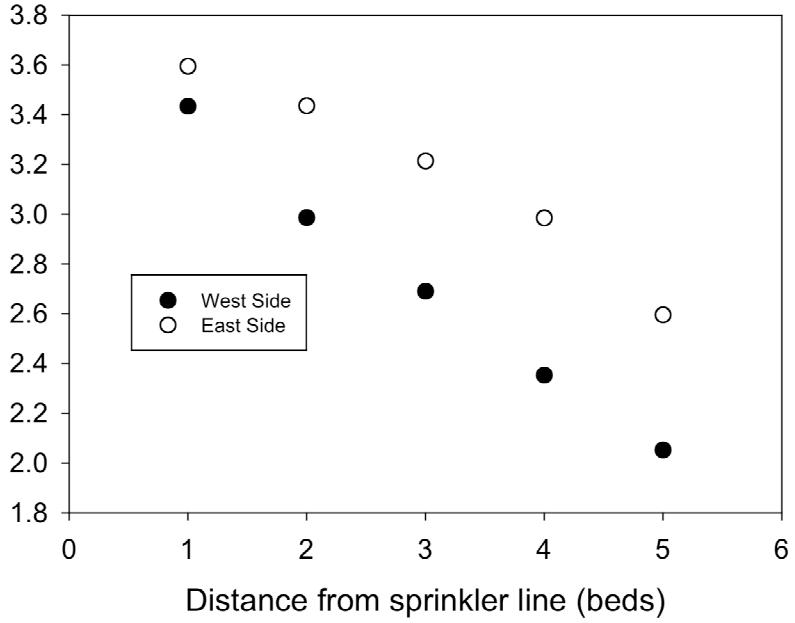

Figure 3. Applied water (inches) for west and east sides of the inoculated small plot experiment.

Table 4. Estimated coefficient values for the multivariate regression models fit to *E. coli* survival data from the small plot study.

	Estimated values of statistically signficant regression coefficie[1]			
				Combined sources
Independent variable	Soil E. Coli	Water E. coli	Plant E. coli	E.coli[2]
Intercept	-0.061	-1.299	-1.451	4.603
Log(day)	-2.379	-1.974	-1.913	-1.004
Inoculum conc. (log(E. coli cfu/ml)	0.615	0.712	0.718	--
Applied water (inches)	-1.426	--	--	-1.495
Cumulative applied water (inches)	0.693	0.344	0.217	--
R^2 of regression model	0.78	0.71	0.77	0.31
p-value of F-test	0.0001	0.0001	0.0001	0.001

[1.] model variables were determined statistically signficant at p <0.15 using step-wise multivariate regression a data collected from 0 to 14 days after inoculation. Dependent regression variable = log(E.coli cfu/ g of soil)

[2.] regression analysis was performed on data collected from 0 to 8 days after inoculation

For all isolates (soil, plant, water, cocktail) bacterial recovery and survival were greater on the wetter east side of the sprinkler line; the dryer west side had lower numbers of recovered bacteria (Table 5). Bacterial numbers were generally higher on beds closer to the central sprinkler line;

moving away from the sprinkler line, the bacterial numbers generally decreased as soil moisture levels decreased. This recovery pattern was similar for all inoculum sources (Table 5).

Table 5. Recovered E. coli (log CFU/gm soil) from inoculated beds in the small plot experiment.

	Beds east of the sprinkler pipe					pipe		Beds west of the sprinkler pipe				
	5	4	3	2	1	0	1	2	3	4	5	
Isolate												
plant log 6	1.07	0.98	1.14	1.37	1.85		0.86	-0.35	-0.35	0.13	-0.35	
plant log 8	2.74	2.71	2.61	2.56	3.28		3.5	2.76	2.8	2.12	2.19	
water log 6	1.25	1.8	1.91	1.49	1.88		0.22	-0.82	0.08	0.72	-0.12	
water log 8	3.4	3.37	3.05	3.64	3.26		2.68	3.2	2.75	1.95	1.48	
soil log 6	1.52	2.02	1.56	1.7	2.67		1.26	1.95	1.08	1.82	1.26	
soil log 8	2.46	3.19	2.74	3	2.57		3.55	3.56	3.09	3.42	3.11	
cocktail log 7.5	3.23	2.85	2.71	4.25	3.01		3.07	2.3	2.76	3.24	2.97	
control	0.73	0.73	0.73	0.73	0.73		0.73	0.73	0.73	0.73	0.73	
	log CFU/gm soil											

2. Generic *E. coli* survival in a Salinas Valley field managed under different irrigation systems.
3. Field survival between generic and attenuated *E. coli* strains.

