Transfer of *Escherichia coli* O157:H7 from Simulated Wildlife Scat onto Romaine Lettuce during Foliar Irrigation

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ABSTRACT

A field trial in Salinas Valley, California, was conducted during July 2011 to quantify the microbial load that transfers from wildlife feces onto nearby lettuce during foliar irrigation. Romaine lettuce was grown using standard commercial practices and irrigated using an impact sprinkler design. Five grams of rabbit feces was spiked with 1.29×10^8 CFU of *Escherichia coli* O157:H7 and placed -3, -2, and -1 days and immediately before a 2-h irrigation event. Immediately after irrigation, 168 heads of lettuce ranging from ca. 23 to 69 cm (from 9 to 27 in.) from the fecal deposits were collected, and the concentration of *E. coli* O157:H7 was determined. Thirty-eight percent of the collected lettuce heads had detectable *E. coli* O157:H7, ranging from 1 MPN to 2.30×10^5 MPN per head and a mean concentration of 7.37×10^3 MPN per head. Based on this weighted arithmetic mean concentration of 7.37×10^3 MPN of bacteria per positive head, only 0.00573% of the original 5 g of scat with its mean load of 1.29×10^8 CFU was transferred to the positive heads of lettuce. Bacterial contamination was limited to the outer leaves of lettuce. In addition, factors associated with the transfer of *E. coli* O157:H7 from scat to lettuce were distance between the scat and lettuce, age of scat before irrigation, and mean distance between scat and the irrigation sprinkler heads. This study quantified the transfer coefficient between scat and adjacent heads of lettuce as a function of irrigation. The data can be used to populate a quantitative produce risk assessment model for *E. coli* O157:H7 in romaine lettuce to inform risk management and food safety policies.

Consumption of produce contaminated with bacterial, parasitic, or viral pathogens remains a major cause of foodborne illness in the United States *(23),* and both industry and regulatory efforts are underway to help mitigate these food safety risks *(5, 26).* For many outbreaks associated with preharvest contamination of produce, the originating source(s) of the foodborne pathogen is unclear, in part due to the wide range of vertebrate hosts for enteric pathogens and the challenges of conducting traceback investigations. For example, although a definitive vertebrate source was not identified for the 2006 *Escherichia coli* O157:H7 outbreak associated with spinach, both wild and domestic animals tested positive for the outbreak strain *(4, 16).*

Domestic animals, wildlife, and humans have all been identified as possible microbial sources of produce-associated foodborne outbreaks during the past decade. Speculation regarding the mechanism of microbial contamination of preharvest produce includes improperly composted soil amendments that contain animal manure, deficient personal hygiene practices during harvest, and contaminated irrigation water *(2, 7, 8, 10, 12, 14, 15, 21).* Wildlife may play a significant role in microbial contamination of preharvest produce when in-field defecation occurs from mammalian or avian wildlife species *(1, 11, 16).* Potential contamination of produce is magnified when wildlife congregate in larger numbers, such as flocks of birds foraging along rows of produce or colonies of rodents establishing burrows in adjacent hedgerows or within the field of produce *(1).* Several mechanisms can facilitate microbial transfer from wildlife feces to produce, e.g., defecation directly onto produce during perching or foraging behavior from avian sources, or dispersal from soil splash subsequent to foliar irrigation when scat is deposited in furrows *(19, 21).* Formally characterizing the mechanisms via which wildlife contaminate produce will help inform the metrics of good agricultural practices, such as how wide of a diameter surrounding scat should be discarded before harvest of leafy greens *(5).*

To date, few studies have quantified the microbial load that transfers from soil or animal feces onto produce during a preharvest irrigation event *(19, 21).* The focus of this study was to determine the proportion of an *E. coli* O157:H7 load that will transfer from wildlife feces to adjacent heads of romaine lettuce due to standard foliar irrigation practices and how such factors as distance between scat and lettuce

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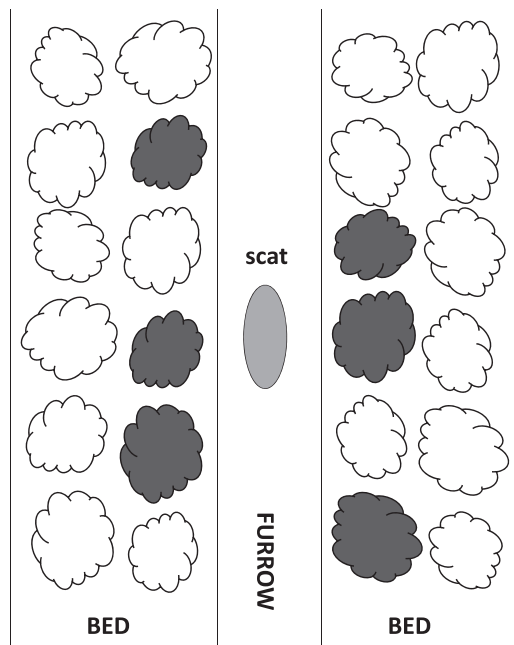
FIGURE 1. *Field design: relationship between feces (rabbit scat) in furrows and a cluster of six heads of romaine lettuce. Only the shaded heads were sampled.*

and age of scat before irrigation influence the magnitude of this transfer coefficient. Air sampling, irrigation water sampling, and in-field rodent testing were also conducted to measure possible environmental movement of *E. coli* O157:H7 that would confound our data.

