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**Research Note**

# Inactivation of *Escherichia coli* O157:H7 on Romaine Lettuce When Inoculated in a Fecal Slurry Matrix

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## ABSTRACT

A field trial was conducted in July 2011 to quantify the inactivation rate of *Escherichia coli* O157:H7 when mixed with fecal slurry and applied to romaine lettuce leaves. Lettuce was grown under commercial conditions in Salinas Valley, CA. One-half milliliter of rabbit fecal slurry, containing $6.3 \times 10^7$ CFU of *E. coli* O157:H7, was inoculated onto the upper (adaxial) surface of a lower leaf on 240 heads of lettuce within 30 min after a 2.5-h irrigation event. Forty-eight romaine lettuce heads were collected per event at 2.5 h (day 0.1), 19.75 h (day 0.8), 43.25 h (day 1.8), 67.25 h (day 2.8), and 91.75 h (day 3.8) postinoculation and were analyzed for the concentration of *E. coli* O157:H7 ($C_t$). *E. coli* O157:H7 was detected on 100% of collected heads in concentrations ranging from 340 to $3.40 \times 10^{10}$ most probable number (MPN) per head. Enumeration data indicate substantial growth of *E. coli* O157:H7 postinoculation (2.5 h), leading to elevated concentrations, 1 to 3 log above the starting inoculum concentration ($C_o$). By the end of the 92-h trial, we observed a net 0.8-log mean reduction of *E. coli* O157:H7 compared with $C_o$; however, after accounting for the substantial bacterial growth, there was an overall 2.3-log reduction by the final sampling event (92 h). On the basis of two different regression models that used either the raw data for $C_t$ or log-transformed values of $C_t/C_o$ during the period 2.5 to 91.75 h postinoculation, there was an estimated 76 to 80% reduction per day in bacterial counts; however, more accurate predictions of MPN per head of lettuce were generated by using non–log-transformed values of $C_t$. This study provides insight into the survival of *E. coli* O157:H7 transferred via splash from a contaminated fecal source onto produce during irrigation. Moreover, these findings can help generate inactivation times following a potential contamination incident.

Key words: *Escherichia coli* O157:H7; Fecal contamination; Field trial; Produce; Slurry; Survival

Over the past several decades (1976 to 2009), consumption of raw or minimally processed vegetables in the United States has increased by 52.7% *(8)*. In parallel with this increase of raw vegetable consumption has been an increase in the occurrence of foodborne outbreaks associated with leafy green vegetables *(13),* including those caused by such pathogens as *Escherichia coli* O157:H7, *Salmonella* Enteritidis, and most recently non-O157 Shiga toxin–producing *E. coli (9, 13)*. Key factors influencing preharvest produce safety include the source(s) of microbial contamination (e.g., domestic or wild animals, soil amendments, and human handlers) and the mode of microbial transmission (e.g., foliar irrigation, source water contamination, and animal foraging habits) that link these pathogen sources to produce commodities *(15, 18, 26)*. A particular challenge for open agricultural systems is in-field defecation by wildlife, given the potential shedding of bacterial and protozoal pathogens in many species of wildlife common to U.S. agricultural regions *(5, 12, 17, 18, 20)*.

Animal fecal material containing *E. coli* O157:H7 deposited in a furrow can contaminate heads of lettuce on adjacent beds owing to fecal splash subsequent to foliar irrigation *(2, 23)*. To account for the potential contamination by feces, the California Leafy Green Marketing Agreement recommends not harvesting leafy green produce within a 5-ft (1.524-m) radius of identifiable scat owing to food safety concerns *(19)*. The proportion of fecal load transferred to nearby heads of lettuce is, in part, a function of factors, such as age of feces, location of fecal deposit within the irrigated quadrant, and distance between fecal load and heads of lettuce *(2)*. What is less clear is the fate and inactivation kinetics of *E. coli* O157:H7 once deposited onto the surface of lettuce and the likelihood of survival under typical growing conditions up to the day of harvest, processing, and final distribution *(1, 3, 4, 6, 16, 28)*. For example, *E. coli* O157:H7 was detected on lettuce irrigated with contaminated water, 22 and 77 days postinoculation by Bezanson et al. *(6)* and Islam et al. *(16),* respectively, with a 5-log difference in inactivation rates after 14 days postinoculation.

