Applied and Environmental Microbiology, July 2011, p. 5044–5049 Vol. 77, No. 14
0099-2240/11/$12.00 doi:10.1128/AEM.02940-10

Evaluation of Physical Coverings Used To Control *Escherichia coli* O157:H7 at the Compost Heap Surface▽†

Marion W. Shepherd, Jr.,[1] Jinkyung Kim,[2] Xiuping Jiang,[2]*
Michael P. Doyle,[3] and Marilyn C. Erickson[3]

Department of Biological Sciences, Clemson University, Clemson, South Carolina 29634-0314[1]; Department of Food, Nutrition, and Packaging Sciences, Clemson University, Clemson, South Carolina 29634-0316[2]; and Center for Food Safety, The University of Georgia, Griffin, Georgia 30223-1797[3]

Received 16 December 2010/Accepted 17 May 2011

Throughout four field trials, compost heaps covered with finished compost maintained temperatures under the physical covering that were ca. 7 to 15.5°C higher, resulting in rapid *Escherichia coli* O157:H7 reduction, than those of the heaps covered with fresh straw or left uncovered. Our results validated recommendations made by the U.S. Environmental Protection Agency for covering fresh compost.

Compost is an excellent soil amendment that has been widely used by both organic and conventional farming worldwide. Composting is a biological decomposition process driven by microbial activities. The elevated temperatures within the compost heap are generally regarded as the most important factor contributing to pathogen abatement in compost. Our previous studies have demonstrated that active composting can be very effective in achieving a 6-log reduction of artificially inoculated pathogens (regular or stress-adapted cultures) inside static composting heaps within 3 weeks of composting, even without turning of the heaps (17), results which are in agreement with several other studies (7, 8, 9, 10, 13). However, results of field studies revealed that pathogenic *Escherichia coli* may experience extended survival at the compost surface and on the periphery of the heaps when unturned (5, 16) or inside the heaps under mesophilic conditions (17).

Peripheral locations of the heap may not reach temperatures necessary for pathogen destruction. For static aerated composting, the use of insulating materials has been recommended to keep compost temperatures up and to help reduce odor emissions from a compost pile (2, 19). Physical coverings become more critical if the outdoor temperature is low, since the heat from the composting heaps can dissipate rapidly. The lack of exposure to elevated temperatures may be a major contributing factor to the persistence of human pathogens at the compost surface. Curtis et al. (4) and Brito et al. (1) both suggest that temperatures achieved during composting with physical coverings such as an insulated pool tarp or a permeable polypropylene covering are high enough to inactivate pathogens. Most research is centered on the application of insulators to prevent the release of ammonia and/or greenhouse gases (3, 6, 11, 18). However, few data are available on how a physical covering, especially common farm materials such as finished compost (FC) or straw, might affect pathogens at the compost surface. Therefore, it is important to develop and validate practical strategies to inactivate pathogens on the surfaces of compost heaps which are not maintained through frequent turning.

The goal of our study was to evaluate the use of common farm materials as physical coverings as a practical method to enhance *E. coli* O157:H7 inactivation at the compost surface.

Field study setup. Four composting trials were performed during the indicated time periods: trial 1, April to August 2008; trial 2, August to December 2008; trial 3, June to August 2009; and trial 4, February to April 2010. In each trial, the composting mixture consisted of a sawdust-cow manure mixture, waste feed, old straw, and fresh straw at a ratio of ca. 8.5:3:3:1. The compost ingredients were mixed thoroughly with the use of a front-end loader and then divided equally into four heaps. Two compost heaps were bisected with clear Palruf polyvinyl chloride (PVC) (Palram Industries Ltd., Kutztown, PA) and covered with either 15 or 30 cm of FC. Half of each heap was covered with FC with a moisture content (MC) of 30 or 50%. One compost heap was covered with ca. 13 cm of fresh straw and held in place with weighted nylon net. The final compost heap was uncovered, serving as the control heap. In trials 3 and 4, an additional uncovered heap was constructed and was turned on days 3, 7, 14, 21, and 30; this served as the turned control heap.

The compost covering treatments were applied to the heaps, and the 15- and 30-cm coverings of FC increased the sizes of the heaps (Fig. 1). The compost samples (described below) were placed in mesh bags, which were secured onto the surface of the compost heaps and then covered with FC or straw.

