Metrics Proposed To Prevent the Harvest of Leafy Green Crops Exposed to Floodwater Contaminated with *Escherichia coli*

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ABSTRACT

The California Leafy Green Products Handler Marketing Agreement (LGMA) requires leafy green crops within 9 m of the edge of a flooded field not be harvested due to potential contamination (California Leafy Green Products Handler Marketing Board, *Commodity Specific Flood Safety Guidelines for the Production and Harvest of Lettuce and Leafy Greens*, 2012). Further, previously flooded soils should not be replanted for 60 days. In this study, the suitability of the LGMA metrics for farms in the Mid-Atlantic region of the United States was evaluated. The upper end of a spinach bed (in Beltsville, MD) established on a –5% grade was flooded with water containing 6 log CFU/ml *Escherichia coli* to model a worst-case scenario of bacterial movement through soil. *Escherichia coli* prevalence in soil and on foliar tissue was determined by most probable number (MPN) analysis at distances up to 9 m from the edge of the flood for 63 days. While *E. coli* was quickly detected at the 9-m distance within 1 day in the spring trial and within 3 days in the fall trial, no *E. coli* was detected on plants outside the flood zone after 14 days. On day 63 for the two trials, *E. coli* populations in the flood zone soil were higher in the fall than in the spring. Regression analysis predicted that the time required for a 3-log MPN/g (dry weight) decrease in *E. coli* populations inside the flood zone was within the 60-day LGMA guideline in the spring but would require 90 days in the fall. Overall, data suggest that the current guidelines should be revised to include considerations of field and weather conditions that may promote bacterial movement and survival.

IMPORTANCE

This study tracked the movement of *Escherichia coli* from floodwater across a horizontal plane of soil and the potential for the contamination of distant leafy green produce. The purpose of this study was to address the validity of the California Leafy Green Products Handler Marketing Agreement recommendations for the harvest of leafy green crops after a flooding event. These recommendations were based on the turning radius of farming equipment but did not take into consideration the potential subsurface movement of pathogens in the water through soil. This research shows that further considerations of field slope, temperature, and additional rainfall events may be necessary to provide appropriate guidelines to prevent the harvest of leafy green crops contaminated by enteric pathogens in floodwaters. This study may be used to provide a framework for comprehensive recommendations to growers for good harvesting practices after a flooding event.

Leafy green vegetables are vulnerable to contamination from a variety of sources, including contaminated manure, soil, irrigation water, and contact with wildlife and/or their feces. This vulnerability is partly due to the fact that edible plant portions grow low to the ground and have no protective layers such as peels, shells, or skins (1). The California Leafy Green Products Handler Marketing Agreement (LGMA) has recommended that farmers not harvest any leafy green crops within a minimum of 30 ft (9 m) from the edge of a flood and that they wait 60 days before replanting the soil that had been underwater (2). The 9-m buffer zone was based on the turning radius of farming equipment to prevent cross contamination, via the tires, between flooded crops and leafy greens that had not contacted the floodwater (2). However, no consideration was made for the potential subsurface dissemination of bacteria from the floodwater through soil.

While the vertical transport of bacteria through soil has been thoroughly examined and modeled (3–7), fewer studies have characterized the subsurface horizontal movement of bacteria across a field of harvestable leafy greens. Further, limited data are available on the effect of floodwater on the microbial safety of leafy green crops. Floodwaters are frequently contaminated with pathogenic bacteria, viruses, helminthes, and protozoa (8). Mi-

crobiological analysis of floodwaters from the category 4, 2005 hurricane Katrina found fecal coliform concentrations in the water which were significantly higher than the U.S. Environmental Protection Agency recreational water quality standards of 126 most probable number (MPN)/100 ml (9). After severe flooding along the Mississippi River in 2001, an increase in gastrointestinal illness after contact with floodwater was observed, especially in children (10). To our knowledge, only one study has examined the potential for leafy green contamination in a field setting after a

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FIG 1 Layout of lysimeter plot (−5% grade) used for flooding experiment, where A to E indicate sampling distances.

flooding event. After a natural flooding event in Spain in September 2012, Castro-Ibanez et al. sampled surface soil, irrigation water, and lettuce heads for 7 weeks to determine the levels of indicator organisms, *Salmonella* spp., and verotoxigenic *Escherichia coli* (VTEC). Levels of coliforms on lettuce decreased from approximately 6 log CFU/g at 1 week after flooding to approximately 3 log CFU/g after 7 weeks (11). *Salmonella* was detected only 1 week after the flooding event and then remained undetectable for the duration of sampling. Non-O157 VTEC was identified by PCR in soil for 3 weeks after the flooding event and for 1 week on lettuce but was not detected in irrigation water (11). Unfortunately, it is difficult to apply these results to the threat of produce contamination from floodwater as it is unclear where the soil and lettuce samples were collected in relation to the flood zone.