The data from this trial can help refine the metrics of good agricultural practices and provide information to validate and parameterize food safety risk models, including, for example, the Produce Risk Assessment Model, developed by the Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, in collaboration with RTI International *(18)*.

MATERIALS AND METHODS

Field site and lettuce plots. The field trial was conducted at a field plot located several miles south of Salinas, CA, at the Agricultural Research Service, U.S. Department of Agriculture. Consistent with commercial farming practices for Monterey County, California, romaine lettuce (*Lactuca sativa* L. var. longifolia) cv. Green Towers seeds were planted and maintained beginning 23 May 2011. The field plot consisted of 12 beds 56 to 61 cm (22 to 24 in.) wide separated by furrows 40 to 46 cm (16 to 18 in.) wide; beds were 102 cm (40 in.) apart from midpoint to midpoint. Within each bed, two rows of seeds were planted in parallel rows 30.5 cm (12 in.) apart. Lettuce plants were thinned at 4 weeks, and the plot was weeded by hand as needed. Overhead impact sprinklers using 46-cm (18-in.) risers and Nelson rotary nozzles were spaced around the field in a pattern (9.12 by 9.12 m [30 by 30 ft]). The field was irrigated every 5 to 7 days as needed. Irrigation ceased during the 4-day field inoculation period. Irrigation commenced at 8:40 a.m. on 14 July 2011 and continued for ~2.5 h, with an estimated 1.25 to 3.85 mm of cumulative water applied to the plot.

Clusters of lettuce were randomly selected within the plot of 20 beds. A cluster was made up of six heads of lettuce, with three

heads in parallel on each side of the furrow (Fig. 1). Each cluster varied in distance and degree (360°) from magnetic north to the stationary sprinkler system within the field plot. The heads of romaine within the clusters also varied in relation to each other, due to planting, thinning, and natural plant death. The distance (centimeters) and degree from each plant to the four closest sprinklers were recorded. Each head of lettuce within a cluster was marked by placing a labeled polyvinyl chloride flag behind the head and was labeled with lettuce identification and date of fecal spiking (−3, −2, −1, and 0 days; described in ''Rabbit scat and inoculation with *E. coli* O157:H7'').

Bacterial strain. We used a rifampin-resistant (100 µg/ml) *E. coli* O157:H7 strain ATCC 700728 that lacked stx_1 and stx_2 genes *(20)*. All bacterial cultures were grown daily as needed in tryptic soy broth (TSB; Difco, BD, San Jose, CA) supplemented with 50 µg/ml rifampin (+R; Gold Biotechnology, St. Louis, MO) and incubated at 37°C for 6 h under orbital rotation (100 rpm). Cell counts were first estimated using a regression equation that extrapolated bacterial concentration from the optical density at 600 nm, and then counts were confirmed by serial dilution in phosphate-buffered saline (PBS; Sigma-Aldrich, St. Louis, MO) and spread plated onto tryptic soy agar (TSA; Difco, BD) supplemented with +R (TSA+R) and incubated at 37°C for 18 to 24 h.

Rabbit scat and inoculation with *E. coli* O157:H7. Laboratory rabbit (*Oryctolagus cuniculus*; New Zealand White) feces were used to simulate Lagomorpha and Rodentia wildlife feces. Spiked fecal scat deposits were constructed by stomaching 50 g of fresh rabbit feces with a 50-ml suspension of *E. coli* O157:H7 in PBS for 5 min at 230 rpm at University of California at Davis. After addition of the *E. coli* O157:H7 suspension, the rabbit feces had a consistency of firm paste. The concentration of the stock suspension of *E. coli* O157:H7 was first estimated using an optical density–based growth curve as described in ''Bacterial strain,'' with a target concentration of ~1 × 10^8 CFU of *E. coli* O157:H7 in a 5-g aliquot of fecal–*E. coli* O157:H7 matrix. The spiked fecal samples were wrapped in sterile plastic and stored under refrigeration overnight until transported the next morning on ice to the field site. The 5-g scat samples were placed on the soil surface of the furrow in the middle of the lettuce cluster at either −3, −2, or −1 days, or immediately before the morning foliar irrigation event (day 0). Before irrigation, the distance and degree from magnetic north of the scat to each head of lettuce within the cluster and to the four closest sprinklers were recorded.