Compost sample preparation, sampling schedule, and temperature measurements. Three strains of avirulent green fluorescent protein (GFP)-labeled *E. coli* O157:H7 (strain B6914, provided by Pina Fratamico, U.S. Department of Agriculture [USDA], Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA; and strains CV2b7 and 6980-2, provided by Michael P. Doyle, The University of Georgia Center for Food Safety, Griffin, GA) were used as surrogates for pathogenic *E. coli* O157:H7. Avirulent strains were used in this

* Corresponding author. Mailing address: 217 P&A Bldg., Department of Food, Nutrition, and Packaging Sciences, Clemson University, Clemson, SC 29634 Phone: (864) 656-6932. Fax: (864) 656-0331. E-mail: xiuping@clemson.edu.

† Supplemental material for this article may be found at http://aem.asm.org/.

▽ Published ahead of print on 27 May 2011.

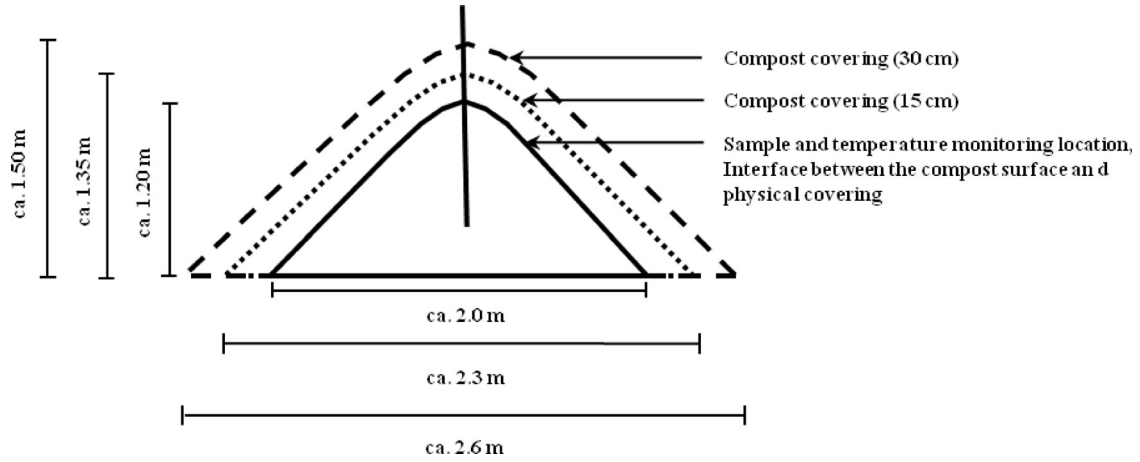

FIG. 1. Schematic depicting dimensions of compost heaps covered with finished compost.

study because the use of virulent strains is prohibited under field conditions, due to the risk of escape into the environment. The compost samples were prepared using a previously described method (17). Approximately 150 g of the inoculated compost mixture was inserted into Tyvek self-seal pouches (8.89 by 13.33 cm; DuPont, Wilmington DE) to be placed under the compost coverings, whereas ca. 150 g of the sample mixture was placed into polystyrene trays with perforated bottoms to be anchored atop of the uncovered heaps, serving as the experimental controls.

Compost samples were obtained at 0, 1, 3, 5, 7, 14, 21, 30, and 60 days after heap construction. In trials 1 and 2, an additional sampling day occurred on day 120 after the onset of composting. Using an OT-21 temperature and oxygen sensor (Demista Instruments, Arlington Heights, IL), heap temperatures and oxygen contents were monitored and recorded at the center of each heap and at each sample location (the interface where the surface of the newly formed compost heap met the FC or straw being used as the physical covering) every day for the first 2 weeks of composting and every sampling day thereafter.

In trial 4, a soil corer with a 30-cm-sample retaining auger (Oakfield Apparatus, Inc., Oakfield, WI) was used to obtain samples of the FC coverings on day 60 of the trial, so that changes in the moisture content of the covering could be recorded. Calculations of the bulk density for the compost used as coverings in trial 4 were performed using guidelines published by the Washington State University Puyallup Research Center (20).

Detection of *E. coli* O157:H7 and *Enterobacteriaceae* in the compost samples. Twenty-five grams of each compost sample was homogenized in 225 ml of universal preenrichment broth (UPB; Acumedia Manufacturers, Inc., Lansing, MI) and serially diluted in 0.85% saline. The methods used for the detection of *E. coli* O157:H7 through direct plating and enrichment were previously described by Shepherd et al. (17). Green fluorescent colonies grown on tryptic soy agar (TSA) (Becton Dickinson, Sparks, MD) supplemented with 100 μg/ml ampicillin (Sigma Chemical Co., St. Louis, MO) (TSA-A) were confirmed as *E. coli* O157:H7 by using the *E. coli* O157 latex agglutination test kit (Oxoid, Basingstoke, Hampshire, United Kingdom). Isolates ($n = 18$) detected in the surface samples in each of the four trials on the final sampling day of the trial were picked, purified, and preserved in tryptic soy broth (TSB) with 20% glycerol at −80°C for pulsed-field gel electrophoresis (PFGE) analysis.