While the new Food Safety Modernization Act *Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption* (12) discusses the potential risks and concerns regarding “pooled water,” it does not provide any quantitative metrics. The LGMA has outlined a 9-m zone of no crop harvest and a waiting period of 60 days before replanting, a requirement that could impact the ability to market leafy greens in California. However, the 9-m buffer zone refers to the turning radius of farming equipment, not the underground movement of bacteria through soil. Such a requirement could have a disproportionate impact on small farms. Because floodwaters are likely to contain zoonotic pathogens from animal fecal matter, there is a significant need to comprehensively study the movement of bacteria from floodwater through soil to determine the specific risk of contamination of nearby crops. Such research can be used to set more specific guidelines and regulations for farmers after a flooding event to prevent the harvest of potentially contaminated crops and protect public health while protecting small farms from unnecessary crop destruction and the associated profit loss. On small farms, such as those common in the Mid-Atlantic region of the United States, the proposed 9-m buffer zone may be a significant portion of the farm’s entire crop field. Such a loss of marketable crops to a flood may be financially devastating to a small farm. The

purpose of this study was to evaluate the transport of bacteria from floodwater through soil in a field setting and to determine if this transport can lead to potential contamination of spinach leaf tissue on growing plants. This study was specifically designed to assess the suitability of the LGMA metrics for farms in the Mid-Atlantic region.

MATERIALS AND METHODS

Field site and plot design. The field used in this experiment was a lysimeter plot with a −5% grade (3° slope) at the Beltsville Agricultural Research Center (BARC), U.S. Department of Agriculture (USDA) Northeast Area (NEA). This slope was selected to model a worst-case scenario where a flood occurs at the top of a field slope (i.e., heavy rainfall causes a stream to overflow its banks) and where the force of gravity promotes water and potentially bacterial movement through the soil. The soil in this lysimeter plot was a clay loam soil with a pH of approximately 6.8.

Nine 10-m-long by 0.5-m-wide rows of spinach (cultivar Racoon; Rijk Zwaan USA, Salinas, CA) were planted in groups of three, with 0.5 m between rows and 2.5 m separating the three groups (Fig. 1). Spinach seed was applied via broadcasting at the standard rate of 42 kg/ha. Over time, seedlings were thinned to a density of one plant every 0.05 m. Prior to flooding, a 1-m by 1-m soil berm was hand built at the upper end of each center row of spinach to ~0.1 m high to contain the floodwater. Additionally, immediately before flooding, the entire plot was saturated with irrigation water to promote standing-water formation. The experiment was performed for an April planting of spinach (henceforth known as the spring trial) and a September planting (fall trial). Daily maximum temperature and total rainfall data for the duration of both trials were collected from the USDA ARS BARC Weather Station 3 Old Beltsville Airport (BARC). Total UV-B radiation data were acquired from the Beltsville, MD, site of the UV-B Monitoring and Research Program of the Natural Resource Ecology Laboratory (Colorado State University [<http://uvb.nrel.colostate.edu/UVB/index.jsf>]).

