

Soil Solarization Reduces *Escherichia coli* O157:H7 and Total *Escherichia coli* on Cattle Feedlot Pen Surfaces[†]

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ABSTRACT

Feedlot pen soil is a source for transmission of *Escherichia coli* O157:H7, and therefore a target for preharvest strategies to reduce this pathogen in cattle. The objective of this study was to determine the ability of soil solarization to reduce *E. coli* O157:H7 in feedlot surface material (FSM). A feedlot pen was identified in which naturally occurring *E. coli* O157:H7 was prevalent and evenly distributed in the FSM. Forty plots 3 by 3 m were randomly assigned such that five plots of each of the solarization times of 0, 1, 2, 3, 4, 6, 8, and 10 weeks were examined. Temperature loggers were placed 7.5 cm below the surface of each plot, and plots to be solarized were covered with clear 6-mil polyethylene. At each sampling time, five FSM samples were collected from each of five solarized and five unsolarized plots. *E. coli* concentrations and *E. coli* O157:H7 presence by immunomagnetic separation and plating were determined for each FSM sample. Initial percentages of *E. coli* O157:H7–positive samples in control and solarized FSM were 84 and 80%, respectively, and did not differ ($P > 0.05$). *E. coli* O157:H7 was no longer detectable by 8 weeks of solarization, but was still detected in unsolarized FSM at 10 weeks. The average initial concentration of *E. coli* in FSM was 5.56 log CFU/g and did not differ between treatments ($P > 0.05$). There was a 2.0-log decrease of *E. coli* after 1 week of solarization, and a >3.0-log reduction of *E. coli* by week 6 of solarization ($P < 0.05$). *E. coli* levels remained unchanged in unsolarized FSM ($P > 0.05$). Daily peak FSM temperatures were on average 8.7°C higher for solarized FSM compared with unsolarized FSM, and reached temperatures as high as 57°C. Because soil solarization reduces *E. coli* O157:H7, this technique may be useful for reduction of persistence and transmission of this pathogen in cattle production, in addition to remediation of *E. coli* O157:H7–contaminated soil used to grow food crops.

Bovine manure is an important source of *Escherichia coli* O157:H7 for either direct or indirect contamination of food, water, and the environment. Heavy rains in the spring of 2000 contributed to the transport of *E. coli* O157:H7 and *Campylobacter* in runoff from a bovine manure-amended field, and the subsequent contamination of the Walkerton, Ontario, Canada, municipal water system (36). The result of this contamination was a huge waterborne disease outbreak, in which an estimated 2,300 people became ill and 7 died. Several other waterborne illness outbreaks associated with manure contamination have been reported (29, 32), and *E. coli* O157:H7 illness also has been linked to foods likely contaminated with manure by runoff from cattle production or manure storage, by application of manure to soil used to grow food, or by other inadvertent contact (11, 16, 26, 35). Produce has become a significant source of *E. coli* O157:H7 foodborne illness (40).

Similarly, manure in the preharvest cattle production environment is a significant source of transmission of *E. coli* O157:H7 for the infection of additional animals. Horizontal fecal–oral transmission of this pathogen among cattle has been indicated in several studies (4, 17, 22, 41). The significance of this transmission route has been highlighted by recent work that has associated the presence of cattle excreting high levels of *E. coli* O157:H7 ($\geq 10^3$ to 10^4 CFU/g of feces) with higher prevalence of fecal shedding and hide contamination among cattle in the pen or herd (2, 4, 15, 18, 33, 45). Results from many of these studies have shown that even a very small proportion of these cattle, dubbed “super-shedders,” can be responsible for a large proportion of the total *E. coli* O157:H7 contamination of the pen environment and other cattle. Furthermore, both generic and pathogenic strains of *E. coli*, including *E. coli* O157:H7, can survive for long periods in feces, manure, and soil; this ability can contribute to the transmission risk associated with these materials (3, 8, 10, 49). For example, the increased persistence of *E. coli* O157:H7 in manure from cattle that are fed distillers grains likely is responsible to some extent for the higher prevalence of the pathogen in feces and on hides that has been observed for cattle fed distillers grains (47, 48, 51). Because *E. coli* O157:H7 fecal

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shedding and hide prevalence are correlated with beef carcass contamination (1, 12, 21), understanding these relationships is critical for reducing the risk of human illness associated with beef production and consumption.