For *Enterobacteriaceae* enumeration, molten violet red bile glucose agar (VRBGA) (Becton Dickinson, Sparks, MD) at 50°C was mixed well with 1 ml of each sample homogenate in sterile petri dishes. After the agar solidified, the VRBGA plates were incubated at 37°C for 24 h. All microbiological analyses were performed in duplicate.

Statistical analyses. Bacterial populations were compared between each sample location on each sampling day. Duplicate temperature recordings from each location in each trial were analyzed by using the PROC MIXED procedure, whereas bacterial populations were analyzed using the PROC GLM procedure. All calculations were performed using the Statistical Analysis System (SAS 2001, Cary, NC) as previously described (17).

Compost heap construction and initial parameters. Four composting trials were performed: two in 2008, one in 2009, and one in 2010. Ambient temperatures, including highs and lows, ranged from 8 to 32°C, 0 to 32°C, 18 to 33°C, and 0 to 23°C in trials 1, 2, 3 and 4, respectively.

The initial parameters of the compost heaps are presented in Table 1. Based on compost guidelines published by the National Organic Standards Board (12), the carbon-to-nitrogen (C/N) ratios of the heaps were acceptable for composting as all were between the prescribed 15:1 to 60:1 range. In trials 1 to 3, the moisture contents of the compost were in the acceptable range for composting, whereas in trial 4, the moisture content was slightly too high to be considered acceptable for composting (Table 1). In addition, the compost mixture of each trial was negative for the presence of *E. coli* O157:H7, even after enrichment culture.

Temperatures and sample moisture contents at the surfaces under the physical coverings and inside the compost heaps. In trials 1 and 4, temperatures at the interface of the compost heaps and the coverings were different ($P < 0.05$) under all five covering treatments. In trial 2, temperatures under the FC of the 15 cm-50% MC (thickness of the covering-moisture con-

TABLE 1. Initial abiotic and biotic parameters of the composting heaps[a]

Trial	Trial dates	C/N ratio	Moisture content (%)	Mesophiles (log CFU/g)	Thermophiles (log CFU/g)	*E. coli* O157:H7[b]
1	April to August 2008	19.41 ± 1.39	63.16 ± 3.04	7.84 ± 0.02	7.49 ± 0.18	−
2	August to December 2008	15.74 ± 0.59	63.57 ± 1.40	7.66 ± 0.05	7.07 ± 0.04	−
3	June to August 2009	24.81 ± 0.66	60.14 ± 0.85	7.80 ± 0.07	7.33 ± 0.06	−
4	February to April 2010	27.24 ± 1.92	67.87 ± 0.72	7.71 ± 0.14	7.06 ± 0.12	−

[a] Values are means ± standard deviations.
[b] −, sample was negative after enrichment culture.

tent) and 30 cm-30% MC and of the 15 cm-50% MC and 30 cm-50% MC cover treatments were not different, with *P* values of 0.075 and 0.432, respectively; the results for all other treatments in trial 2 were different. In trial 3, the 15 cm-30% MC and 15 cm-50% MC covering treatments were the only ones that were not different from each other ($P = 0.8291$). In each composting trial, the internal location of the compost heaps reached the thermophilic phase, achieving temperatures of ≥50°C for 8 to 12 days (Table 2). Importantly, many of these days occurred within the initial 2 weeks of composting.

The moisture contents under the coverings in each trial increased during certain periods between days 3 to 60 in trials 1 and 2 and between days 1 to 60 in trials 3 and 4 (see Table S1 in the supplemental material). With a few exceptions, most of the covered samples maintained elevated moisture contents during the trials, and in some samples the final moisture content was higher than the initial moisture of the compost mixture, which was most likely due to the condensation which accumulated inside the sampling bags. In each trial, the samples at the compost surface of the control heaps exhibited the most variation in moisture contents during composting. This is because these samples were exposed to periods of desiccation and precipitation.