Simulating foliar irrigation with inoculated water has been studied in multiple growing regions throughout the world *(4, 6, 11, 16, 24)*. Despite variation in reported results, field-based studies have shown that *E. coli* O157:H7 is rapidly inactivated within the first 1 to 5 days postinoculation when applied to produce in an aqueous suspension *(10,*

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*16, 24, 27)*. For example, Moyne et al. *(24)* found that aqueous suspensions of *E. coli* O157:H7 applied to the upper surface of heads of romaine lettuce in the Salinas Valley, CA, resulted in rapid inactivation of the bacterial load, with up to a 3-log reduction within 2 h. We conducted the following study to determine the rate of inactivation for *E. coli* O157:H7 when applied as a simulated fecal splash onto the surface of romaine lettuce *(2)*. Data obtained from this inactivation study will (i) generate better insight into the survival behavior of *E. coli* O157:H7 within a fecal matrix and under typical environmental conditions and (ii) provide an estimate of bacterial inactivation (die-off) kinetics if a contamination incident has occurred in the preharvest environment.

## MATERIALS AND METHODS

**Field site and lettuce plot.** This field trial was conducted in tandem with another experiment, as described in Atwill et al. *(2)*, with field conditions relatively uniform, except for the commencement of each trial. Briefly, romaine lettuce (*Lactuca sativa* L. var. *longifolia*) was grown under commercial conditions in the Salinas Valley, CA; plant thinning, weed removal, and irrigation were performed as needed up until the field was inoculated. The lettuce plants were 60 days old from seed sowing in ground and were in the head formation stage. The field plot (18.3 m wide by 90.5 m long) consisted of beds (56 to 61 cm wide) separated by furrows (40 to 46 cm wide); each bed contained two parallel rows of lettuce, separated by 30.5 cm, at time of planting. A subplot of 20 adjacent beds within the experimental field plot were allocated for this survival study. Lettuce heads were randomly selected for inoculation and marked by placing a a color-coded stake flag, labeled with the lettuce head identification, directly behind the head. Prior to inoculation, the field was irrigated for 2.5 h beginning at 8:40 a.m. on 14 July 2011; field disturbances (e.g., irrigation, plant thinning, and weed removal) were halted for the remainder of the experiment.

**Weather data.** Hourly weather data were logged and collected through the California Irrigation Management Information System (httpp://www.cimis.water.ca.gov) whose weather station 89 (latitude 36°36′34.0″N, longitude 121°31′45.9″W) was chosen based on proximity, within 2.5 km, of the field site (latitude 36°37′41.8″N, longitude 121°32′26.4″W). No rain occurred during the trial.

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

**Fecal slurry and spiking with *E. coli* O157:H7.** Fresh feces were collected from laboratory rabbits (*Oryctolagus cuniculus*; New Zealand White), housed at the University of California, Davis, Teaching and Research Animal Care Services facilities. Feces used in the experiments were confirmed to be negative for *E. coli* O157:H7 and rifampin–resistant non-O157 *E. coli*. A rabbit fecal–PBS suspension was constructed by combining feces and PBS (gram:milliliter, 1:2.2) in a 55-oz Whirl-Pak filter bag (Nasco, Fort Atkinson, WI) and stomaching in a Seward Stomacher 80 Lab Blender (Seward Laboratory Systems Inc., Bohemia, NY) for 5 min at 230 rpm; the liquid portion was separated by hand squeezing, combined in a sterile flask, and vortexed for 1 min. The optical density–estimated *E. coli* O157:H7 suspension was added to the vortexed liquid (herein referred to as slurry) and its concentration determined through serial dilution followed by spread plating onto TSA+R (in triplicate). The slurry was stored for 12 h at 4°C and then transported on ice to the field site. Immediately prior to lettuce inoculations, the slurry was removed from the cooler, and the concentration of *E. coli* O157:H7 within the slurry was again determined through serial dilutions and spread plating, as stated previously. By using a calibrated Rainin Lite LTS Pipette L-1000 (Rainin Instrument, LLC, Oakland, CA), 0.5 mL of slurry containing approximately $6.3 \times 10^7$ CFU of *E. coli* O157:H7 ($C_o$), was spiked directly onto the adaxial (upper) surface of a lower outer leaf on 240 lettuce heads. At the commencement of collection, the slurry was placed in a cooler on ice for transport to the laboratory; this treatment allowed the slurry to experience the same ambient temperatures as the lettuce samples. Bacterial concentrations of the slurry were again determined, as described previously, 12 h postinoculation, while being held at 4°C.