Inoculum preparation and field flooding. Nalidixic acid-resistant strains of nonpathogenic *Escherichia coli* (MW416, MW423, and MW425), isolated from sewage (13) and previously shown to be suitable surrogates for *E. coli* O157:H7 attachment to leafy greens (14), were grown separately overnight at 37°C in 150 ml of tryptic soy broth (Neogen, Lansing, MI) supplemented with 50 µg/ml nalidixic acid (TSBN) (Sigma-Aldrich, St. Louis, MO). Cultures were combined with liquid dairy ma-

nure diluted 1:10 in sterile water to serve as the inoculated floodwater. Liquid dairy manure was obtained from a solid/liquid extractor from the USDA ARS NEA BARC dairy herd the day of inoculation. Specifically, 33 ml of each *E. coli* culture was combined with 1.8 liters of liquid dairy manure in 16.1 liters of sterile water for a final *E. coli* population of ~ 6 log CFU/ml. A separate inoculum was prepared for each of three individual floods applied to the center row of each group of spinach (Fig. 1) in each season's trial. Using a spigot to control flow, approximately 11 liters of the inoculum was applied to the area inside the soil berm to create a flood zone of standing water at the top of the 4-week-old spinach plants. A small amount of floodwater (approximately 0.5 liters) was allowed to overflow the berm; the edge of this water was marked as the edge of the flood and is here referred to as the edge of the flood zone.

Sample collection. Immediately after flooding, soil and spinach samples were collected inside the flood zone and at 0.5 m, 1.5 m, 4.5 m, and 9 m from the edge of the flood zone. At each of these distances, five samples were collected from each flooded spinach row: surface soil within the spinach row (0 to 5 cm deep), rhizosphere soil (5 to 10 cm deep), surface soil between the spinach rows (0 to 5 cm deep), bulk soil (5 to 10 cm deep), and spinach leaf tissue. Bulk soil was collected from soil not planted with spinach, immediately adjacent to soil containing spinach rows. Rhizosphere soil was collected from soil within the spinach rows, immediately adjacent to spinach plant roots. The soil samples were collected using 2.5-cm diameter soil probes (Oakfield Apparatus, Fond du Lac, WI), with three soil samples taken at each distance for both bulk and rhizosphere soils. The soil probes were rinsed in water and sanitized using 70% ethanol between collections of individual samples. A single soil core removed from the probe was divided into a surface (0 to 5 cm) sample and a subsurface (5 to 10 cm) sample using alcohol-sanitized plastic knives. The three surface samples collected at the same distance were combined into one Whirl-Pak bag (Nasco, Ft. Atkinson, WI), and subsurface samples were handled similarly. Spinach leaf tissue was collected at each distance by harvesting the leaves of 3 to 5 spinach plants (depending on size) with alcohol-sanitized scissors and placed into sterile Whirl-Pak bags (Nasco).

Soil and spinach samples were collected on days 0, 1, 3, 7, 14, 21, 28, 35, 42, 49, 56, and 63 postflooding. Spinach tissue samples were also collected on day 9 when no soil samples were obtained. Additionally, soil and spinach tissue samples were collected on the day before the start of the study to confirm the absence of nalidixic acid-resistant bacteria in the plot. Samples were stored in coolers on ice packs for transport to the USDA ARS Environmental Microbial and Food Safety Laboratory, with processing initiated within 3 h of sample collection.

Microbial analysis of soil and spinach samples. *E. coli* populations in soil samples were quantified using a mini-most probable number (MPN) assay. A portion of each soil sample (30 g) was diluted 1:5 in buffered peptone water (BPW; Neogen) in filtered Whirl-Pak bags (Nasco) and then hand massaged for 2 min. A mini-MPN procedure was performed in 48-well blocks (VWR, Radnor, PA) by adding 1 ml of each filtered soil homogenate to 1 ml of double-strength TSBN (this represents a 1:10 dilution of the original soil sample). Samples were then serially diluted 1:10 in quadruplicate in 1.8 ml of TSBN and incubated at 37°C for 24 h. The number of dilutions required was determined by the results of the previous sampling day. A 1- μ l aliquot of each dilution was then streaked onto MacConkey agar (MAC; Neogen) supplemented with 50 μ g/ml nalidixic acid (MACN). Spinach leaf tissue was weighed and diluted 1:10 in TSBN, stomached for 1 min, and incubated at 37°C for 24 h. A 50- μ l aliquot of each enrichment was spread onto MACN plates in duplicate.