Immediately after placing the scat samples in the field, replicate stock scat samples (feces and *E. coli* O157:H7) were serial diluted and spread plated to determine the concentration of *E. coli* O157:H7 at the time of spike. All field serial dilutions and spread plates were completed inside a van at the field site. Three 1-g aliquots of stock scat were serially diluted in PBS and then spread plated in triplicate using three dilutions. These dilutions were plated on TSA+R and incubated 18 to 24 h at 37°C. On average, the 5-g scat deposits contained 1.29 × 10^8 CFU at the time of placement in the field. This high concentration of *E. coli* O157:H7 represents a worst-case scenario of bacterial shedding levels among wildlife, which we are assuming did not influence the conclusions of this study.

Sampling lettuce. All prelabeled heads of lettuce within each cluster were harvested immediately after foliar irrigation, transported to the laboratory under refrigeration, and processed that afternoon and evening for bacterial enumeration (within 12 h of

being harvested). Two harvesting teams with two individuals per team collected 192 samples; 144 samples were intact heads of lettuce. An additional 24 heads of lettuce were collected by first removing the inner one-third (heart) section and then removing the outer two-thirds section separately to allow us to determine where the microbial contamination was located (inner or outer leaves). Each team consisted of a bagger and a harvester. The harvester collected the lettuce by putting on a new pair of gloves, decontaminating the lettuce knife with 70% ethanol, cutting through the base of the lettuce head, and placing the head into a sterile prelabeled Bitran collection bag (24 by 24 in. [ca. 61 by 61 cm]; Fisher Scientific, Pittsburgh, PA). The bagger also changed gloves between each head and was responsible for opening and sealing each sample bag to ensure sterility. The 24 collected heads were split into inner and outer sections according to the aforementioned protocol, with the following modifications: the harvester first removed the inner 30% of the head by severing the inner stock and placing this section in the sample bag as described above, followed by disinfecting the knife, changing of gloves, and then removing the remaining lower section of the head as before. Sample bags were placed in a container with ice until laboratory processing occurred. Ten negative control samples were collected after irrigation from a plot adjacent to the experimental plot.

Enumeration of *E. coli* O157:H7 on lettuce: high concentration assay. Samples were immediately processed upon arrival in the laboratory at University of California at Davis within a 12-h hold time from collection to completion of the assay. Processed samples were stored at 4°C until results were final. Five hundred milliliters of PBS was added directly into each collection bag containing the head of lettuce. The lettuce heads were then washed by hand for 1 min by vigorous shaking and massaging, being careful not to shred or tear the leaves. The concentration of *E. coli* O157:H7 in the washate was estimated by a most-probable-number (MPN) procedure: 1 ml of washate was transferred into the first two wells of a 12-well reservoir plate (VWR International, Radnor, PA) containing 9 ml of TSB+R. The broth suspensions were homogenized via pipette; replicate dilutions were serially diluted by transferring 100 µl of each dilution into 9.9 ml of TSB+R (100-fold dilution) to a final dilution of 10^{-11}. This multiple-tube design (2 by 6) allowed us to enumerate a wide range of potential concentrations of *E. coli* O157:H7 per head of lettuce. Each reservoir plate was incubated for 24 h at 37°C with orbital rotation at 50 rpm. After incubation, 3 µl from each well was streaked onto ChromAgar-O157 medium (DRG International, Inc., Springfield, NJ) supplemented with +R (O157+R) and incubated at 37°C for 18 to 24 h. Magenta-colored colonies indicate the presumptive presence of *E. coli* O157:H7. Results were recorded and calculated using MPN Calculator Build 23, created by Mike Curiale (available at: http://i2workout.com/mcuriale/mpn/index. html). This assay was designed to enumerate elutable concentrations of *E. coli* O157:H7 of ≥340 MPN per head of lettuce.

Enumeration of *E. coli* O157:H7 on lettuce: low concentration assay. Approximately 50% of lettuce heads that tested negative using the high concentration assay were retested using a low concentration assay with a much higher sensitivity. Fifty micrograms per milliliter of rifampin was aseptically added directly into the remaining 498 ml of washate along with 15 g of sterile TSB powder. The lettuce head was washed again by vigorously shaking until the TSB powder was fully dissolved. The MPN procedure was used to estimate the concentration of bacteria: washate was divided into triplicate tubes with each set containing 100-, 10-, 1-, and 0.1-ml aliquots followed by overnight incubation

at 37°C with shaking at 50 rpm. Detection of positive tubes was determined as described in ''Enumeration of *E. coli* O157:H7 on lettuce: high concentration assay.'' This assay was designed to enumerate elutable concentrations of *E. coli* O157:H7 ≥2 MPN per head of lettuce. Heads that were still negative using the low concentration assay were enriched in 500 ml of TSB+R at 37°C and 50 rpm for 24 h; the enrichment was plated on O157+R and incubated for 24 h at 37°C. Results were recorded and calculated using MPN Calculator Build 23.