**Collection of lettuce.** Five collection events occurred over approximately 92 h, at 2.5 h (day 0.1), 19.75 h (day 0.8), 43.25 h (day 1.8), 67.25 h (day 2.8), and 91.75 h (day 3.8) after initial bacterial inoculation. A total of 48 heads of lettuce were sampled per event by using methods described by Atwill et al. *(2)*. Two negative control samples were collected after each sampling event from a plot immediately adjacent to the experimental plot. Inoculated and negative control samples were placed on ice (about 4°C) and transported to the laboratory for analysis.

**Bacterial enumeration and confirmation of *E. coli* O157:H7.** Samples were immediately processed upon arrival at the laboratory, within 4 ± 0.5 h of sampling. Samples were processed by using the high concentration assay method described by Atwill et al. *(2)*. Briefly, the lettuce was washed in 500 mL of PBS by using vigorous shaking and massaging for 30 s, being careful not to tear the leaves. The concentration of *E. coli* O157:H7 was determined by transferring 1 mL of the PBS washate into the first two positions of a 12-well-deep reservoir (VWR, Radnor, PA) containing 9 mL of TSB+R ($10^{-1}$), followed by duplicate 100-fold serial dilutions ($10^{-3}$ to $10^{-11}$) into the remaining 10 wells containing 9.9 mL of TSB+R, followed by an incubation step at 37°C for 24 h with orbital rotation of 50 rpm. Enriched washate was then selectively grown and isolated on CHROMagar O157 (CHROMagar, Paris, France) supplemented with +R (O157+R), according to the manufacturer's instructions. Washed lettuce heads were stored at 6°C until results were final. All samples that tested negative by using the high concentration assay were further tested for the presence of *E. coli* O157:H7 by enriching the entire lettuce head in 500 mL of TSB+R at 37°C, with orbital rotation of 50 rpm for 24 h, followed by plate confirmation on O157+R *(2)*. Because of the high inoculum concentration, this assay was designed to enumerate elutable concentrations of *E. coli* O157:H7, ranging from 340 to $3.47 \times 10^{12}$ most probable number (MPN) per head of lettuce. Results ($C_t$) were recorded and calculated by using MPN Calculator Build 23, created by Mike Curiale (http://i2workout.com/mcuriale/mpn/index.html.) A subset of suspect isolates (10%)

TABLE 1. *Summary of weather statistics recorded by a nearby California Irrigation Management Information System weather station over the 92-h trial in central coastal California, 2011*

| Variable | Mean | SD | Minimum | Maximum | Model A[a] (*P* value) | Model B[a] (*P* value) |
|---|---|---|---|---|---|---|
| Solar radiation (W/m$^2$) | 238.5 | 309.6 | 0.0 | 896.0 | 0.14 | 0.16 |
| Air temperature (°C) | 15.0 | 2.9 | 8.8 | 20.9 | 0.14 | 0.44 |
| Evapotranspiration (mm) | 0.2 | 0.2 | 0.0 | 0.7 | 0.14 | 0.23 |
| Relative humidity (%) | 67.2 | 10.7 | 48.0 | 84.0 | 0.15 | 0.23 |
| Wind speed (m/s) | 3.7 | 1.4 | 0.8 | 6.2 | 0.14 | 0.41 |
| Wind direction (degrees) | 279.1 | 95.9 | 0.1 | 348.7 | 0.27 | 0.91 |
| Soil temperature (°C) | 21.1 | 0.4 | 20.3 | 21.9 | 0.16 | 0.03 |

[a] Association of each weather variable to the outcome [$\log(C_t/C_o)$] for model A; $C_t$ for model B) in multiple regression models with days postinoculation or time ($t$) included as a covariate.

was confirmed by PCR, followed by genetic similarity comparison by using pulsed-field gel electrophoresis, as described by Atwill et al. *(2)*.