This information suggests that preharvest control strategies aimed at reducing *E. coli* O157:H7 on cattle feedlot pen surfaces may reduce the transmission of this pathogen among cattle, in addition to reducing transmission to food crops, water, and the environment (9). Soil solarization is a technique used in crop production for preplanting pathogen and pest control, which utilizes solar energy to heat the soil. Typically, the soil is covered with transparent polyethylene plastic sheets for a period of several weeks, and the resultant heat can inactivate bacterial and fungal pathogens, nematodes, weeds, and weed seeds in the covered soil (30, 31, 43). The objective of this work was to determine the ability of soil solarization to reduce populations of *E. coli*, including *E. coli* O157:H7, from feedlot pen surface material.

MATERIALS AND METHODS

Soil solarization. The study was conducted at the 6,000-head capacity feedlot at the U.S. Meat Animal Research Center near Clay Center, Nebraska, from July to September 2009. In late June, feedlot surface material samples from candidate feedlot pens containing cattle were collected and screened to identify a pen with high prevalence of *E. coli* O157:H7, using procedures described below for determination of the presence of this pathogen. The selected pen was approximately 30 by 90 m, with a 4% slope from the feed bunk to the bottom of the pen, and stocked with 75 head of cattle, which were removed from the pen on the day the experiment began (6 July 2009).

Forty plots 3 by 3 m were laid out in a 4-plot by 10-plot grid pattern. Plots were randomly assigned such that there were five plots of each of the solarization times of 0 (unsolarized control), 1, 2, 3, 4, 6, 8, and 10 weeks. Assignments in the grid were reviewed to confirm that plots of the same solarization times were distributed throughout the pen. WatchDog 100 Series button temperature loggers (Spectrum Technologies, Inc., Plainfield, IL) were placed 7.5 cm below the surface in the center of each plot to continuously monitor feedlot surface material (FSM) temperature. Plots to be solarized were covered with sheets of 6-mil clear polyethylene 3.6 by 3.6 m. Corners and edges were staked, and the edges covered with fill dirt to prevent flapping and tearing. Unsolarized control plots were left uncovered. The site was regularly inspected to look for free edges, holes, or other problems. Air temperature and rainfall volumes at the feedlot were monitored and recorded at 15-min intervals by an on-site, all-season weather station.

Sample collection and analyses. At each sampling period, five separate FSM samples (approximately 50 to 100 g each) were collected at random locations in each of five solarized and five unsolarized plots (the same five unsolarized control plots were sampled throughout the study). FSM was sampled to a depth of 7.5 cm by using a small metal spade, which was wiped off, sanitized with 70% isopropanol, and wiped dry with a clean lab tissue between uses. Samples were placed in separate Ziploc bags and transported to the laboratory for immediate processing.

The FSM samples were processed and analyzed to determine both the concentrations of total *E. coli* and the presence of *E. coli* O157:H7, using procedures previously described (10). Briefly, 10 g of each FSM sample was weighed into separate, sterile filtered

stomacher bags. Ninety milliliters of tryptic soy broth (TSB; Difco, BD, Sparks, MD) was added to each 10-g sample, and the bag contents were mixed well by massaging the bag. For determination of total *E. coli* concentration, a 1-ml volume was removed, diluted further as needed in 2% buffered peptone water (Difco, BD), and spiral plated onto CHROMagar ECC (DRG International, Inc., Mountainside, NJ) with an Autoplate 4000 spiral plater (Spiral Biotech, Inc., Norwood, MA). The CHROMagar ECC plates were incubated at 37°C for 22 to 24 h, and blue *E. coli* colonies were counted and recorded.

For determination of the presence of *E. coli* O157:H7, the FSM samples in TSB were incubated for 7 h at 37°C and then held at 4°C overnight. After this enrichment incubation, 500 µl of each FSM sample was added to separate sample wells of a deep-well, 96-well block, each containing 500 µl of phosphate-buffered saline with Tween (PBS-Tween; Sigma, St. Louis, MO) and 20 µl of immunomagnetic anti-O157 Dynabeads (Invitrogen Corp., Carlsbad, CA). After 30 min of shaking at room temperature, the beads were magnetically removed from the sample, washed twice in 1 ml of PBS-Tween, and concentrated into 100 µl of PBS-Tween. Fifty microliters of each concentrated sample was spread plated onto CHROMagar O157 (DRG International, Inc.) containing 5 mg/liter novobiocin and 2.5 mg/liter potassium tellurite (ntCHROM). The plates were incubated at 37°C for 22 to 24 h. Presumptive colonies were tested with *E. coli* O157 latex agglutination reagents (Oxoid Ltd., Basingstoke, UK) and confirmed by multiplex PCR for the *E. coli* genes *eaeA*, *slt-I*, *slt-II*, *fliC*, and *rfbE* (27). The *fliC* primer sequences were those reported by Gannon et al. (24). PCR conditions were those of Paton and Paton (38).