Rabbit scat samples postirrigation. After irrigation and harvest, the fecal samples were collected from each cluster. One-tablespoon (15-ml) Sterileware sampling spoons (Bel-Art, Pequannock, NJ) were used to scoop the scat and an ~1.27-cm (0.5-in.) depth of soil directly beneath the scat. The scat–soil matrix was placed into a prelabeled 510-g (18-oz) Whirl-Pak bag (Nasco, Salida, CA) and processed by adding 90 ml of TSB+R. The samples were hand massaged for 1 min, and the concentration of *E. coli* O157:H7 (MPN per gram wet weight of scat and soil) was determined by the MPN procedure as described in ''Enumeration of *E. coli* O157:H7 on lettuce: high concentration assay.''

Rodent trapping and sample processing. Four Sherman rodent traps (model XLK; H.B. Sherman Traps, Tallahassee, FL) were set out daily until harvest in the four quadrants of the experimental field. Institutional Animal Care and Use Committee protocol 15546 was followed when collecting rodent samples. In brief, each trap contained cotton balls for warmth in the event of captured rodents. Some rodents can enter the trap without releasing the trap door; therefore, all traps were inspected carefully for the presence of fecal matter. The contents of the traps were collected daily at dusk and aseptically placed in a Whirl-Pak bag labeled with the trap identification number. If a rodent was captured, then fresh feces was collected using a rectal swab, along with any voided fecal samples and the cotton ball, and placed in transport media. All trapped rodents were tagged and released. Cages were reused if there were no sign or indication of visitation. Contaminated cages were sprayed with Lysol, washed, and autoclaved before further use. Rodent fecal samples were processed the same day that a rodent was trapped. One hundred milliliters of TSB+R was added to the Whirl-Pak bag containing all cotton ball nests, rectal swabs, and any collected rodent feces. Samples were incubated for 18 to 24 h at 37°C shaking at 50 rpm; the suspension was then streaked for isolation onto O157+R and incubated for 18 to 24 h at 37°C. All magenta-colored colonies were confirmed by PCR, and its pulsed-field gel electrophoresis (PFGE) pattern was compared against the attenuated *E. coli* O157:H7 ATCC 700728 strain used for this project.

Air sampling. Air samples were taken to determine whether the rifampin-resistant attenuated *E. coli* O157:H7 strain was being aerosolized, dislocated by wind, or both. Each day, one air sample was collected upwind at the northern edge of the field plot, while downwind samples were taken at the southern edge of the field plot (air samplers were >3 m from nearest lettuce cluster). Using an Air Sampler (EMD Millipore, Gibbstown, NJ), 500 liters of ambient air was sampled directly onto an O157+R plate. Seventy percent ethanol was used to clean the air sampler between each sample. The O157+R plates were incubated at 37°C for 18 to 24 h and checked for the presence of *E. coli* O157:H7 as described in ''Rodent trapping and sample processing."

Irrigation water sampling. Twenty liters of irrigation water was collected from a low-pressure sample port located adjacent to

field plot and transported on ice to University of California at Davis. Water samples were concentrated to 500-ml samples (retentate) using ultrafiltration *(13)*. Sterile TSB powder +R was added directly into the retentate. Samples were then incubated for 24 h at 37°C and with rotation at 50 rpm. After enrichment, samples were cultured as described in ''Rodent trapping and sample processing.''

DNA confirmation of *E. coli* O157:H7. Due to use of a +R-resistant strain of *E. coli* O157:H7 combined with the use of selective agars, we elected to confirm 10% of presumptive-positive samples isolated from the lettuce samples using traditional PCR as described by Paton and Paton *(24)*. In brief, DNA was extracted using a simple boiling method; bacterial cells were incubated at 100°C for 20 min, followed by centrifugation for 10 min at 5,000 rpm (model 5417C, Eppendorf, Hauppauge, NY). Each PCR reaction contained 48 µl of master mixture and 2.0 µl of DNA. The master mixture was composed of $1 \times$ buffer, 0.4 µM forward and reverse primers, 200 µM deoxynucleoside triphosphates, 1.5 mM MgCl$_2$, and 1.25 U of *Taq,* and the remaining volume was adjusted with DNase-free water to a final volume of 48 µl per reaction. The PCR assays were performed using a thermocycler (Eppendorf) with an initial denaturation at 95°C for 1 min, followed with 30 cycles of 94°C for 15 s, 55°C for 15 s, and 72°C for 1 min, and with a final extension at 72°C for 1 min. PCR products were visualized on 2% agarose gel stained with GelRed (Biotium, Hayward, CA), and the 259-bp amplicon was measured with Low Mass Ladder (Invitrogen, Carlsbad, CA).