**Statistical analysis:** Two different approaches were used to model the rate of bacterial inactivation of *E. coli* O157:H7 on heads of romaine lettuce over the 92-h trial by using STATA 14 software (StataCorp LP, College Station, TX). The outcomes of interest were concentration of surviving bacteria ($C_t$) as a function time ($t$), which could then be used to calculate $C_t/C_o$ or $\log(C_t/C_o)$, with $C_o$ set at $6.3 \times 10^7$ MPN of *E. coli* O157:H7 per head.

## RESULTS AND DISCUSSION

Weather data were collected hourly over the 92-h trial, beginning at time of inoculation and ending at time of harvest. The seven weather variables monitored included cumulative solar radiation (watts per square meter), air temperature (degrees Celsius), evapotranspiration (millimeters), relative humidity (percentage), wind speed (meters per second), wind direction (degrees), and soil temperature (°C; Table 1); precipitation (millimeters) was dropped from analysis owing to lack of rain during the trial. All weather variables were significant ($P < 0.05$) in both models A and B during univariate analysis; however, all variables were dropped from the final regression models owing to lack of significance ($P > 0.05$) once the covariate of time was introduced (Table 1). Interestingly, soil temperature was significant ($P < 0.05$) in model B, which generated a lower Akaike information criterion score compared with our final chosen model, but upon further investigation, the mean difference between the predicted outcomes and actual outcomes was much greater when soil temperature was kept in the model, so it was subsequently removed from the final model (Table 2).

TABLE 2. *Regression models for the rate of inactivation of* E. coli *O157:H7 on heads of romaine lettuce during a 92-h field trial in central coastal California, 2011*

| Factor[a] | Coefficient | 95% confidence interval | *P* value |
|---|---|---|---|
| Model A: $[\log(C_t/C_o) = \alpha + \beta X]$ | | | |
| Duration (per day) | –0.691 | –0.815, –0.567 | <0.001 |
| Intercept | 0.381 | 0.097, 0.665 | 0.009 |
| Model B: $[C_t = e^{(\alpha+\beta X)}]$ | | | |
| Duration (per day) | −1.434 | −1.617, −1.250 | <0.001 |
| Intercept | 21.34 | 20.915, 21.765 | <0.001 |

[a] Model A regressed onto $\log(C_t/C_o)$ as the outcome variable; model B regressed onto $C_t$. A quadratic term was nonsignificant ($P > 0.05$) for both models, indicating a relatively linear inactivation rate as a function of time over the 92-h trial.

All heads of lettuce had detectable concentrations of bacteria during the trial. Although a relatively uniform inoculum of $6.3 \times 10^7$ MPN of *E. coli* O157:H7 was applied to each head of lettuce, a wide range of bacterial concentrations were recovered during each sampling event across the 92 h duration (Fig. 1). The arithmetic mean concentration of bacteria per head of lettuce increased rapidly during the first few hours after field inoculation (mean $C_t/C_o = 30.2$ at 2.5 h) and remained above the initial inoculum dosage for at least three sampling events over 2 days ($t = 43$ h; mean $C_t/C_o = 3.9$) and did not fall below the inoculum dosage until days 3 and 4 (67 and 92 h, respectively; Table 3 and Fig. 2). Given that 4 h were needed to transport the lettuce samples from the field site to the laboratory, additional bacterial replication may have occurred during this period, resulting in slightly elevated MPN estimates of $C_t$ for all samples; this potential effect of harvested romaine lettuce on bacterial growth was not determined immediately prior and after the 4-h transport. Instead, we determined that the bacterial concentration in the slurry matrix used for inoculations was not significantly ($P > 0.05$) different immediately prior to and after transportation, suggesting that minimal growth or inactivation occurred because of the effect of transport. This lack of change for the concentration of bacteria in the slurry matrix suggests that the combination of leaf surface, field conditions, and presence of the slurry provided favorable conditions for rapid bacterial replication on the lettuce.

