CENTER for PRODUCE SAFETY

CPS 2010 RFP
FINAL PROJECT REPORT

Project Title

Escherichia coli O157:H7 in bioaerosols from cattle production areas: evaluation of proximity and airborne transport on leafy green crop contamination

Project Period

January 1, 2011 – December 31, 2012

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Objectives

1. Determine if *E. coli* O157:H7 can be transferred by dust or wind from cattle production environments to leafy green produce crops.

2. Determine the impacts of environmental conditions and proximity on the transmission of *E. coli* O157:H7 by dust or wind from cattle production environments to leafy green produce crops.

3. Determine the impacts of environmental conditions and proximity on the density of flies and *E. coli* O157:H7-positive flies in a leafy green produce crop.

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FINAL REPORT

Abstract

Recent *Escherichia coli* O157:H7 outbreaks linked to the consumption of spinach and lettuce have fueled the need for information regarding *E. coli* O157:H7 dissemination from cattle production facilities. The overall goal of this project was to evaluate the impact of proximity to a beef cattle feedlot on the *E. coli* O157:H7 contamination of a leafy green produce crop, and focused on the potential for *E. coli* O157:H7 dissemination by bioaerosols and cattle pest flies. In each of two years, beginning in May, leafy greens were planted to nine plots which were sited at 200, 400, and 600 feet from a cattle feedlot (3 plots each distance). In order to have leaf growth above ground during the entire project season, additional subplots were planted every few weeks. As a means of understanding the effects of proximity on transmission of *E. coli* O157:H7 and/or total *E. coli* from the feedlot to the leafy green crop, four sample types were collected and analyzed throughout each project season from June to September: feedlot surface manure (FSM), leafy greens, air samples, and flies. The percentage of *E. coli* O157:H7-positive FSM samples was high at each sampling, ranging from 47% to 98%, with an average prevalence of about 72% in both years. *E. coli* O157:H7 was recovered in leafy greens at low rates, but was found in samples at all three plot distances tested, including 600 feet. Although *E. coli* O157:H7 was not recovered from 1000-liter air samples at any location, total *E. coli* was recovered from air samples at the edge of the feedlot and all three plot distances, which indicates that airborne transport of the pathogen can occur. On some sampling days, decreases in total *E. coli* concentrations in air samples were suggested as distance from the feedlot increased. The primary cattle pest fly species captured at the edge of the feedlot and at the leafy green plots included house flies (*Musca domestica*), face flies (*Musca autumnalis*), stable flies (*Stomoxys calcitrans*), flesh flies (Sarcophagidae), and blow flies (Calliphoridae). Over both years, the percentage of *E. coli* O157:H7-positive fly pools was highest ($P < 0.05$) at the edge of the feedlot (18.5%), although the pathogen was found in 10.4, 8.5, and 9.5% of fly pools at 200, 400, and 600 feet from the feedlot, respectively. This information is critical for understanding the risks associated with growing leafy greens crops in close proximity to cattle production, and for determining safe distances between cattle feedlots and crop production. The results of this study suggest that the current leafy green field distance guidelines of 400 feet may not be adequate to limit the occurrence of *E. coli* O157:H7 in crops planted near concentrated animal feeding operations.

Background

Recent *E. coli* O157:H7 outbreaks linked to the consumption of spinach and lettuce have focused attention on cattle as potential sources of contamination, and fueled the need for information regarding *E. coli* O157:H7 dissemination from cattle production facilities. Manure containing this pathogen may contaminate produce crops when manure is used as a soil amendment, or because of other inadvertent contact (Besser et al., 1993; Beuchat and Ryu, 1997; Cieslak et al., 1993; Suslow et al., 2003). Runoff from animal feeding operations or manure storage may contaminate crops or water that is used for human consumption or irrigation, further increasing the risk for water- or foodborne illness (Jackson et al., 1998; Johnson et al., 2003; Licence et al., 2001; O'Connor, 2002; Suslow et al., 2003). Proper containment and treatment of runoff from livestock production areas will reduce the possibilities for contamination of crops and water with pathogens from manure (Berry et al., 2007). Likewise, fencing of produce fields could exclude livestock and large wildlife that may shed *E. coli* O157:H7 and other zoonotic pathogens. However, information and guidelines are limited

for reducing contamination risk from other potential pathogen sources that are difficult or impossible to exclude, such as windborne particulates or insects. Furthermore, the significance or magnitude of the risk of transport of *E. coli* O157:H7 from animal production facilities to produce crops by wind, dust emissions, or insects is unknown.