PFGE. The Centers for Disease Control and Prevention's PulseNet standard procedure *(25)* was used for PFGE. The PFGE patterns from all PCR-confirmed isolates were compared against the PFGE pattern from the positive control strain *E. coli* O157:H7 ATCC 700728. The gel was stained with ethidium bromide and visualized using Gel/Chemi Doc (Bio-Rad, Hercules, CA). The genetic similarity (100%) between the sample isolates and positive control was determined using GelCompar II software (Applied Maths, Austin, TX).

Statistical analyses. Both a McNemar test and Fisher exact test were used to determine whether the presence of *E. coli* O157:H7 was significantly higher for the outer leaves section compared with the inner heart section of the heads of lettuce. In addition, a negative binomial regression (Stata 9; StataCorp LP, College Station, TX) was used to test the association between the various environmental factors and the concentration of *E. coli* O157:H7 per head of lettuce, with the 95% confidence interval and *P* values adjusted for potential correlated bacterial data within each cluster of six adjacent heads of lettuce. Environmental factors that were evaluated for their association with *E. coli* O157:H7 per head of lettuce included ambient air temperature (daily maximum and minimum, 24-h mean), 24-h mean humidity, maximal wind speed, distance between feces and lettuce head, age of feces before irrigation, mean distance between feces and the four sprinklers impacting the feces, and aspect of lettuce relative to furrow and wind direction (lettuce down- or upwind of fecal sample, which equates to lettuce on north side of bed versus south side of bed).

RESULTS AND DISCUSSION

After 2.5 h of foliar irrigation, an estimated 38% of the heads of romaine lettuce within 70 cm of the fecal deposit had detectable *E. coli* O157:H7. The concentration of *E. coli* O157:H7 per positive head ranged from 1.30

MPN to 2.30×10^5 MPN of *E. coli* O157:H7, with a weighted arithmetic mean of 7.37×10^3 MPN of *E. coli* O157:H7 per positive head of lettuce. This large range of bacteria levels is, in part, the consequence of using high concentrations of bacteria in the scat deposit to simulate a worst-case scenario. This estimate of 38% positive heads was generated by a pair of assays used in serial (high then low concentration enumeration assays) to generate an overall estimate of the positive prevalence of lettuce contamination. Specifically, using the high concentration assay, which was capable of detecting concentrations ≥340 MPN of *E. coli* O157:H7 per head of lettuce, 9 (5.4%) of 168 romaine heads tested positive for *E. coli* O157:H7, with concentrations ranging from 340 MPN to 2.30×10^5 MPN of *E. coli* O157:H7 per head and an arithmetic mean of 5.18×10^4 MPN of *E. coli* O157:H7 per positive head of lettuce. Retesting 81 presumptive-negative heads using the low concentration assay (capable of detecting ≥2 MPN per head), 28 (34.6%) of 81 of these lettuce heads tested positive, with concentrations ranging from 1.30 to 385 MPN of *E. coli* O157:H7 per head and an arithmetic mean of 87.97 MPN of *E. coli* O157:H7 per head. This suggests that 34.6% of the 159 lettuce heads that tested negative with the initial high concentration assay were false negatives due to low concentrations of bacteria. Hence, in addition to the 9 positive heads originally detected, there were an estimated 55 additional positive heads ($0.346 \times 159 = 55$), leading to an overall positive prevalence of 38.1% (64 of 168). The ratio of the weighted arithmetic mean concentration of 7.37×10^3 MPN of *E. coli* O157:H7 per positive head after irrigation to the original spiked total load of 1.29×10^8 CFU of *E. coli* O157:H7 in the 5 g of feces equals 0.000057. This suggests that, on average, ~0.006% of the fecal load transferred to the positive heads of lettuce, keeping in mind that the initial load of 1.29×10^8 CFU of *E. coli* O157:H7 in fresh scat may have changed for the scat that was 1 to 3 days old and thereby influenced this calculation. Moreover, it is likely that an even greater amount of fecal material was transferred during the 2.5 h of irrigation, but a proportion was rinsed off before the end of irrigation. In total, four negative controls were collected, and all of them tested negative for *E. coli* O157:H7 using both assays. All water and air samples tested negative for *E. coli* O157:H7.

None of the inner sections (0 of 24) and only one of the outer sections (1 of 24) of lettuce tested positive for *E. coli* O157:H7 when tested using the high concentration assay. Following up with the more sensitive assay to retest 12 pairs of inner and outer sections of lettuce, none of the inner and only five of the outer sections tested positive for *E. coli* O157:H7. Using a McNemar test, which assumes lack of independence for each inner and outer pair, we detected a significant difference in the positive prevalence between inner and outer sections (one-sided exact *P* value = 0.03). Alternatively, using a Fisher exact test, which assumes independence between the inner and outer sections for each pair, we also detected a significant difference in the positive prevalence (one-sided exact *P* value = 0.02). Within the constraints of this study and the observed location of fecal splash, we speculate that removing the outer leaves of

TABLE 1. *Negative binomial regression models for the concentration of* E. coli *O157:H7 per head of romaine lettuce, based on data from the high concentration assay[a]*