TABLE 2. Combined internal and interface temperature data during composting

Trial	Treatment			Interface[b]		Internal	
	Type[a]	Thickness (cm)	Cover moisture (%)	Max temp (°C)	No. of days with temp ≥45°C	Max temp (°C)	No. of days with temp ≥50°C
1	Ctrl	0	N/A	33	0	55	8
	Hay	12.5	9	41	0	55.5	8
	FC	15	30	48	6	55.5	9
		15	50	42.5	0	55.5	9
		30	30	41	0	53	9
		30	50	40	0	53	9
2	Ctrl	0	N/A	37	0	57.5	12
	Hay	12.5	9	45	3	56.5	12
	FC	15	30	50	13	56	12
		15	50	47	6	56	12
		30	30	48.5	9	56.5	12
		30	50	47.5	6	56.5	12
3	Ctrl	0	N/A	37	0	52.5	9
	Hay	12.5	9	38.5	0	51.5	9
	FC	15	30	46	5	52	9
		15	50	46	6	52	9
		30	30	46	6	52	10
		30	50	48	8	52	10
4	Ctrl	0	N/A	27	0	56	10
	Hay	12.5	9	27.5	0	55.5	11
	FC	15	30	46	6	57	10
		15	50	48.5	10	57	10
		30	30	50.5	7	56	10
		30	50	52.5	9	56	10

[a] Ctrl, control; FC, finished compost.
[b] Where the surface of the newly formed compost heap met the FC or straw being used as the physical covering.

Determination of the moisture content and bulk density of the FC used in trial 4 revealed that the FC covering with 50% moisture had a much larger bulk density than the FC covering with 30% moisture (see Table S2 in the supplemental material). Also, during 60 days of field composting, the FC applied to the heaps at a thickness of 30 cm retained its moisture near the interface with the newly formed compost heaps better than the FC applied at a thickness of 15 cm, regardless of the initial moisture content. Additionally, there was a moisture gradient observed, with the highest value at the base and the lowest at the surface of the physical covering.

Survival of *E. coli* O157:H7 at the surface or interface of compost heaps. Initial *E. coli* O157:H7 inoculation levels were in the range of 7.1 to 7.8 log CFU/g in the four composting trials (Fig. 2A to D). Under the straw covering, the populations of *E. coli* O157:H7 were reduced below the detection limit within 120, 7, 30, and 60 days of composting in trials 1, 2, 3, and 4, respectively. In samples under FC treatments with 30% moisture, *E. coli* O157:H7 was below the limit of detection within 21, 5, 21, and 14 days when the FC covering was 15 cm thick in trials 1, 2, 3, and 4, respectively, compared to 21, 5, 21, and 7 days when the FC covering thickness was 30 cm. *E. coli* O157:H7 fell below the detection limit within 120, 7, 21, and 14 days in trials 1, 2, 3, and 4, respectively, when the FC covering with 50% moisture was 15 cm thick, compared to 120, 7, 14, and 7 days, respectively, when the cover was 30 cm thick.

E. coli O157:H7 was inactivated more rapidly in FC coverings of the same thickness with 30% MC than with 50% MC in trials 1 and 2, which was evident after observing the slope of the log-linear portions of the survival curves (see Table S3 in the supplemental material). During trials 3 and 4 using FC coverings with the same moisture contents, coverings that were 30 cm thick performed slightly better than those 15 cm thick in terms of the *E. coli* O157:H7 inactivation rate, with the exception being the results between the 15 cm-50% MC and 30 cm-50% FC coverings in trial 3, as the slopes of the log-linear portions of those survival curves were −2.001 and −1.232, respectively. When reductions in bacteria are observed in the samples covered with straw, it can be seen that the inactivation rate of *E. coli* O157:H7 was the lowest of all the covered