Guidelines provided in the Leafy Greens Marketing Agreement (LGMA, 2012) propose an interim guidance distance of 400 feet between concentrated animal feeding operations and leafy green crops, but admit that there is lack of science supporting this guidance distance. Although the airborne transport of generic *E. coli* in particulates and bioaerosols has been observed, data regarding emission rates from livestock production or transport distances is limited (Duan et al., 2009; Hutchison et al., 2008; Millner and Suslow, 2008; as reviewed by Millner, 2009). Millner and Suslow (2008) collected 1000 liter bioaerosol samples at distances of 30 to 400 feet downwind of a feedlot; they recovered high *E. coli* concentrations from air at 30 feet, lower *E. coli* concentrations at 100 feet, and did not detect *E. coli* at distances of 200 feet or greater. Recent experiments staged fresh spinach bundles at distances of 0, 60, and 150 feet downwind from the load out area of a cattle feedyard, an area where substantial dust can be generated when cattle are being moved (Yanamala, 2011). Air sampling did not detect airborne pathogens; however, *E. coli* O157:H7, *Salmonella*, and generic *E. coli* were found on the spinach following dust generation by cattle, with little effect of distance from the load out area on the numbers of pathogen-positive samples or the levels of generic *E. coli* on the spinach. Further research is needed to determine set-back distances or buffer zones that will effectively reduce the risk of airborne *E. coli* O157:H7 contamination of produce crops.

Similarly, additional research is needed to understand the risk for produce contamination by insects bearing pathogens. Pest flies are ubiquitous in cattle production environments, and their potential role in transmission of *E. coli* O157:H7 among cattle has been explored. Detection of *E. coli* O157:H7 in flies collected from both dairy and beef cattle environments has been reported (Alam and Zurek, 2004; Hancock et al., 1998; Iwasa et al., 1999; Keen et al., 2006; Shere et al., 1998). Several fly species have been shown to harbor this pathogen; however, house flies (*Musca domestica*) are most frequently implicated (Iwasa et al., 1999; Keen et al., 2006; Moriya et al., 1999; Talley et al., 2009). Cattle manure is an important habitat for flies, and flies can subsequently contaminate other surfaces with pathogens from manure by regurgitation, fecal deposition, or mechanical transfer (Graczyk et al., 2001). House flies have been implicated in the transmission of *E. coli* O157:H7 from cattle to humans via food contamination (Moriya et al., 1999) and transmission of this pathogen to cattle via flies has been demonstrated (Ahmad et al., 2007). More recently, Talley et al. (2009) detected *E. coli* O157:H7 genes in filth flies captured in a leafy green crop that was located near a cattle production area, and further demonstrated in laboratory studies that house flies can transfer this pathogen to spinach leaves. Further work is warranted to clarify a role for flies in the preharvest produce contamination with *E. coli* O157:H7, and to determine if proximity to cattle influences risk of contamination by flies.

Research Methods and Results

Experimental design and approach

In both 2011 and 2012, beginning in May, leafy greens were planted in each of nine plots which were sited at 200, 400, and 600 feet from the nearest row of pens at the 6,000-head capacity USMARC beef cattle feedlot. The plots were fenced with seven-foot panels to exclude deer, with poultry netting to exclude rabbits. The plots were located in a field north of the feedlot in order to take advantage of the prevailing south winds that are typical during the late spring and

summer in this region. In order to have leaf growth above ground during June through September, additional subplots within each plot were planted every few weeks, with the last planting occurring in August of each year. As a means of understanding the effects of proximity on transmission of *E. coli* O157:H7 and/or total *E. coli* from the feedlot to the leafy green crop, three sample types were collected and analyzed throughout each project season from June to September: leafy greens, air samples, and flies. In addition, for the assessment of *E. coli* O157:H7 prevalence in the source material, each time that leafy greens samples were collected, feedlot surface manure samples also were collected from each of the ten feedlot pens that are immediately adjacent to the field containing the plots. Cattle were housed in these pens continuously from May through mid-September. Additionally, weather data were collected throughout the study period. The on-site all-season weather station was managed by project collaborator Bryan Woodbury, and monitored and recorded rainfall volumes and intensity, air temperature, wind direction and speed, and relative humidity at 15-minute intervals. Because the prevalence of *E. coli* O157:H7 shedding by cattle can vary from year to year, as can climatic conditions such as temperature and rainfall, the study was conducted in each of two years to ensure that we gathered the data needed to accomplish the research objectives.