Factor	Coefficient	95% CI[b]	P value[b]
		Model 1 (AIC[c] = 264)	
Intercept	119.1	84.8, 153.3	<0.001
Distance between feces and lettuce (cm)	−0.305	−0.38, −0.23	<0.001
Distance between sprinklers and feces (cm)[d]	−0.133	−0.17, −0.09	<0.001
Aspect of wind relative to bed			
Downwind[e]	0.0		
Upwind	−6.88	−9.46, −4.31	<0.001
		Model 2 (AIC = 262)	
Intercept	87.9	42.5, 133.3	<0.001
Age of feces before irrigation (h)	−0.134	−0.18, −0.08	<0.001
Distance between sprinklers and feces (cm)[d]	−0.100	−0.16, −0.04	0.001
Aspect of wind relative to bed			
Downwind[e]	0.0		
Upwind	−4.87	−8.45, −1.30	0.008

[a] The assay used for enumerating *E. coli* O157:H7 per head of lettuce was designed to estimate ≥340 MPN per head.

[b] The 95% confidence interval (CI) and *P* values adjusted for potential correlated bacterial data within each cluster of six adjacent heads of lettuce that surround a rabbit fecal deposit with an average bacterial load of 1.29×10^8 CFU of *E. coli* O157:H7.

[c] AIC, Akaike Information Criterion.

[d] Mean distance of the four sprinklers to the fecal deposit for each cluster of six heads of romaine.

[e] Referent category; lettuce head was downwind of the fecal deposit (lettuce located on north-facing, windward edge of bed).

romaine lettuce during harvest or packing substantially reduces the risk of bacterial contamination resulting from fecal and soil splash that can occur during foliar irrigation. In contrast, Oliveria et al. *(21, 22)* found that bacterial pathogens were located on both the inner and outer portions of lettuce heads as a result of irrigating with inoculated water. However, our experiment used irrigation water that contained no detectable levels of *E. coli* O157:H7 contamination.

Various environmental factors were associated with the transfer of *E. coli* O157:H7 from feces to lettuce, such as the distance between feces and the lettuce, age of feces before irrigation, and mean distance between feces and the four irrigation sprinklers that impacted the feces. Using data generated from the high concentration assay (9 of 168 positive heads), two different regression models generated similar Akaike Information Criterion values (Table 1). Both regression models included the mean distance between the four sprinklers and the fecal deposit in the furrow as factors in the transfer of *E. coli* O157:H7 to lettuce. The peak transfer of *E. coli* O157:H7 from feces to nearby heads of lettuce occurred at shorter mean sprinkler distances of 690 to 710 cm or ~7 m (Figs. 2 and 3). Given that the four impact sprinklers were set at the corners of a square with
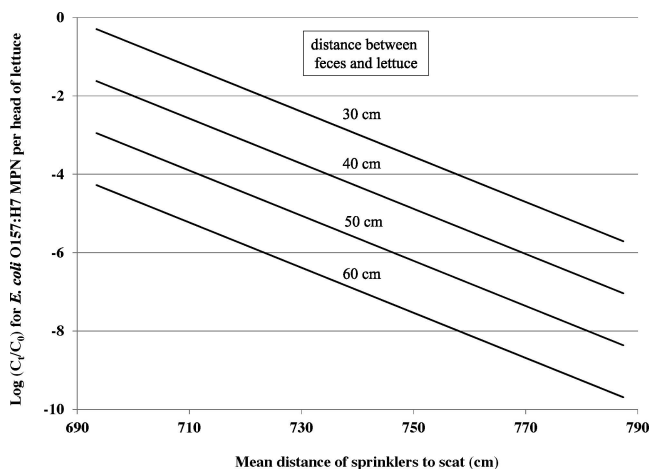

FIGURE 2. *Model 1: predicted concentration of* E. coli *O157:H7 per head of romaine lettuce on the windward (north-northwest facing) side of the bed as a function of distance to feces and sprinklers, based on data from the high concentration assay.*
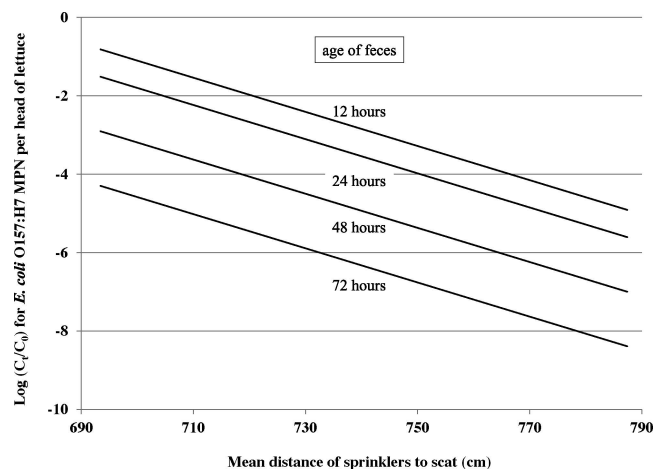

FIGURE 3. *Model 2: predicted concentration of* E. coli *O157:H7 per head of romaine lettuce on the windward (north-northwest facing) side of the bed as a function of age of feces and distance to sprinklers, based on data from the high concentration assay.*

9.12-m edges, the location that generates a mean 7.0-m distance from all four sprinklers is located about a meter inside the edges of the grid. For example, the middle of the square grid generates a mean distance of 6.44 m from all four sprinklers. In contrast, as the fecal location approaches any of the four sprinklers, the mean distance exceeds 7.6 m.