The pH of solarized and unsolarized control FSM was measured for samples collected at week 10. Approximately 1 g of FSM sample was placed into a glass tube, and 2 ml of distilled H₂O was added and mixed thoroughly by vortexing. The pH was measured in the diluted FSM with a 6-mm general purpose, gel-filled pH electrode (Broadley-James Corp., Irvine, CA) and a UB-10 pH meter (Denver Instrument, Arvada, CO).

Statistical analyses. *E. coli* concentrations were transformed to log CFU per gram of FSM for statistical analyses. For samples in which *E. coli* concentrations were below the lower limit of detection of 2.3 log CFU/g, the value was set at 2.0 log CFU/g. The number of FSM samples in each plot that were positive for *E. coli* O157:H7 for each sample week were reported as a percentage. The unit of observation was the plot. Least-squares means of bacterial data were analyzed with the general linear model procedure (SAS Institute Inc., Cary, NC). The model included the effects of treatment (solarized versus unsolarized control), time, treatment × time, and plot nested within treatment. Differences were considered significant when *P* values were less than 0.05, and were considered tendencies when *P* values were less than 0.10 but greater than 0.05.

RESULTS AND DISCUSSION

Most *E. coli* O157:H7 preharvest control research efforts have focused on procedures aimed at reducing the prevalence and/or levels of this pathogen that are shed by cattle, including vaccination (34, 42); feeding probiotics or direct-fed microbials (44, 53); and oral administration of bacteriophages, sodium chlorate, or other dietary supplements (5, 13, 14). Although many of these procedures are now available to cattle producers and have shown promise for reducing this pathogen, preharvest interventions that substantially reduce *E. coli* O157:H7 shedding in cattle have

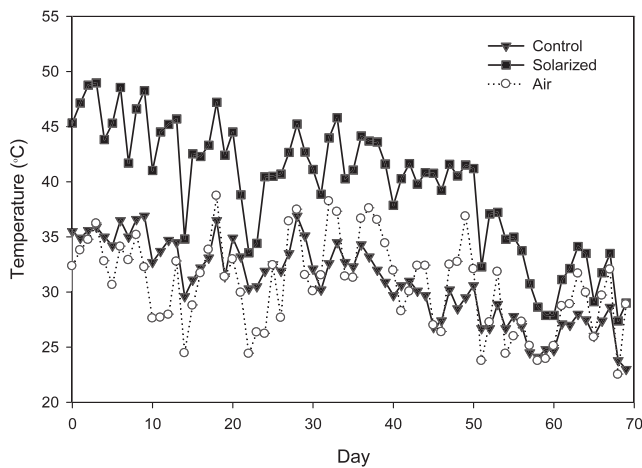


FIGURE 1. Daily peak air temperature and average daily peak temperature (in degrees Celsius) of feedlot surface material (FSM) for control (unsolarized) and solarized plots over time of the study, which was initiated on 6 July 2009, and ended after 70 days on 14 September 2009. For FSM, temperature loggers were placed ca. 7.5 cm below the surface in the center of each plot.

not been clearly demonstrated. As we discussed in a recent review, reducing this pathogen may require reducing both the shedding of *E. coli* O157:H7 by cattle and its survival in manure, thereby breaking its transmission cycle in cattle production (9). Potential interventions to inactivate pathogens in manure on feedlot pen surfaces include treatment with carbonate and alkali (20, 37) or plant essential oils (46, 50). Barriers to the adoption of these or similar antimicrobial treatments of feedlot pens include cost, lack of practical application procedures, and/or a rapid loss of antimicrobial activity, in addition to the need to demonstrate efficacy of these treatments in production-scale studies. In this study, we investigated the potential for soil solarization to reduce manure-borne zoonotic pathogens on the surface of feedlot pens. Soil solarization is used to inactivate a variety of bacterial, fungal, and nematode plant pathogens from soil, typically before planting, of an assortment of crops including food, field, and ornamental crops, and fruit and nut trees (30, 31, 43). Inactivation of these soil pests is primarily achieved by passive solar heating, with additional benefits including the control of certain weeds and insects. The use of soil solarization is thought to be increasing as the use of the chemical fumigant methyl bromide is phased out, and as the growth of organic crop production increases (43). However, only limited work has explored the ability of this technique to eliminate human pathogens or fecal bacteria from soil (6, 52).