All MACN plates from soil homogenate MPN or spinach enrichment assays were incubated at 37°C for 24 h and examined for presence/absence of *E. coli*. The MPN/gram was calculated for soil samples using the MPN Calculator Build 23 (version VB6 [<http://i2workout.com/mcureale/mpn/index.html>]). The limit of detection for the MPN assay was 1.1 MPN/g (0.04 log MPN/g), as calculated by the software. All soil samples that were negative for the presence of *E. coli* by MPN assay were nonselectively

enriched by incubating the entire soil homogenate at 37°C for 24 h in BPW (standard protocol for our laboratory). A 10- μ l aliquot was then streaked onto MACN to determine the presence/absence of *E. coli*.

The *E. coli* population per gram of dry weight of soil was calculated by adjusting the MPN/gram of wet weight with the moisture factor (gram of wet weight/gram of dry weight) determined from drying 10 g of each soil sample at 105°C for 24 h and subtracting the residual weight from the wet weight. All MPN/gram values are reported as MPN/gram (dry weight) of soil.

Confirmation of isolates. To confirm that the *E. coli* bacteria recovered from the soil were the same nalidixic acid-resistant *E. coli* strains present in the inoculated floodwater, DNA was extracted from a random sample of *E. coli* isolates recovered from MACN plates from the MPN analysis of soil homogenates on sampling days 56 and 63 in both trials using Insta-Gene matrix (Bio-Rad, Hercules, CA). DNA was also extracted from pure cultures of each of the three inoculated *E. coli* strains. Repetitive extragenic palindromic-PCR (REP-PCR) was performed using primer sequences described previously (15) using a Mastercycler ProS (Eppendorf, Hauppauge, NY) (referred to as BOX-PCR). The reaction mixtures (25 μ l) consisted of 2 μ l of template DNA, 50 pM of the BOX-A1R primer, 4 μ l of 5 $\times$ MyTaq reaction buffer (Bioline, Taunton, MA) containing 3 mM MgCl₂ and 1 mM deoxynucleoside triphosphates (dNTPs), and 0.5 units of MyTaq HS DNA polymerase (Bioline). The amplification conditions consisted of an initial denaturation at 95°C for 10 min, 30 amplification cycles (94°C for 1 min, 53°C for 1 min, and 72°C for 4 min), and a final extension at 65°C for 10 min. The amplification products were separated by electrophoresis on 2% agarose gels containing GelRed nucleic acid gel stain (Biotium, Hayward, CA) and 1 $\times$ lithium borate buffer (Faster Better Media, LLC, Hunt Valley, MD) at 250 V. Gels were visualized using a Gel Doc EZ Imager (Bio-Rad).

Statistical analysis. The USDA Integrated Pathogen Modeling Program (IPMP) (U.S. Department of Agriculture, 2013 [<http://www.ars.usda.gov/Main/docs.htm?docid=23355>]) was used to generate a linear regression, using the survival curve program, of the population of *E. coli* at each measured distance over time. JMP Pro, version 11.0.0 (SAS Institute, Inc., Cary, NC), software was used to perform an analysis of variance (ANOVA), and Tukey's test was used to determine significant differences between soil sample types (bulk, rhizosphere, surface, or subsurface) and between maximum populations and rates of decline of *E. coli* populations at different distances and between the two seasons. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

Enumeration of *E. coli* bacteria from soil. No nalidixic acid-resistant *E. coli* bacteria were detected in soil or on spinach 1 day prior to inoculation for either trial. Results of statistical analysis showed no significant differences between the MPN/gram (dry weight) values for *E. coli* between the four soil samples (bulk surface and subsurface and rhizosphere surface and subsurface) taken at the same distances on the same days (*P* = 0.99 in spring; *P* = 0.46 in fall). As such, for subsequent analyses, mean populations from the bulk and rhizosphere soil samples were averaged to yield one soil *E. coli* population value (MPN/gram of dry weight) for each sampling distance in each replicate on each day.

Mean *E. coli* populations at each sampling distance during the spring and fall trials are shown in Fig. 2. In the spring trial, *E. coli* (0.82 log MPN/gram of dry weight [gdw]) was recovered from soil at the 4.5-m sampling distance in one row and from one soil sample (~ 1.82 log MPN/gdw) 9 m from the edge of the flood zone immediately following inoculation (day 0). An increase in populations (from <1 log MPN/gdw to 3 log MPN/gdw) outside the flood zone was seen on day 1, followed by a generally linear decline at all distances (Fig. 2A). By day 42, *E. coli* populations could not be quantified by MPN assay outside the flood zone, and presence/

FIG 2 *Escherichia coli* population present in soil inside the flood zone ($\blacktriangle$) and at 0.5 m ($\blacksquare$), 1.5 m ($\bullet$), 4.5 m ($\blacklozenge$), and 9 m (bar) from the edge of the flood zone in the spring trial, June to August 2014 (A), and the fall trial, October to December 2014 (B). Each data point represents the average of 12 samples, i.e., the four soil samples collected at each distance from the three rows of spinach. The limit of detection for enrichment (dashed line) was -1.48 log CFU/g. Error bars indicate the standard errors of the means.