E. coli^{rif} was recovered from soil for up to 30 days post inoculation (Fig. 4). Both strains (generic and attenuated O157:H7) declined rapidly in the soil at similar rates during the first 14 days after inoculation. After 14 days, bacterial counts continued to decline, and by 30 days after inoculation, bacterial counts were close to the detection limit used in the trial (Fig. 4).

No significant differences in *E. coli* populations were detected in the different fertilizer (standard vs. supplemental) treatments (Fig. 4). In addition, similar bacterial counts were recovered in drip and sprinkler irrigated plots (Fig. 5).

For the first sampling, *E. coli*^{rif} was recovered at low levels from soil in uninoculated control beds, indicating some drift occurred during the inoculation of the plots; however, this recovery level was significantly lower than treated beds, and bacterial levels were below the detection level by 3 days after inoculation (Fig. 4).

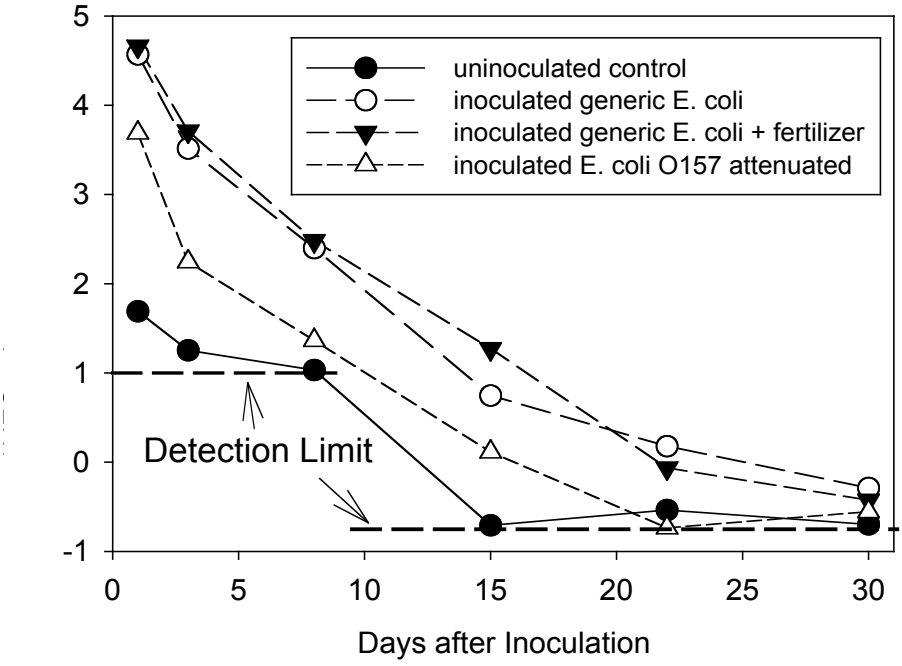

Figure 4. Recovery of generic and attenuated O157:H7 *E. coli*^{rif} from soil samples collected at the large plot trial.

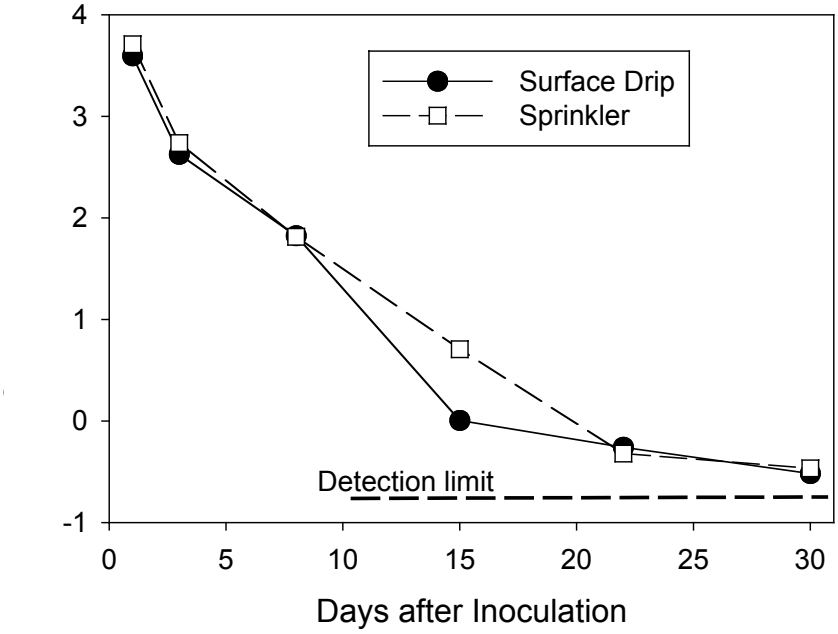

Figure 5. Recovery of generic *E. coli*^{rif} from soil samples collected from plots irrigated with either sprinklers of surface drip.