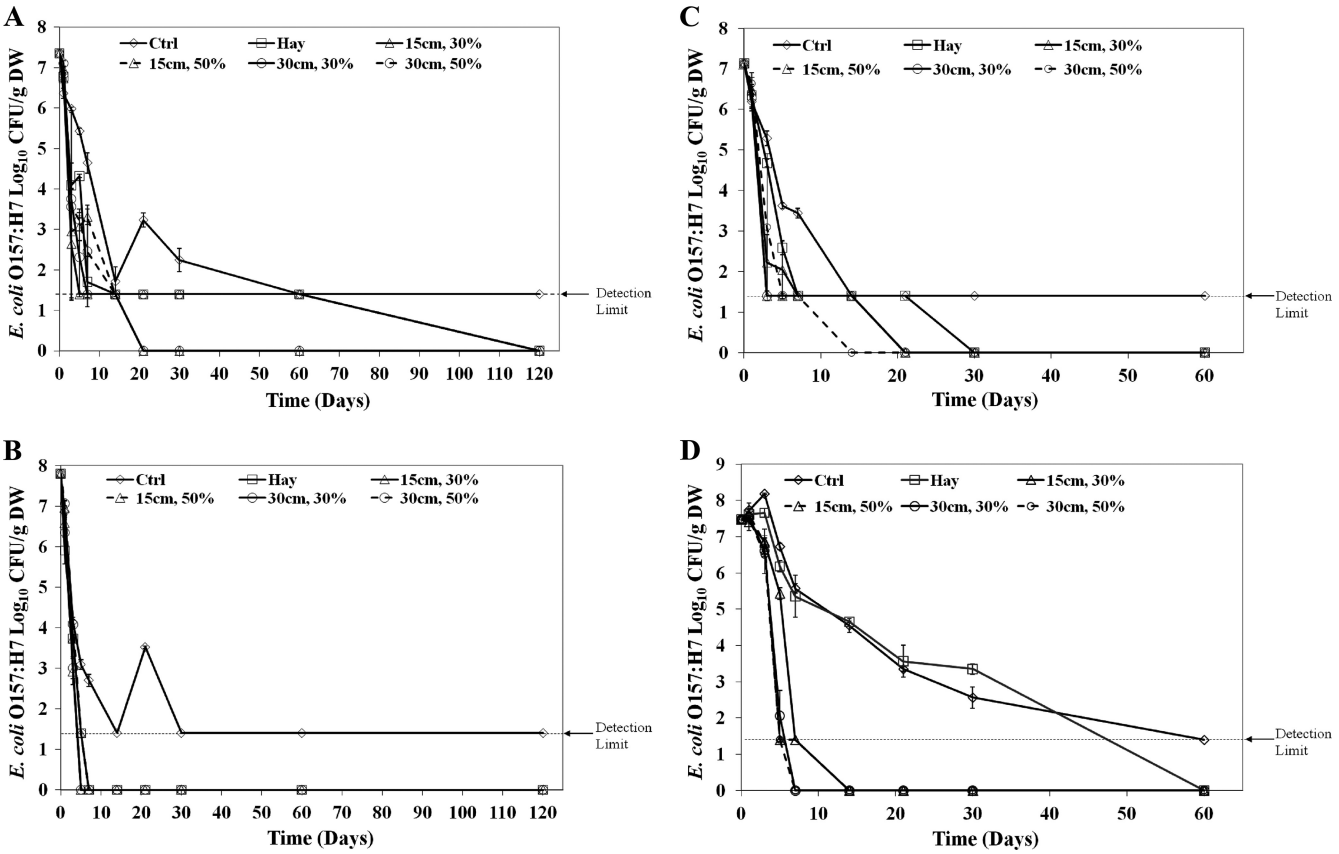

FIG. 2. Survival of *E. coli* O157:H7 at the interface between the physical coverings and newly formed composting heaps in trial 1 (A), trial 2 (B), trial 3 (C), and trial 4 (D). Each data point is the average of four replicates.

samples in each trial. Importantly, in the control (uncovered) samples, *E. coli* O157:H7 was detected after enrichment culture in the samples on the final sampling day in each trial (day 120 in trials 1 and 2 and day 60 in trials 3 and 4), regardless of whether the heaps were turned. In trials 3 and 4, an uncovered heap which was turned was used as a secondary control; however, the data collected on pathogen survival were not significantly different from those of the unturned control heap (data not shown).

Additionally, using PFGE (14), we identified *E. coli* O157:H7 strain B6914 as the predominant strain with extended survival at the surface of the uncovered heap (data not shown).

***Enterobacteriaceae* populations in compost surface samples.** Initial populations of *Enterobacteriaceae* were in the range of ca. 7.4 to 8.0 log CFU/g over the four composting trials (Table 3). In the control heaps, the populations of *Enterobacteriaceae* were gradually reduced ca. 2.6 to 3 log CFU/g in the surface samples over all trials on the final sampling day. Regrowth occurred soon after precipitation events in the control samples in all trials. On the final sampling day over the four trials, the populations of *Enterobacteriaceae* ranged from ca. 4.6 to 5.1 log CFU/g in the control surface samples. Reductions of the indicator bacteria in straw-covered heap samples in trials 1, 3, and 4 generally followed the same trend as those observed in the control samples (data not shown).