We observed substantial growth of *E. coli* O157:H7 during the initial 2 days (≤43.25 h) postinoculation. This observation is in contrast to the rapid bacterial inactivation observed when applied via an aqueous solution, as demonstrated by McKellar et al. *(21)*. Although environmental factors and strain variations may explain some discrepancies in field-based inoculation trials, the Moyne trials, described by McKellar et al. *(21)*, used the same strain of attenuated *E. coli* O157:H7 at the same field location (Salinas, CA) as this study, yet had substantially higher

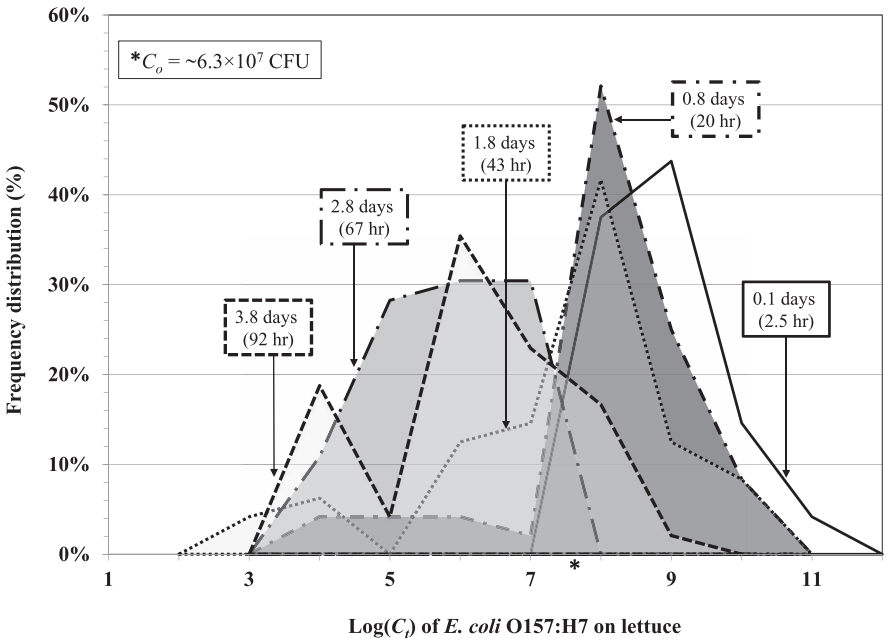


FIGURE 1. *Distribution of* E. coli *O157:H7 MPN per head of romaine lettuce recovered during five sampling events compared with the initial inoculum dose during a 92-h inactivation trial. Events distinguished by different shades of gray and borders. Samples to the right of the inoculum dose (*) indicate bacterial growth, while samples to the left of inoculum dose represent bacterial inactivation.*

inactivation rates compared with our trial. The key difference was the inoculum matrix, with much of the prior work using an aqueous suspension of bacteria applied to heads of lettuce compared with our study that used a fecal slurry matrix. One possible explanation for the observed bacterial growth in our study compared with McKellar et al. *(21)* was the presence of nutrients in the rabbit slurry, which could have supported bacterial replication, in contrast to the nutrients available in an aqueous solution used in earlier work.

Approximately 40% of the inoculated heads of lettuce exhibited bacterial growth above the original inoculum dosage during the first 2 days of the trial, as indicated by the mode of the frequency distribution for bacterial concentration per head of lettuce being greater than $10^8$ MPN for 2.5, 19.75, and 43.25 h (Fig. 1). McKellar et al. *(21)* used a Cerf (biphasic) regression model of pooled data from multiple independent studies to predict *E. coli* O157:H7 inactivation on lettuce leaf surfaces. They *(21)* estimated that over 99.99% of *E. coli* O157:H7 exhibited rapid inactivation within 2 days, with less than 0.01% of the bacteria exhibiting tailing survival behavior after this rapid die-off. In contrast, the data shown in Figures 1 and 2 indicate that in the presence of rabbit fecal material, the ATCC 700728 strain of *E. coli* O157:H7 on romaine lettuce leaves exhibited a wide range of replication and inactivation trajectories that ranged from sustained growth to rapid inactivation across the 92-h trial, with up to a 7-log difference in the observed concentration of bacteria per head of lettuce per period (Fig. 1). Bezanson et al. *(6)* had similar heterogeneity per period ($\sigma^2 \pm 2$ log) when spiking ATTC 700728 strain of *E. coli* O157:H7, suspended in distilled water, onto romaine lettuce.