We recovered *E. coli*^{rif} strains in runoff water from the sprinkler plots for a 30 day period after spraying inoculum on the soil. At 14 days post-inoculation, *E. coli*^{rif} was recovered from sprinkler runoff from generic/standard fertilizer, generic/high nutrient, and attenuated plots (Fig. 6). Low populations were detected in run-off from uninoculated control plots. Highest populations were measured in the generic *E. coli* receiving the high fertilizer treatment. By 30 days post-inoculation, all plots tested positive at low levels. After 40 days, all bacterial populations were at or below the detection level. Total water applied to the entire crop was 18 inches for the sprinkler areas and 11 inches for the drip areas (Fig 7).

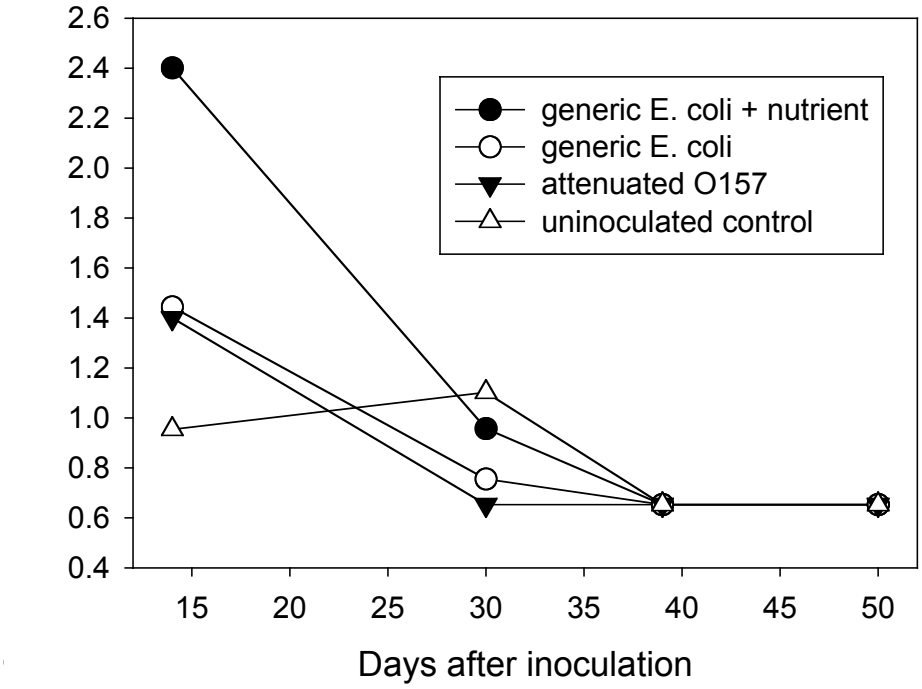

Figure 6. *E. coli* concentrations recovered from sprinkler runoff at large plot trial. Detection limit = $10^{0.7}$ CFU/100 ml.