Except in trial 1, the FC coverings were shown to reduce *Enterobacteriaceae* populations at least 5 logs within 7 days (see Table S4 in the supplemental material). However, on some sampling dates in trials 1, 3, and 4, regrowth of *Enterobacteriaceae* was observed in samples under at least one of the cov-

TABLE 3. Survival of *Enterobacteriaceae* in uncovered compost heap samples

Trial[a]	No. of *Enterobacteriaceae* (log CFU/g of compost) after indicated days of composting									
	0	1	3	5	7	14	21	30	60	120[b]
1	7.93 ± 0.00	7.70 ± 0.03	7.25 ± 0.10	6.88 ± 0.15	6.83 ± 0.17	5.67 ± 0.04	4.20 ± 0.13	5.01 ± 0.03	4.53 ± 0.05	5.13 ± 0.10
2	8.00 ± 0.00	6.96 ± 0.05	6.07 ± 0.03	7.10 ± 0.03	7.13 ± 0.14	6.53 ± 0.05	7.94 ± 0.07	4.97 ± 0.03	4.65 ± 0.07	5.03 ± 0.10
3	7.68 ± 0.00	6.22 ± 0.14	6.23 ± 0.26	5.15 ± 0.22	5.13 ± 0.04	3.03 ± 0.09	4.35 ± 0.25	4.35 ± 0.13	5.11 ± 0.25	NA
4	7.64 ± 0.00	7.76 ± 0.11	8.18 ± 0.02	7.10 ± 0.10	6.71 ± 0.10	6.74 ± 0.20	6.37 ± 0.26	6.60 ± 0.30	4.62 ± 0.20	NA

[a] Greater than 5 mm of precipitation occurred on days 2, 22, 32, 45, 64, 91, 115, and 116 of composting in trial 1, on days 10, 19, 20, 22, 62, 77, and 117 in trial 2, on days 2, 3, 19, and 54 in trial 3, and on days 1, 10, 19, 27, 28, 29, 38, 45, and 56 in trial 4.

[b] NA, not applicable.

TABLE 4. *P* values with potential correlation between *E. coli* O157:H7 and *Enterobacteriaceae* populations in compost samples

Treatment[a]	Treatment thickness (cm)	Treatment MC (%)	*P* value for survival in composting trial[b]:			
			1	2	3	4
Ctrl	0	NA[c]	0.0155	0.1794	0.0027	<0.001
Hay	12.5	9	0.1007	0.8014	0.0111	<0.001
FC	15	30	0.2800	0.8363	0.7390	0.7672
	15	50	0.6012	0.9076	0.2124	0.7725
	30	30	0.1463	0.8774	0.4051	0.0908
	30	50	0.1050	0.9351	0.5106	0.7311

[a] Ctrl, control; FC, finished compost.
[b] A *P* of >0.05 indicates that there was no significant difference between the survival of *Enterobacteriaceae* and that of *E. coli* O157:H7.
[c] NA, not applicable.

ering treatments (data not shown). In each trial, an analysis of covariance was performed comparing the populations of *E. coli* O157:H7 to total *Enterobacteriaceae* using an α of 0.05 (Table 4). The results revealed that the two populations were different in trial 1 ($P = 0.0155$), trial 3 ($P = 0.0027$), and trial 4 ($P < 0.0001$), but not in trial 2, in the uncovered (control) samples. In the samples covered with straw, populations of *E. coli* O157:H7 and *Enterobacteriaceae* were different only in trials 3 ($P = 0.0111$) and 4 ($P < 0.0001$). Importantly, populations of the two groups were not different in any of the samples covered with FC during the four trials conducted during this study ($P > 0.05$).

Due to the heterogeneous nature of the composting process, methods that are implemented in composting need to be optimized to enhance pathogen inactivation. Composting using windrows or static aerated heaps usually involves the addition of oxygen in the composting process, either by physically turning the heaps with machinery or by forcing air into the heaps via perforated PVC piping. However, many small farms may not have the resources to maintain their compost heaps through turning or aeration using forced air. As such, pathogens, especially on the compost heap surface, may remain viable in the compost materials that are exposed to ambient temperatures, as has been demonstrated in previous studies (5, 16, 17).