The prevalence of *E. coli* O157:H7 shedding by cattle typically is highest during the warmer months of summer and early fall, so the study was initiated in July to increase the probability of finding a feedlot pen surface with high rates of *E. coli* O157:H7 (7). In addition, conducting the study from July to September allowed us to test soil solarization during a period of high temperature and intense solar radiation, when this technique is most effective (30, 31, 43). Figure 1 shows the average daily peak temperature

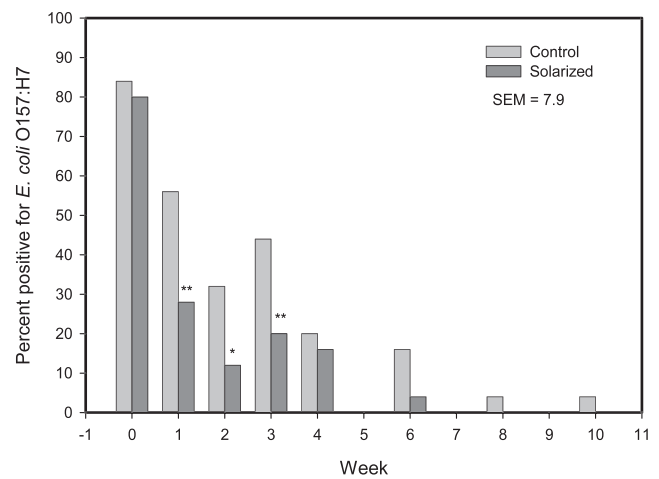


FIGURE 2. Percentage of feedlot pen surface material (FSM) samples positive for *Escherichia coli* O157:H7 for control (unsolarized) and solarized plots at weeks 0, 1, 2, 3, 4, 6, 8, and 10 of the study ($n = 25$). Within week, solarized treatment least-squares means labeled with asterisks are significantly different (** $P < 0.05$) or tend to differ (* $P < 0.10$) from the control least-squares means. The noted SEM is the standard error of the least-squares means.

of FSM for both control (unsolarized) and solarized plots, and the daily peak air temperature over the course of the experiment. Average daily peak temperatures of unsolarized FSM ranged from 23.0 to 36.9°C, with a high individual temperature logger recording of 40.0°C. In contrast, average daily peak temperatures of solarized FSM ranged from 27.4 to 49.0°C, with a high individual temperature logger recording of 57°C. Average daily peak temperatures were $\geq 40.0^\circ\text{C}$ in solarized FSM for 43 days of the 70-day study. On a daily basis, the average difference in peak temperatures between solarized and unsolarized FSM ranged from 3.1 to 14.0°C, with an average difference of 8.7°C over the entire study. These solarized FSM temperatures are similar to values that have been reported for solarized crop soil at similar soil depths (31, 43). Differences in daily peak temperatures of air and FSM (both solarized and unsolarized) became smaller as air temperatures decreased during the late weeks of the study (Fig. 1).

In preliminary screening, 100% of the FSM samples collected in the selected pen were positive for *E. coli* O157:H7, at concentrations that were < 200 CFU/g of FSM. Initial percentages of *E. coli* O157:H7-positive FSM samples at week 0 were 84 and 80% for unsolarized control and solarized FSM, respectively, and did not differ ($P > 0.05$) (Fig. 2). Overall, soil solarization reduced *E. coli* O157:H7 in FSM compared with unsolarized FSM ($P < 0.05$). After 1 week of solarization, the proportion of *E. coli* O157:H7-positive samples was lower ($P < 0.05$) at 28% in solarized FSM, compared with 56% in unsolarized FSM. The percentage of *E. coli* O157:H7-positive samples tended to be lower (12 versus 32%, $P < 0.1$) after 2 weeks, and was lower (20 versus 44%; $P < 0.05$) after 3 weeks of solarization. The percentages of *E. coli* O157:H7-positive samples for both solarized and unsolarized FSM decreased during the study; however, *E. coli* O157:H7 was no longer