We speculate that several processes may be occurring that maximize the transfer of *E. coli* O157:H7 from the fecal load to the nearby heads of lettuce at these 6.9- to 7.1-m locations. First, we visually observed during the irrigation trial that the irrigation water with the largest droplet size and presumably highest kinetic energy impacted at this approximate location (several feet inside each plot), maximizing both the erosive forces of irrigation water on the fecal deposit and resulting fecal dispersal onto nearby heads of lettuce. Second, the impact sprinklers located along the periphery of the experimental plot had a road guard installed that redirected the stream of irrigation water back into the plot when the Nelson rotary head rotated to the outside of the plot. This resulted in a much higher rate of irrigation water impacting several feet in from the outer edge of the experimental plot, further enhancing fecal dispersal onto the lettuce. Monagahn and Hutchison *(19)* suggested that irrigation water could transfer pathogens from soil surface onto field crops and that sprinkler design could play an important role in reducing field contamination. Kincaid *(17)* demonstrated that impact energy associated with sprinkler heads is directly proportional to droplet size. Modifications to the operating pressure or nozzle type and diameter that lead to less erosive droplets might be a possible food safety intervention if this association is in fact causal.

The second variable that both regression models contained was whether the lettuce was downwind or upwind of the fecal deposit, such that lettuce heads located on the north-facing and windward edge of the bed (i.e., downwind of the fecal deposit) had on average 130- to 970-fold higher counts (i.e., 2 to 3 log increase) of *E. coli* O157:H7 compared with heads located upwind of the feces ($e^{4.87} = 130$; $e^{6.88} = 970$). This is a biologically meaningful difference in the bacterial load on heads of raw produce for virulent pathogens such as *E. coli* O157:H7, suggesting that irrigating lettuce before peak wind velocity, which can occur in this region during the afternoon, may function to reduce bacterial contamination and subsequent human exposure to contaminated produce.

Regarding regression model 1, distance between the lettuce and feces was negatively associated with *E. coli* O157:H7 per head, such that with each additional ca. 8 cm of distance there was an approximate log reduction in the concentration of *E. coli* O157:H7 per head ($e^{-0.776 \times 3} = 0.10$) (Fig. 2). With respect to regression model 2, there was negative association between the age of feces and *E. coli* O157:H7 per head of lettuce (Fig. 3). Specifically, for each additional 24 h between fecal deposition and irrigation there was a 0.04-fold reduction (~1.4 log reduction) of *E. coli* O157:H7 per head ($e^{-0.134 \times 24} = 0.04$).

Lastly, the remaining load of *E. coli* O157:H7 in the rabbit fecal deposits immediately after irrigation was highly
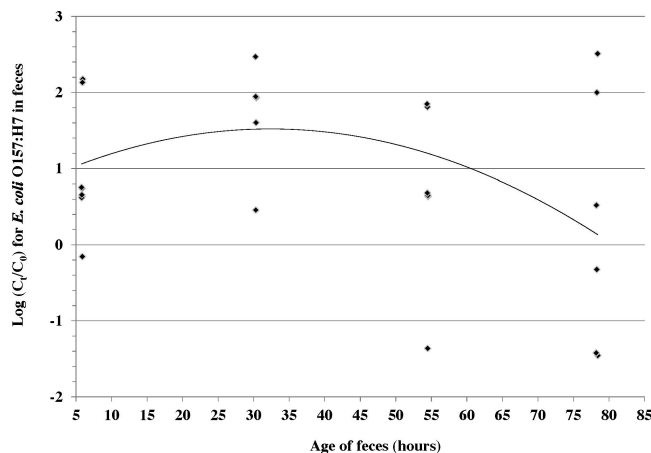

FIGURE 4. *Effect of age of feces on amount of* E. coli O157:H7 *per fecal deposit remaining after irrigation (*C_t*), relative to initial load (*$C_0 = 1.29 \times 10^8$ *CFU per fecal deposit).*

variable, ranging from -1.5 to $+3.6$ log shift in concentration (Fig. 4). Bacteria amplified in the majority of fecal deposits, whereas a minority exhibited levels below the initial inoculum of 1.29×10^8 CFU of *E. coli* O157:H7, possibly due to elution subsequent to foliar irrigation, furrow surface runoff, or environmental inactivation of the bacterial load. Given the high variability, it is not surprising that this factor, *r*, was not significantly associated ($P > 0.05$) with duration based on a $\log(C_t/C_0)$ transformation for bacterial counts (Fig. 4).