The application of a physical covering to compost involves methods that have been evaluated but not for the control of pathogens at the compost surface. Several studies have investigated how covering compost heaps with a physical covering (synthetic or natural, permeable or nonpermeable) affects the emissions of ammonia and greenhouse gases (3, 6, 11, 18). The U.S. Environmental Protection Agency (EPA) suggests that covering heaps can control odors and maintain high temperatures throughout the compost heaps (19). The California Department of Resources Recycling and Recovery (CDRRR) suggests that 6 to 12 in. (15 to 30 cm) of insulating materials be applied to static aerated composting heaps, and California's Leafy Green Marketing Agreement (LGMA) suggests that static aerated composting heaps should be covered with at least 12 in. of insulating materials (2). In spite of these assertions by these important governmental and trade agencies, there is a dearth of published scientific evidence demonstrating that the use of a physical covering on compost heaps results in pathogen inactivation.

A few studies have suggested that the application of physical coverings to composting heaps could create conditions suitable for the elimination of pathogens. Curtis et al. (4) and Brito et al. (1) both suggest that at locations within the heap, temperatures achieved during composting with a physical covering applied to the heap (an insulated pool tarp and a permeable polypropylene covering, respectively) would be elevated to levels suitable for pathogen elimination (≥55°C). This is a plausible assertion, as heat escapes from compost heaps through convection and radiation, and the use of an insulator would aid in trapping heat at the compost surface. However, temperature data at the interface where the physical covering meets the surface of the newly formed heap were not reported in either of the previously mentioned studies. As reported from our field compost trials, temperature elevation beneath the FC coverings ranged from 7 to 15.5°C throughout the study. Wu et al. (21) investigated the fate of *E. coli* in uncovered soil and soil covered with transparent polyethylene sheets. That study found that covering the soil increased soil temperatures and that *E. coli* fell below the detectable limit after enrichment (<0.08 CFU/g dry weight) after 4 weeks, whereas 2 log CFU/g (dry weight) was present in uncovered soil after 6 weeks. In that study, the daily high temperature in the covered soil was at least 40°C for 8 days in the second and third weeks of the experiment, whereas soil temperatures were at or below 35°C throughout the experiment with soil that was not covered.

The ideal physical covering will maintain the heat generated by microorganisms at the compost surface while allowing an adequate amount of oxygen to permeate in order to prevent the creation of anaerobic conditions. In the current study, we used finished compost (FC) and fresh straw as the physical coverings, as they are materials that may be readily available on farms that compost wastes and are inexpensive for the farmers. Based on the results from the four trials with five covering treatments, the FC that had a 30% moisture content and that was 30 cm thick resulted in the most rapid reduction of *E. coli* O157:H7 bacteria at the interface between the physical covering and the newly formed compost heap surface, whereas straw was the least effective in rapid pathogen inactivation, except in trial 2, out of all covering treatments. Although the maximum temperatures at the interface between the newly formed compost heaps and physical coverings were less than 55°C, the high moisture content in the samples maintained by the physical coverings may have contributed to the inactivation of *E. coli* O157:H7. It is also possible that competitive activity among microbial populations which were naturally present in the compost material contributed to the decline of *E. coli* O157:H7 in the samples.

Another important aspect of this study is the revelation that *Enterobacteriaceae* survival was correlated with the survival of *E. coli* O157:H7 when FC was used as the covering, as this suggests that *Enterobacteriaceae* could be used as suitable indicator microorganisms for *E. coli* O157:H7. This is significant, because it shows that simple enumeration can be performed and that reliable results can be elucidated regarding the presence or absence of this pathogen at the compost surface. Underscoring the importance of validating the use of physical coverings as treatment methods for the compost surface are

data revealing that *E. coli* O157:H7 remained viable in the uncovered samples on the compost surface for at least 2 months, in agreement with previous studies (5, 16, 17).

Our data suggest that the application of a physical covering to newly formed composting heaps can accelerate the reduction of *E. coli* O157:H7 when composting under all seasonal conditions. Moreover, the use of finished compost with a moisture content of 30% and at a thickness of 30 cm is most effective in reducing *E. coli* O157:H7 at the compost surface, as a ca. 7 log CFU/g (dry weight) reduction occurred within 21 days from the onset of composting, whereas straw was the least effective covering used in the study. Data in our study also revealed that *Enterobacteriaceae* are suitable indicators for *E. coli* O157:H7 when FC is used as the covering. Based on the data presented, covering the newly formed compost heaps with FC may be a practical approach for small-scale composting in cold climates, where temperature elevation is compromised.

We thank Steve Waggoner, Ricky Tingle, and Ronnie Ducworth of the LaMaster Dairy Farm at Clemson University for assisting with the collection of materials for, and the construction of, the compost heaps.