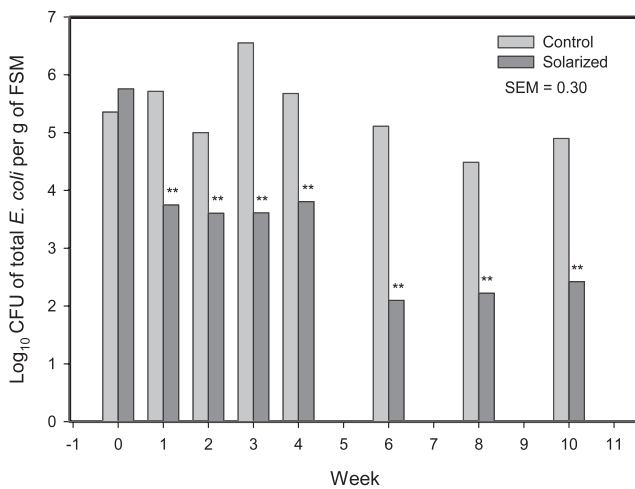


FIGURE 3. *Escherichia coli* concentrations (in log CFU per gram) in feedlot pen surface material (FSM) for control (unsolarized) and solarized plots at weeks 0, 1, 2, 3, 4, 6, 8, and 10 of the study ($n = 25$). The lower limit of detection was 2.3 log CFU/g of FSM. Within week, solarized treatment least-squares means labeled with asterisks are significantly different (** $P < 0.05$) or tend to differ (* $P < 0.10$) from the control least squares means. The noted SEM is the standard error of the least-squares means.

detectable in solarized FSM by week 8 of solarization, but was still detected in unsolarized FSM at week 10.

Total *E. coli* concentrations in FSM are shown in Figure 3. At week 0, *E. coli* concentrations were 5.76 and 5.35 log CFU/g in solarized and unsolarized control FSM, respectively, and were not different ($P > 0.05$). One week of solarization decreased *E. coli* in solarized FSM by 2.0 log CFU/g ($P < 0.05$). Populations of *E. coli* remained at levels of ca. 3.6 to 3.8 log CFU/g in solarized FSM through week 4, before dropping an additional 1.7 log CFU/g ($P < 0.05$), to levels near or below the lower limit of detection of 2.3 log CFU/g of FSM by week 6. *E. coli* was reduced below detectable levels in 23 of the 25 solarized FSM samples collected on week 6. In contrast, *E. coli* concentrations remained at high levels ($P > 0.05$) in unsolarized FSM during the 10-week study period (Fig. 3). In fact, the only significant change in *E. coli* levels in unsolarized FSM ($P < 0.05$) was a 1.5-log CFU/g increase observed at week 3 of the study, which could have occurred in response to rainfall events occurring during the preceding week, which contributed a total of 2.77 cm of precipitation (Table 1). No rain fell during the first week of the study, and 0.89 cm of rain fell during the second week. Our previous work demonstrated that *E. coli*, including *E. coli* O157:H7, can multiply in feedlot soil if the moisture content is adequate and the soil environment is aerobic (8). However, rainfall ample enough to produce anaerobic, fermentative conditions in the FSM can inhibit growth or reduce viable *E. coli* populations (8).

Our results are similar to those reported by two works studying soil solarization as a means of reducing fecal bacteria from manure-amended soil. Barbour et al. (6) examined the impact of soil solarization to reduce bacteria in greenhouse soil amended with chicken manure. After

TABLE 1. Rainfall before and during the solarization study period

Wk of solarization	Precipitation (cm) ^a
0	0.00
1	0.00
2	0.89
3	2.77
4	1.75
5	0.76
6	0.81
7	0.18
8	6.43
9	0.13
10	7.19

^a Total precipitation during the preceding week.

6 weeks of solarization, fecal coliform counts were reduced by 92.6% (1.1-log reduction) in the treated soil. In recent work, Wu et al. (52) inoculated a laboratory-grown nonpathogenic bovine strain of *E. coli* into cow manure and then incorporated this manure into the soil of an open field. Soil solarization reduced initial *E. coli* concentrations of 5.30 log CFU/g of dry soil by 2.5 log in 1 week, and *E. coli* was not detected by enrichment and plating after 4 weeks of solarization (52).