Environmental factors such as temperature, solar radiation, and wind were not significantly associated with bacterial loads on lettuce due to their uniformity during the short field trial. Measurements were recorded hourly by the California Irrigation Management Information System Salinas South Station no. 89 (available at: http://www.cimis.water.ca.gov/). Solar radiation peaked between 12 p.m. and 2 p.m. at ~900 W/m². Average wind speed was 4.67 m/s, ranging from 2.8 to 6.7 m/s. Wind direction during irrigation was typically 317.5° from magnetic north. Soil temperature averaged 21°C and ranged from 20.6 to 21.6°C. Average air temperature was 14.6°C and ranged from 11.4 to 18.8°C. Humidity ranged from 52 to 84%, with an average of 68.3%. Environmental factors effecting survival on the lettuce after splash-mediated transfer were not measured because all samples were harvested immediately after irrigation. In a similar field study, Barker-Reid et al. *(2)* suggested that persistence of *E. coli* O157:H7 on lettuce was influenced by environmental conditions. Alternatively, Bezanson et al. *(3),* using the same *E. coli* O157:H7 strain ATCC 700728 as in this study, found that environmental conditions had no significant effect on the survival of *E. coli* O157:H7 on lettuce or in soil. Further research should be done to evaluate how long *E. coli* O157:H7 can survive on lettuce after a splash-mediated event, taking into account environmental conditions such a solar radiation, humidity, and air temperature.

During the 9 days of rodent collections that flanked the irrigation experiment (5 days before and 4 days after irrigation), deer mice (*Peromyscus maniculatus*) were
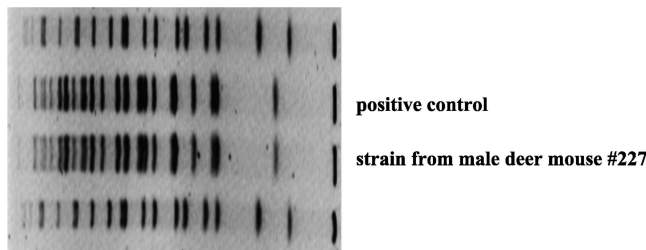
positive control

strain from male deer mouse #227

FIGURE 5. *Molecular confirmation of* E. coli *O157:H7 positive control strain ATCC 700728 that was isolated from a deer mouse trapped inside the experimental plot.*

trapped nine times, along with two cage visitations at night without tripping the snap door of the trap. Of the nine live trappings, three were recaptures of the same adult female mouse tag no. 226 and four were recaptures of the same adult male mouse (tag no. 277). All rodent samples were negative for *E. coli* O157:H7 ATCC 700728, except for the third capture of the adult male tag no. 277. This rodent was negative for the first two captures, positive on the third capture, and negative again on the fourth capture. Figure 5 shows identical PFGE patterns from the strain isolated from mouse tag no. 277 compared with the original strain used during the experiment. Given that our protocol combines both feces and cotton balls (used for bedding) for bacterial culture, we cannot distinguish whether the adult male shed the *E. coli* O157:H7 ATCC 700728 in his feces or had contaminated the cotton ball after contacting the inoculated rabbit feces.

The data generated by this study provide a critical component for produce risk assessment models because they explicitly quantify key contamination transfer parameters and influencing environmental factors during the production stage of romaine lettuce. Models intended to capture the relationships in environmental phenomena are populated with available data, and often extrapolation from laboratory studies to the field environment is required, which may oversimplify or not accurately capture the studied phenomena. The generation of data based on actual environmental events and field trials in the lettuce production environment is a valuable step toward understanding what actually occurs in the field under certain contamination scenarios and, when used for risk modelling, will provide an improved understanding of how to mitigate the risks involved.

This field trial demonstrated that *E. coli* O157:H7 is readily transferred from fecal deposits (scat) to heads of mature lettuce during foliar irrigation by using typical commercial farming practices for central coastal California. Although the mean transfer coefficient was only 0.00573% of the original bacterial load, bacterial concentrations ranged from 1 MPN to 2.30×10^5 MPN per head of lettuce, a biologically meaningful difference when compared against the infectious dose for humans *(6, 9).* The transfer of bacteria during foliar irrigation appeared limited to the outer leaves of the head that are typically discarded during harvesting or processing, thereby reducing the microbial load on the finished product. Heads of lettuce in proximity

to fresh fecal deposits, especially in the areas where the irrigation water impacts with higher kinetic energy, functioned to maximize the transfer of bacteria onto lettuce. The data generated from real-world field trials, such as this collaborative effort between the U.S. Food and Drug Administration and the Western Center for Food Safety, can be used in the development of effective, on-farm intervention strategies for produce.

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