TABLE 1 Average peak concentration and rate of decline of *E. coli* in soil at each sampling distance over time^a

Distance from edge of flood (m)	Maximum population recovered (log MPN/gdw)			Rate of decline (log MPN/gdw/day) ^a			Time to 3-log decrease in recovery (days)		
	Spring	Fall	<i>P</i> value ^b	Spring	Fall	<i>P</i> value	Spring	Fall	<i>P</i> value
Inside flood zone	5.8 A	4.8 A	<0.01	−0.15 AB	−0.03	<0.01	20 B	90	0.01
0.5	3.6 B	2.0 B	0.24	−0.18 B	−0.04	0.02	18 B	105	0.02
1.5	3.1 BC	1.1 BC	0.02	−0.18 B	−0.02	0.01	18 B	172	0.02
4.5	1.4 CD	−0.4 C	0.01	−0.07 A	−0.03	0.04	47 A	112	0.18
9	1.2 D	0.3 BC	<0.01	−0.11 A	−0.01	<0.01	28 B	250	<0.01

^a Different letters within each column (spring and fall) indicate significant differences in the regression parameters or time to 3-log decline, as calculated using one-way ANOVA and Tukey's test. There were no significant differences in the rates of decline of *E. coli* at the different distances from the edge of the flood or in the time to a 3-log decrease in the fall season.

^b *P* values were determined for the difference between values for spring and fall trials at each distance using one-way ANOVA. For all *P* values, boldface indicates significant (*P* < 0.05) differences.

absence could be determined only by enrichment. By day 63, no *E. coli* could be recovered from outside the flood zone.

In the fall trial, *E. coli* populations were detected by enrichment from soil samples at 1.5 m, 4.5 m, and 9 m from the edge of the flood zone immediately after the flooding event (day 0). On day 1, there was a significant increase in recovery of *E. coli* populations at the 0.5-m distance from the edge of the flood compared to the level at day 0 (from 1.5 log MPN/gdw to 4 log MPN/gdw), but such an increase was not seen at the 1.5-m distance until day 3 (from below the limit of detection for the MPN assay to 1.5 log MPN/gdw) (Fig. 2B). Throughout the fall trial, *E. coli* was generally detected only by enrichment at the 4.5-m distance from the flood zone edge; however, populations were detected by MPN analysis from some 9-m soil samples through day 49. On day 63, *E. coli* populations in the flood zone soil had declined by only approximately 2 log MPN/gdw. Outside the flood zone, *E. coli* was detected on day 63 only by enrichment at the 1.5-m, 4.5-m, and 9-m distances from the edge of the flood zone. On day 63, *E. coli* populations in the flood zone soil were significantly higher in the fall (2.8 log MPN/gdw) than in the spring (0.4 log MPN/gdw) (*P* < 0.01).

Regression analysis of *E. coli* populations in soil over time.

The populations of *E. coli* recovered at each distance over time were individually fit to linear regressions using the IPMP software. A regression from the maximum population of *E. coli* recovered to the detection limit value over time was calculated for each sampling distance. Table 1 summarizes the average maximum population and rate of decline of *E. coli* bacteria at each sampling distance over time for each season. One-way ANOVA revealed significant effects associated with distance (*P* < 0.01) and season (*P* < 0.05) on the maximum population of *E. coli* recovered at each sampling distance. Further analysis showed the maximum populations of *E. coli* to be significantly greater in the spring trial than in the fall trial at all distances except 0.5 m from the flood zone edge (Table 1). The rate of decline was significantly affected by season, with a lower rate of decline in the fall than in the spring (*P* < 0.01), but not by sampling distance (*P* = 0.56).