Soil solarization has been most successfully applied in regions of the world that have high summer air temperatures and intense solar radiation (30, 31, 43). Durations of 4 to 6 weeks typically are used to achieve adequate heating for the control of soilborne plant pathogens, nematodes, and weeds. However, longer solarization time may be needed for different seasons and/or geographic locations (39). This study was designed to examine weekly solarization time up to 10 weeks. The study site is located at 40.6°N latitude, 98.2°W longitude, and has an average elevation of 562 m; our region has a climate that is described as a transition zone between semi-humid and semi-arid climates (28). While we anticipated that air temperature and solar intensity would be adequate for effective solarization, the feedlot pen on which the study was conducted has a 4% descending slope from south to north, which may have reduced the intensity of solar irradiation at the soil surface compared with a level plot. In addition, the surface of a cattle feedlot pen is quite different from that of a crop plot in terms of composition and compaction, and can have a large load of the target microorganism *E. coli* (25). With regard to reducing populations of *E. coli* from the FSM, there was no additional benefit to extending solarization time beyond 6 weeks (Fig. 3). However, 1 FSM sample among 25 was positive for *E. coli* O157:H7 after 6 weeks of solarization, and no *E. coli* O157:H7 was recovered from any FSM samples after 8 weeks of solarization, suggesting that extended solarization time may be needed for more thorough reduction of this organism.

Because pathogen inactivation by solarization is more effective when the soil is wet, soil typically is watered prior to covering with the polyethylene sheets (30, 31, 43). During the 3 weeks preceding the solarization experiment,

rainfall events occurred that totaled 5.77 cm. In addition, moisture in feces and urine was added to the feedlot pen surface on a continual basis by the cattle that occupied the pen. For these reasons, we chose not to wet the pen surface further; however, additional water may have further enhanced or hastened *E. coli* reduction in the solarized FSM.

While thermal destruction is the primary means of reducing soil pests during solarization, additional mechanisms contribute to the inactivation of pathogens. The anaerobic or microaerobic conditions that can be produced in moist soil under the polyethylene plastic film can reduce or inhibit some microorganisms (30, 43). In addition, the biological changes that occur in solarized soil could cause shifts in the microbial ecology in favor of antagonists of certain pathogens (30, 43). Furthermore, the decomposition of organic materials during solarization can result in an accumulation of volatile antimicrobial compounds in soil (23, 30, 43). The generation of ammonia from decomposing animal manure and other organic material during solarization has been reported to improve control of plant pathogens in soil (23, 30, 39, 43), and manure decomposition may have played a role in *E. coli* inactivation during solarization of FSM in the current study. The average pH of solarized FSM at week 10 was 8.05, and was higher ($P < 0.05$) than the unsolarized FSM pH of 7.66 (data not shown). We did not measure ammonia concentrations in the FSM; however, the higher pH suggests that ammonia may have accumulated to toxic levels in the solarized FSM.

Results of this study suggest that soil solarization can be used to reduce *E. coli* O157:H7 on feedlot pen surfaces. Further work is needed to determine if reducing this pathogen on the feedlot surface can also reduce its transmission in the cattle production environment, thereby reducing *E. coli* O157:H7 prevalence in cattle. As reviewed by Stapleton (43), disadvantages of soil solarization in crop production include the confinement of its use to the warmer seasons of the year, the removal of land from production for a period of several weeks, and the costs associated with the purchase and disposal of the plastic film. These same disadvantages would also be drawbacks of soil solarization in cattle production scenarios. However, the effectiveness of this technique to inactivate *E. coli* O157:H7 in feedlot soil suggests other applications of soil solarization for reducing the risk of foodborne and waterborne illnesses. An environmental survey of soil, water, animal feces, and plants for *E. coli* O157:H7 in a major leafy greens production region in California identified surface water as a vehicle of transmission of this pathogen, and linked the increased incidence of *E. coli* O157:H7 to heavy rain events and flooding (19). Soil solarization may be an option for treating produce fields compromised by flooding or by other means, such as amendment with untreated manure. Reducing *E. coli* O157:H7 from this soil would not only reduce the risk for pathogen transmission to the food crop, but also reduce the risk for further transmission by runoff. Thus, soil solarization is a potential preharvest control strategy not only for reducing the transmission of *E. coli* O157:H7 among cattle, but also for reducing its transmis-

sion to food crops, water, and the environment. As a final note, the ability of soil solarization to reduce *E. coli* O157:H7 suggests this technique may be effective for reducing other human and zoonotic pathogens such as *Salmonella*, *Campylobacter*, or *Mycobacterium* spp. from feedlot surfaces and crop soil, although further work is needed for confirmation.

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