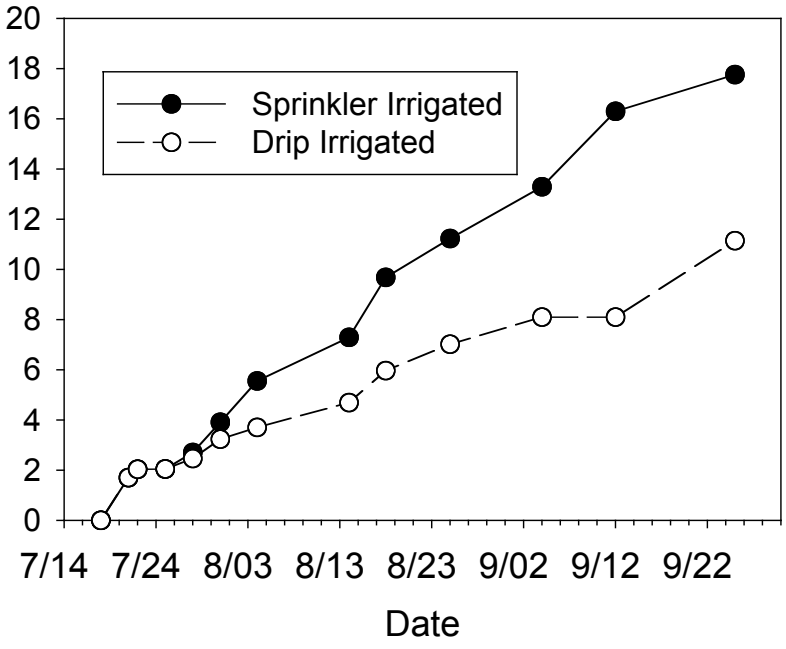

Figure 7. Cumulative water applied by sprinkler and drip irrigated plots.

We did not recover *E. coli*^{rif} from lettuce plants, growing in inoculated beds, at any stage of growth. Bulked and 5-plant lettuce seedling samples failed to test positive for the applied isolates and therefore gave no evidence for epiphytic populations or internalization of *E. coli*^{rif} (detection limit = 0.26 log CFU/root or stem). An enrichment detection method used on lettuce seedlings also resulted in no recovery (detection limit = -0.70 log CFU/stem). Plants sampled at rosette stage and just prior to harvest were also negative for the inoculation isolates. *E. coli*^{rif} was not recovered from romaine in uninoculated control beds.

Soil moisture tension ranged between 0 and 10 kPa at the 8 and 18 inch depths during the first 4 weeks of the crop (Fig. 8), indicating that soil moisture was near field capacity up to the date that *E. coli*^{rif} was no longer detected in the soil. Although more water was applied to the crop by sprinklers than by drip, no difference in soil moisture was measured between the sprinkler and drip irrigated plots during the 30 day period after inoculation. Soil moisture tensions increased at the 8 inch depth after 30 days (Fig. 8), indicating that the soil was drying as the water use of the lettuce crop increased.

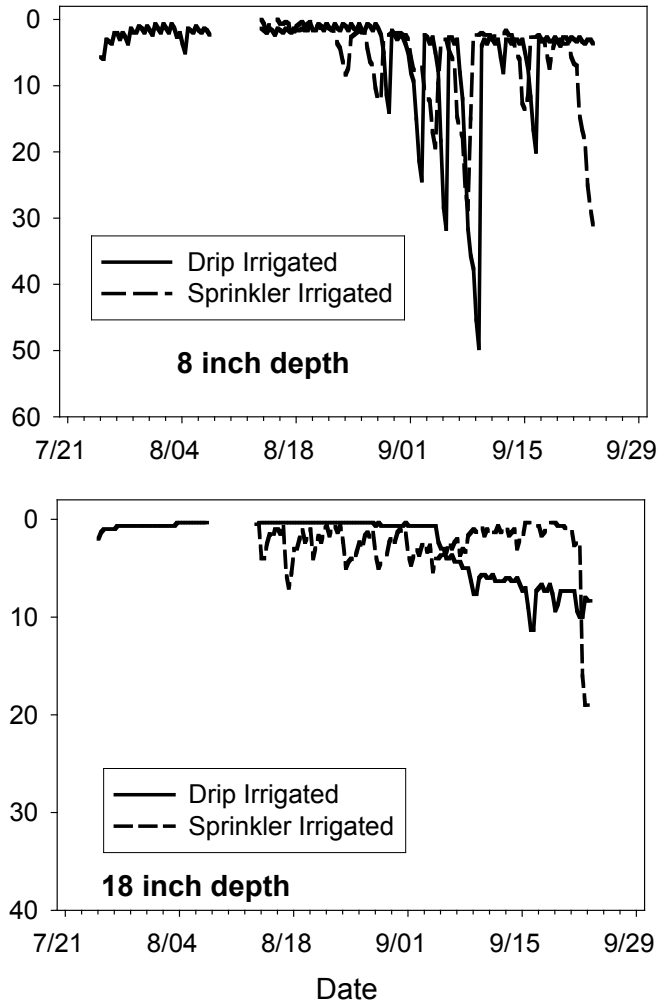

Figure 8. Soil moisture tension data from Spence field trial. Data missing from sprinkler plots are between 7/24/080 and 8/13/08.