The time required for a 3-log decrease in *E. coli* populations was calculated for each distance using the linear regression equation generated in IPMP (Table 1). One-way ANOVA showed that the time required for a 3-log decrease was significantly affected by season (*P* < 0.01) but not by distance (*p* = 0.43). Further analysis showed that the time required for a 3-log reduction in *E. coli* populations was significantly longer in the fall than in the spring at

all sampling distances except 4.5 m from the edge of the flood zone (Table 1).

Contamination of spinach foliar tissue. During the spring trial, spinach foliar samples were collected over a 14-day period before the plants bolted. In the fall trial, spinach samples were collected through day 42. The number of spinach enrichments positive for *E. coli* during the spring planting is shown in Table 2. No spinach samples were positive by enrichment outside the flood zone on day 0. At just 1 day postflooding, one of three spinach samples was positive for *E. coli* by enrichment 9 m from the edge of the flood zone, and two were positive at the same distance on day 9 in the spring trial. Inside the flood zone, all spinach samples were positive for *E. coli* throughout the 14 days of sampling. However, outside the flood zone, *E. coli* could be detected from only one spinach enrichment on day 14 (Table 2).

The number of spinach enrichments positive for *E. coli* during the fall trial is shown in Table 2. Inside the flood zone, all spinach samples were positive for *E. coli* by enrichment for the duration of sampling. The number of positive samples peaked on day 3 for all distances outside the flood zone. After day 14, only 6 enrichments were positive for *E. coli* outside the flood zone, and this number declined until only one spinach enrichment outside the flood zone was positive for *E. coli* on day 42 (Table 2).

TABLE 2 Number of spinach tissue samples positive by enrichment for *E. coli* out of three replicates in the spring and the fall trials

Trial ^a and distance from edge of flood zone (m)	No. of positive samples at the indicated day ^b									
	0	1	3	7	9	14	21	28	35	42
Spring										
0	3	3	3	3	3	3				
0.5	0	3	3	3	2	0				
1.5	0	2	1	1	1	0				
4.5	0	3	2	0	0	1				
9	0	1	1	0	2	0				
Fall										
0	3	3	3	3	3	3	3	3	3	3
0.5	2	2	3	0	3	1	1	1	0	0
1.5	0	0	3	1	2	0	0	1	0	0
4.5	0	0	1	1	1	0	0	1	1	1
9	0	0	2	1	1	0	0	0	0	0

^a Spring trial, June to August 2014; fall trial, October to December 2014.

^b Spinach samples were not collected beyond day 14 in the spring trial.

FIG 3 Daily maximum temperature (A), total UV-B radiation (B), and total rainfall (C) data for spring (black) and fall (gray) trials.

Temperature and rainfall. Fig. 3 shows the maximum temperature, total UV-B radiation, and total rainfall each day for the spring and fall trials. Student's *t* test showed that the average maximum daily temperature was significantly lower ($P < 0.01$) in the

fall trial (13°C) than in the spring (29°C) (Fig. 3A). Student's *t* test also determined that the average total UV-B radiation per day was significantly lower ($P < 0.01$) during the fall trial (5.6 kJ/m² per day) than in the spring (11.3 kJ/m² per day) (Fig. 3B). There was no significant difference in the average rainfall amounts during the two seasons although a noticeably greater amount of rain occurred during the first 3 days in the spring trial (28.7 mm total rainfall) than in the fall trial (18.0 mm total rainfall) (Fig. 3C). Correlation analysis was used to evaluate potential associations between peaks in recovery of *E. coli* over time to heavy rainfall events or to peaks in the daily maximum temperature. However, no significant correlations were found.

Isolate confirmation. All *E. coli* isolates ($n = 30$ for each trial) collected during the last 2 weeks of sampling (days 56 and 63) in both trials were confirmed by BOX-PCR to be one of the three nalidixic acid-resistant *E. coli* strains inoculated into the floodwater on day 0.