Assuming that variation around each time point is not entirely stochastic, it would be worthwhile to identify the key plant or environmental factors driving this high amount of interplant variation of the inactivation rate to better understand this observation and to model this process. Although not measured in this study, similar studies found reduced inactivation rates when lettuce leaves were injured, possibly owing to released nutrients from the leaf or additional protection from environmental conditions *(1, 4)*. Avery et al. *(3)* demonstrated that *E. coli* O157:H7 was capable of surviving and persisting in multiple water sources, with an increase in survival after the addition of feces. In contrast, in the trials described by McKellar et al. *(21),* the inoculum was applied in a matrix of sterile deionized water or peptone water or both, which may not provide sufficient protection or nutrition to support sustained bacterial replication. Although extreme or fluctuating

TABLE 3. *Mean concentrations of* E. coli *O157:H7 per head of romaine lettuce during a 92-h field trial in central coastal California, 2011*[a]

| Days (h) postinoculation | No. of heads sampled | Mean $C_t$ (SD) | Mean ($C_t/C_o$) | Log of mean ($C_t/C_o$) | Mean of log($C_t/C_o$) | Difference[b] |
|---|---|---|---|---|---|---|
| 0.1 (2.5) | 48 | $1.9 \times 10^9$ ($6.8 \times 10^9$) | 30.21 | 1.48 | 0.50 | −0.98 |
| 0.8 (20) | 48 | $2.9 \times 10^8$ ($6.3 \times 10^8$) | 4.58 | 0.66 | −0.36 | −1.02 |
| 1.8 (43) | 48 | $2.4 \times 10^8$ ($6.4 \times 10^8$) | 3.88 | 0.59 | −0.94 | −1.53 |
| 2.8 (67) | 46 | $8.1 \times 10^6$ ($1.0 \times 10^7$) | 0.13 | −0.89 | −1.57 | −0.68 |
| 3.8 (92) | 48 | $9.9 \times 10^6$ ($3.6 \times 10^7$) | 0.16 | −0.80 | −2.18 | −1.38 |

[a] With $C_o = 6.3 \times 10^7$ CFU.
[b] Difference between the mean of log($C_t/C_o$) and the log of the mean of ($C_t/C_o$).