During the first 3 days after inoculation, relative humidity of the air one-foot above the beds averaged approximately 80% and ranged between 40% and 50% during the day (Fig 9). Air temperature generally ranged between 20 and 25 °C during the day (Fig. 10).

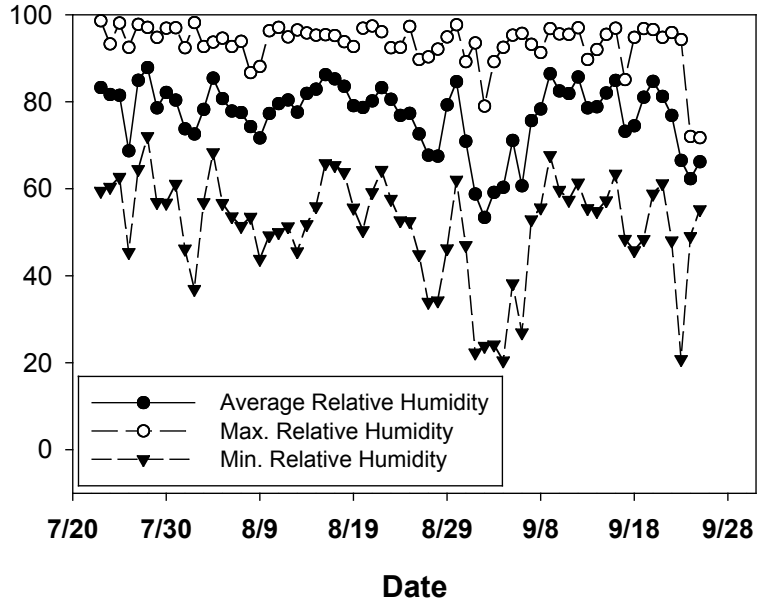

Figure 9. Relative humidity at the large plot trial site 2008.

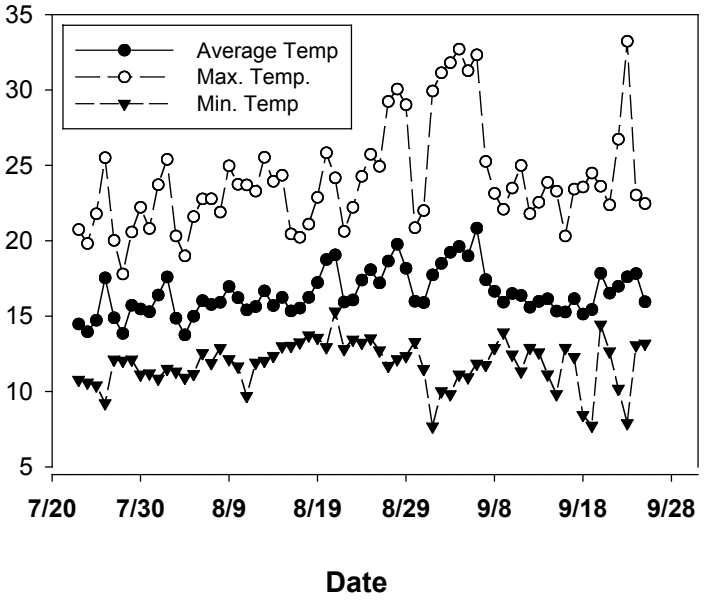

Figure 10. Air temperature at the large plot trial site 2008.

Discussion

These 2008 experiments simulated a one-time, high level contamination event of irrigation water containing various *E. coli* strains. In general, both generic *E. coli* (strains from various sources) and a non-pathogenic attenuated O157:H7 *E. coli* strain survived in soil for relatively short periods (ranging from 14 to 23 days post inoculation). *E. coli* also persisted for a relatively short period (up to 30 days) in surface water runoff from sprinkler irrigation plots. Production factors such as irrigation method (overhead sprinklers vs. surface drip) and fertilizer rates (standard vs. supplemented) did not appear to result in differing levels of E. coli survival. The romaine crop grown in the experimental plots did not test positive for *E. coli*^{rif} for the duration of the experiment. The duration of survival of applied *E. coli* strains in 2008 was longer than the survival period for applied *E. coli* in our 2007 experiments. This slightly prolonged survival could be attributed to the increased pre-application soil moisture present in the 2008 field plots; wetter bed tops in these plots likely assisted in survival of the applied inoculum. Also, climatic conditions were cooler for 2008, which again likely helped the *E. coli* to survive for a longer period of time. The small plot field study demonstrated an association between wetter soils and increased irrigation water with *E. coli* survival in the field.

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