DISCUSSION

In this study, the movement of *E. coli* through soil and the subsequent contamination of spinach leaf tissue were examined using a lysimeter plot with a -5% grade. The *E. coli* was inoculated into water containing fresh dairy liquid manure at the upslope portions of spinach rows. This introduced a potential enhancement of the horizontal movement of *E. coli* through the soil as the force of gravity promoted the percolation of water down the slope. However, the experimental design used in our experiments simulates a flood that may occur when a heavy rainfall event causes river or creek water contaminated with animal manure to overflow its banks and cause flooding on cropland at the higher end of a field slope. Therefore, this study provides evidence for the movement of bacteria through soil from a worst-case scenario where the force of gravity augments the potential transport of enteric bacteria through soil and, subsequently, the risk of contamination of produce crops.

Within 1 day of the flood event, *E. coli* was quickly detected in soil and on leaf tissue beyond the edge of the flood zone. In the spring trial, approximately 1.5 log MPN/gdw *E. coli* was recovered from soil at the farthest sampling distance, 9 m from the edge of the flood zone, on day 1. On the same day, one spinach enrichment (out of 3) was positive for *E. coli* presence. Similarly, in the fall trial, but on day 3, *E. coli* was detected at ~ 1 log MPN/gdw in soil at 9 m and on two of the three spinach tissue samples at the same distance. Potentially, this could have been the chance detection of an *E. coli* that was not from the inoculum. However, composite soil and leaf tissue samples taken the day prior to flooding in both trials showed no presence of nalidixic acid-resistant *E. coli* in the plot. Heavy rainfall after the flooding event in both seasons made it difficult to determine if this relatively rapid detection of *E. coli* at the 9-m distance was a result of transport of the bacteria through soil or simply overland runoff down the slope to the farthest sampling distances. However, the recovery of the inoculated *E. coli* in soil from both surface and subsurface samples at the 9-m distance provides strong evidence for the subsurface lateral transport of *E. coli* contributing to the contamination of spinach foliar tissue at that distance. Rain splash of contaminated soil onto the spinach leaves during the heavy rainfall in the first few days after the initial flooding event may be the cause of the recovery of *E. coli* from the spinach leaves as rain splash has been shown to be a

significant method of dispersal of bacteria in soil to plant tissue (16).

Populations of *E. coli* in soil were quantified separately for bulk and rhizosphere soils at surface and subsurface depths. Previous research has shown that the transport of pathogens through soil is significantly retarded in the presence of plant roots. In 2005, Roodsari et al. investigated the effect of vegetated filter strips on the transport of fecal coliforms down a hillslope and concluded that there was a significant decrease in the populations of fecal coliforms recovered from surface runoff in vegetated plots (17). These results suggest that the presence of plants decreases the movement of water, and therefore bacteria, through a field. A study investigating the effect of plant roots on percolation of *Salmonella enterica* serovar Typhimurium and *E. coli* O157:H7 found that in soils containing lettuce roots, neither species percolated to a depth beyond the roots. Additionally, populations of the two pathogens were significantly higher in rhizosphere soils than in bulk soils at the same depths (7). Researchers suggested that enhanced bacterial survival in the presence of plant root exudates may have contributed to the increased populations recovered from rhizosphere soil relative to those from bulk soil (7). Several experiments investigating the downward movement of bacteria have concluded that the population of bacteria in soil is greatest at the soil surface, where filtration of bacteria is most efficient (3, 18). In contrast to these results, the current study found no significant differences between the populations of *E. coli* recovered from the bulk and rhizosphere soils or between surface and subsurface soils in either season. This lack of effect may have occurred because the relatively high clay content of the soil (~40%) led to the formation of macropores in the soil that promoted the spread of the bacteria beyond the plant roots (5, 19).

The populations of *E. coli* recovered from soil over time at each sampling distance was fit to a linear regression from the highest recovery to when the populations reached the limit of detection (1.1 MPN/gdw). In both trials, the maximum population recovered from soil dropped significantly as the distance from the flood zone increased, but there was no definitive pattern for the rate of decline at the different sampling distances for either season (Table 1). However, the maximum populations of *E. coli* recovered were significantly higher in the spring than in the fall at all sampling distances, except for 0.5 m (Table 1). At 0.5 m from the edge of the flood zone, the maximum population was higher in the spring than in the fall, but the difference was not statistically significant, possibly because of high variability between replicates in the fall. These results suggest that greater numbers of *E. coli* bacteria traveled out of the flood zone and to farther distances in the spring than in the fall. This greater mobility is likely the result of greater rainfall amounts in the first few days postflooding in the spring than in the fall (Fig. 3), which mobilized more bacteria out of the flood zone through the soil, similar to the effect of rainfall on bacterial transport through soil observed in other experiments (5).