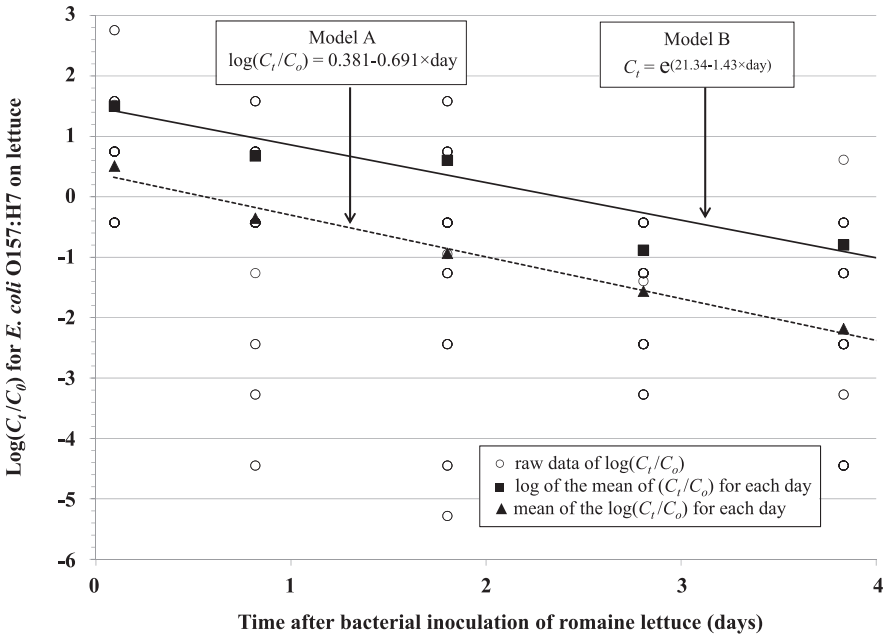


FIGURE 2. *Comparison of regression models for the inactivation rate of* E. coli *O157:H7 on heads of romaine lettuce in central coastal California for bacteria applied to the surface of lettuce.*

environmental conditions can play an important role in the ability for *E. coli* O157:H7 to survive *(1, 30, 32),* the wide range of replication-inactivation trajectories observed in this study do not seem to be associated with differential exposure to solar radiation or wind, in terms of cellular degradation or desiccation. Heads of lettuce with inoculated leaves that were, on average, downwind (leeward) of prevailing winds and facing south-southeast, with more solar exposure, did not have significantly higher inactivation rates ($P > 0.05$) compared with bacteria on heads of lettuce with inoculated leaves that were upwind (windward) and facing north-northwest, with more shade surrounding the plant. Erickson et al. *(11)* found that survival rates of *E. coli* O157:H7 inoculated onto the surface of a lettuce leaf were also not affected by the presence of shade or solar protection. A possible explanation for the lack of UV inactivation or the broad range of recovered bacterial concentrations in our samples is a cellular repair mechanism known as photoreactivation *(29, 31, 34)*; researchers have demonstrated that pathogenic bacteria can regain viability after exposure to harmful UV irradiation, if also being irradiated by visual light *(29, 31, 34)*.

Depending on the structure of the data (i.e., the presence of linear inactivation, a lag phase, or significant tailing for the survival of *E. coli* O157:H7), a first-order kinetic or more complicated biphasic or sigmoidal model would be appropriately used on the outcome variable *(33)*. Our data were normalized by using log transformation [$\log(C_t/C_o)$], then regressed with time, referred to as model A. Despite the utility of such models for predicting thermal and nonthermal bacterial inactivation in food and environmental matrices *(33),* one shortcoming is that regressing on log-transformed values for bacterial concentrations can lead to underestimations of the predicted mean concentrations of bacteria at time ($t$) *(7)*. This occurs when the estimate for the mean of either $\log(C_t)$ or $\log(C_t/C_o)$ is backtransformed to $C_t$ or $(C_t/C_o)$; these predicted bacterial concentrations can be biologically meaningful when considering the outcome is exposure risk to human health. This potential negative bias for concave functions is known as Jensen's inequality *(7),* which predicts that the mean of log-transformed values, when back-transformed to the original scale of the raw data, is always less than or equal to the mean of nontransformed or raw values:

$$\log\left(\frac{\sum_{i=1}^{n} x_i}{n}\right) \geq \frac{\sum_{i=1}^{n} \log(x_i)}{n} \qquad (1)$$

To rectify this potential problem of underestimation from logged values, we used negative binomial regression to construct a second regression model (model B) that could estimate the mean concentration of surviving bacteria ($C_t$) as a function time ($t$) as opposed to $\log(C_t/C_o)$ as was done for model A. Negative binomial regression is well suited to handle the variance of highly right-skewed and overly dispersed bacterial count data compared with Poisson regression, which constrains the variance to equal the mean *(14)*. Negative binomial regression has been used successfully to predict microbial concentrations for a variety of food matrices and environmental samples, such as *E. coli* O157:H7 concentrations on heads of lettuce due to fecal splash from foliar irrigation *(2),* concentrations of *Giardia duodenalis* in dairy farm runoff *(22),* and *E. coli* concentrations in irrigation water from leafy green produce farms *(5)*.