This research was supported by a grant from the USDA-NIFSI.

REFERENCES

1. **Brito, L. M., J. Coutinho, and S. R. Smith.** 2008. Methods to improve the composting process of the solid fraction of dairy cattle slurry. Bioresour. Technol. **99:**8955–8960.
2. **California Leafy Green Products Handler Marketing Agreement (LGMA).** 2010. Commodity specific food safety guidelines for the production and harvest of lettuce and leafy greens. http://www.wga.com/DocumentLibrary/scienceandtech/California%20GAPs%20-%20metrics%20011210%20_1.pdf. Accessed 16 April 2010.
3. **Chadwick, D. R.** 2005. Emissions of ammonia, nitrous oxide and methane from cattle manure heaps: effect of compaction and covering. Atmos. Environ. **39:**787–799.
4. **Curtis, M. J., W. A. Kleiner, V. P. Claassen, and R. A. Dahlgren.** 2005. Differences in a composted animal waste and straw mixture as a function of three compost methods. Compost Sci. Util. **13:**98–107.
5. **Fremaux, B., S. Raynaud, L. Beutin, and C. Vernozy-Rozand.** 2006. Dissemination and persistence of Shiga toxin-producing *Escherichia coli* (STEC) strains on French dairy farms. Vet. Microbiol. **117:**180–191.
6. **Hansen, M. N., K. Henriksen, and S. G. Sommer.** 2006. Observations of production and emission of greenhouse gases and ammonia during storage of solids separated from pig slurry: effects of covering. Atmos. Environ. **40:**4172–4181.
7. **Jiang, X. P., J. Morgan, and M. P. Doyle.** 2003. Fate of *Escherichia coli* O157:H7 during composting of bovine manure in a laboratory-scale bioreactor. J. Food Prot. **66:**25–30.
8. **Jiang, X. P., J. Morgan, and M. P. Doyle.** 2003. Thermal inactivation of *Escherichia coli* O157:H7 in cow manure compost. J. Food Prot. **66:**1771–1777.
9. **Lemunier, M., et al.** 2005. Long-term survival of pathogenic and sanitation indicator bacteria in experimental biowaste composts. Appl. Environ. Microbiol. **71:**5779–5786.
10. **Lung, A. J., et al.** 2001. Destruction of *Escherichia coli* O157:H7 and *Salmonella* Enteritidis in cow manure composting. J. Food Prot. **64:**1309–1314.
11. **Maeda, K., R. Morioka, and T. Osada.** 2009. Effect of covering composting piles with mature compost on ammonia emmission and microbial community structure of composting process. J. Environ. Qual. **38:**598–606.
12. **National Organic Standards Board.** 2002. Compost Task Force Recommendations, as amended by the NOP. http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=stelprdc5058852. Accessed 19 January 2010.
13. **Pourcher, A. M., et al.** 2005. Decrease of enteric micro-organisms from rural sewage sludge during their composting in straw mixture. J. Appl. Microbiol. **99:**528–539.
14. **Ribot, E. M., et al.** 2006. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. Foodborne Pathog. Dis. **3:**59–67.
15. Reference deleted.
16. **Shepherd, M. W., Jr., P. Liang, X. Jiang, M. P. Doyle, and M. C. Erickson.** 2007. Fate of *Escherichia coli* O157:H7 during on-farm dairy manure-based composting. J. Food Prot. **70:**2708–2716.
17. **Shepherd, M. W., Jr., R. Singh, J. Kim, and X. Jiang.** 2010. Effect of heat-shock treatment on the survival of *Escherichia coli* O157:H7 and *Salmonella enterica* Typhimurium in dairy manure co-composted with vegetable wastes under field conditions. Bioresour. Technol. **101:**5407–5413.
18. **Sommer, S. G.** 2001. Effect of composting on nutrient loss and nitrogen availability of cattle deep litter. Eur. J. Agron. **14:**123–133.
19. **U.S. Environmental Protection Agency.** 2008. Aerated static pile composting. http://www.epa.gov/osw/conserve/rrr/composting/static.htm. Accessed 5 April 2010.
20. **Washington State University Puyallup.** 2010. Calculating compost bulk density. http://www.puyallup.wsu.edu/soilmgmt/BulkDensity.htm. Accessed 5 April 2010.
21. **Wu, S., et al.** 2009. Inactivation of *Escherichia coli* in soil by solarization. Soil Sci. Plant Nutr. **55:**258–263.