Inside the flood zone, *E. coli* populations were significantly higher on day 63 in the fall (2.8 log MPN/gdw) than in the spring (0.4 log MPN/gdw) ($P < 0.01$). While the recovery of *E. coli* was significantly higher on day 0 in the spring than in the fall, the rate of population decline over time was significantly greater in the spring than in the fall (Table 1). Additionally, the significantly greater rate of decline in the spring than in the fall was observed at all sampling distances. This higher rate of bacterial die-off in the

spring than in the fall supports previous studies that have shown that survival of bacteria in soil is significantly greater at cooler temperatures (3, 20–23). The differential survival is likely a function of ultraviolet radiation (UV), specifically, UV-B radiation (320 to 290 nm), as the UV-B radiation is the region of solar radiation responsible for the majority of bacterial cell death and is greater at warmer temperatures (24). In this study, both the average daily maximum temperature and UV-B radiation were significantly higher in the spring trial than in the fall.

For this study, floodwater was contaminated with ~6 log CFU/ml *E. coli*. This level of contamination is generally higher than the levels of bacteria in manure that may leach out to contaminate floodwater. A survey of cow feces at the time of slaughter found 75% of samples to have equal to or less than 10^3 log CFU/g *E. coli* O157 (25). As such, the time for a 3-log decrease in population levels was calculated for each sampling distance in each season. The number of days required for a 3-log decrease in the population of *E. coli* was significantly longer in the fall than in the spring at all sampling distances, except for at 4.5 m from the flood edge (Table 1). At the 4.5-m distance, the time to a 3-log decrease was noticeably greater in the fall, but variation between the rates of decline for each row, due to the very low level of recovery of *E. coli*, is likely the reason the difference did not reach significance.

The purpose of this study was to investigate the movement of bacteria from floodwater through a spinach field to determine if the LGMA-proposed metrics are sufficient to prevent the harvest of contaminated spinach crops. The LGMA suggests that farmers refrain from harvesting all crops within 30 ft (9 m) of the edge of a flood zone. In both seasons, *E. coli* was detected from spinach leaf tissue from at least one sample at the 9-m sampling distance, indicating that there is the potential for bacteria mobilized by floodwater to contaminate leafy green crops throughout the 9-m buffer zone of crop destruction suggested by the LGMA. However, in both trials, heavy rainfall occurred within 24 h of the initial flooding event, potentially confounding results. The rainfall may have promoted the rapid subsurface transport of bacteria through the soil but may have also washed contaminated soil across the field with surface runoff. In both cases, rain splash may have led to the contamination of the spinach leaves. However, recovery of *E. coli* from subsurface soil samples provides strong evidence that the inoculated bacteria were transported by infiltrating water through the soil to the 9-m distance. The negative slope of the field likely contributed substantially to the rapid transport of *E. coli* observed in these experiments. The force of gravity likely promoted the movement of water down the slope, and the main transport mode of bacteria through soil is by water infiltration (19). The effects of field slope, as well as of soil texture and initial soil moisture, on the transit of bacteria through soil in floodwater conditions require further investigation.

In addition to the 9-m buffer zone, the LGMA suggests waiting 60 days before replanting previously flooded fields, provided the soil sufficiently dries during this period. In this experiment, *E. coli* in the flood zone could be recovered only by enrichment after 42 days in the spring trial, whereas in the fall, there was only a ~2-log decline in the flood zone population over the entire 63 days of sampling. In fact, linear regression analysis predicted that the time required for a 3-log decrease in the population of *E. coli* in the flood zone was 90 days in the fall trial but only 20 days in the spring. Further research is needed to determine the potential for contamination of subsequent crops of spinach planted in the

flood zone before the *E. coli* populations in the soil decrease below the detection limit. In summary, the results of this study suggest that the LGMA-proposed metrics should be revised to include considerations of soil and ambient temperatures, possibly incident solar radiation, additional rainfall events, and field slope in determining planting waiting periods and buffer zones to prevent the harvest of contaminated leafy greens after a flooding event.

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