Both models A and B reasonably approximate their respective means as a function of time, with model A regressed onto $\log(C_t/C_o)$ and model B regressed onto $C_t$; however, the residuals of model B are, on average, smaller than the residuals on model A. Both models are shown on a $\log(C_t/C_o)$ scale for the $y$ axis for convenience and comparability (Fig. 2). A quadratic term for time was nonsignificant for either model ($P > 0.05$), indicating a relatively linear inactivation rate over the course of the trial,

once the initial growth phase during the first few hours had ended. It is possible that if the trial had been extended beyond 4 days, some degree of tailing for bacterial survival may have been observed. For model A, each additional day was associated with a 0.691 reduction in $\log(C_t/C_o)$, or an 80% reduction per day when backtransformed to $C_t$ (i.e., $10^{-0.691} = 0.20$). For model B, each additional day was associated with a 76% reduction in $C_t$ (i.e., $e^{-1.434} = 0.24$), which is similar to model A's prediction. The results of our trial show an overall 2.3-log reduction between time point 2.5 h, where there had been a mean 30-fold increase ($C_t/C_o$) in concentration compared with $C_o$ (Table 3), and the final time point at 92 h. Similarly, Barker-Reid et al. *(4)* observed an overall 2.2-log reduction in *E. coli* CFU per gram of lettuce over a 92-h duration when using recycled sewage water that was spiked with $1.3 \times 10^7$ CFU/mL *E. coli,* which equates to about a 64% reduction per day when backtransformed to $C_t$ ($10^{-2.2/5} = 0.36$). If the final mean concentration on day 4 is compared with the initial inoculum concentration at $C_o$, there was only a 0.8-log reduction over the 92-h trial ($9.9 \times 10^6$ CFU/$6.3 \times 10^7$ CFU).

Although the estimated mean bacterial inactivation rate is similar between models A and B, the two models differ substantially in their predictions of bacterial concentration per head of lettuce owing to the large difference in their intercept values (Table 2). Comparison of the mean of $\log(C_t/C_o)$ to the mean of $C_t/C_o$ for the data from each period indicates that the mean of $\log(C_t/C_o)$ underestimates the true bacterial concentration ($C_t$) by 1.12 log on average (range of 0.68 to 1.53 log), or about a 92.5% underestimate of $C_t$ (Table 3). This underestimation of $C_t$ from the use of regression models for bacterial inactivation that regress on $\log(C_t/C_o)$ or $\log(C_t)$ is expected, as predicted from Jensen's inequality, as described previously. As a consequence, we recommend the use of count-based exponential regression models, such as negative binomial regression, to estimate the first-order kinetic bacterial inactivation rate (i.e., linear survival curves on the log scale) to avoid underestimation of $C_t$.

Putting these results into a preharvest food safety perspective, we and others have shown that foliar irrigation can act as a vector to transfer bacterial contamination in the form of fecal splash onto the leaves of romaine lettuce when scat or other fecal matrices are present in the furrow or on the soil surface of beds of lettuce *(2, 23)*. Results from this study suggest that *E. coli* O157:H7, when in the presence of a fecal splash, may grow an additional 1 to 3 log over the initial inoculum and result in substantial microbial risk, if harvest occurs several days after irrigation. During this experiment, there were substantial log reductions (>2 log) after 4 days compared with the bacterial concentration reached following an initial growth phase (2.5 h), however, only a 0.8-log reduction compared with the starting concentration ($C_o$) of the bacteria inoculum. Although our starting bacterial concentration may be relatively high for wildlife scat or, for example, super shedder cattle who can shed $>10^4$ *E. coli* O157:H7 CFU/g feces *(25),* caution should be used when determining the appropriate time for adequate bacterial inactivation, if there is a potential for fecal splash. Using the coefficients from model B to cautiously generate extrapolated predictions, a 5- and 7-day interval between a potential fecal splash incident and harvest would generate on average a 3- and 4-log reduction per head of lettuce, respectively. Bacterial inactivation, combined with removal of the outer lower leaves from the head of lettuce during harvest and reasonable due diligence to remove fresh scat prior to final irrigation, would substantially reduce the risk of microbial contamination for harvested romaine lettuce *(2)*.

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