We initially proposed to conduct all experiments using spinach. However in 2011, we found spinach production to be a challenge when temperatures were very hot during July and August. The hot temperatures substantially inhibited the germination and growth of the spinach that was planted during this period (Renegade F1). Thus, in 2012 we explored the use of mustard greens (Tendergreen) and turnip greens (Purple Top White Globe) as alternative trap crops. Both mustard and turnips germinated and grew well compared to spinach when temperatures were hot. However, both mustard and turnips contain glucosinolates and glucosinolate breakdown products, which can have potent antimicrobial properties. To confirm that these antimicrobial properties did not affect the ability to recover *E. coli* O157:H7 from mustard and turnips, we conducted an inoculation and recovery experiment, as follows: twenty samples each of spinach, mustard greens, and turnip greens were inoculated with a low level of a 5-strain cocktail of bovine *E. coli* O157:H7 (4.8 cells per gram of leafy greens), and subjected to our enrichment and immunomagnetic separation (IMS) procedure described below. *E. coli* O157:H7 was recovered from 100% of each sample type, thus demonstrating that mustard greens and turnip greens could be used as alternative trap crops in substitution of spinach.

Leafy greens and feedlot surface manure

Leafy greens and feedlot surface manure (FSM) were collected and analyzed six times in each of 2011 (spinach) and 2012 (spinach, mustard, and/or turnips): once in June, twice in July, twice in August, and once in September. At each sampling period, up to thirty leafy green samples were collected from each plot (target for each sampling period: 270 leafy green samples (90 samples each at 200, 400, and 600 feet from the feedlot) and ten FSM samples were collected from each of the ten feedlot pens (100 FSM samples each sampling period). *E. coli* O157:H7 presence were determined for each leafy green and FSM sample by non-selective enrichment at 37°C for 7 h, IMS using anti-*E. coli* O157 Dynabeads (Invitrogen Corp., Carlsbad, CA), and plating onto CHROMagar O157 (DRG International) containing 5 mg/liter novobiocin and 2.5 mg/liter potassium tellurite (ntCHROM O157; Berry and Wells, 2008). The *E. coli* O157:H7 levels were determined for each FSM sample by spiral-plating onto ntCHROM O157. In addition, subsamples of primary enrichment cultures of both leafy greens and FSM were applied to FTA cards and processed for subsequent real time-polymerase chain reaction (RT-PCR) detection of genes of O157 and non-O157 Shiga toxin-producing *E. coli* strains (TPEC screen; Total Pathogenic E. coli) by project collaborators Trevor Suslow and Gabriela Lopez-Velasco. Suspect *E. coli* O157:H7 were isolated, confirmed by multiplex PCR for the *E. coli* O157:H7

genes *rfbE*, *fliC*, *eaeA*, *stx*$_1$, and *stx*$_2$, and frozen. Confirmed *E. coli* O157:H7 were subjected to pulsed-field gel electrophoresis (PFGE) analysis using the Centers for Disease Control and Prevention PulseNet protocol and the restriction endonuclease *Xba*I (Ribot et al., 2006), to show linkages between leafy greens and FSM isolates.

Data regarding the percentages of *E. coli* O157:H7-positive FSM and leafy greens in 2011 and 2012 are shown in Tables 1 and 2, respectively. The percentage of *E. coli* O157:H7-positive FSM samples was high at each sampling, ranging from 47% to 98%, with an average prevalence of about 72% in both years. *E. coli* O157:H7 was recovered in spinach and other leafy greens at low rates, but was found in samples at all three plot distances tested, including 600 feet. While *E. coli* O157:H7-positive samples appear to decrease with distance from the feedlot, the means of the total percentages were not always significantly different due to distance (Tables 1 and 2). In 2011, 2% of all spinach samples collected at 200 feet were positive for the pathogen and this was significantly higher than the 0.4% at 400 feet, but not different than the 0.7% at 600 feet (Table 1). In 2012, 4.5, 4.1, and 2.8% of leafy green samples collected at 200, 400, and 600 feet were positive for *E. coli* O157:H7, respectively, but percentages were not significantly different (Table 2). The higher percentage of *E. coli* O157:H7-positive leafy green samples collected on 9/10/2012 compared to the other sample collection dates is notable, and occurred four days after movement of new cattle into eight of the pens immediately adjacent to the field containing the leafy greens plots, a process generating substantial dust. *E. coli* O157:H7 isolates with identical PFGE types were found in both FSM and leafy greens.

Table 1. Percentage of *E. coli* O157:H7-positive samples (%) among feedlot surface manure collected in the feedlot pens and spinach collected at 200, 400, and 600 feet from the feedlot, at each sampling period--2011 project season. Standard error of the least squares means = 0.62.

Sampling period	Feedlot Surface Manure	Spinach		
		200 feet	400 feet	600 feet
6/28/2011	50.0	0	0	1.1
7/5/2011	68.0	5.5	2.2	0
7/20/2011	67.0	3.3	0	1.1
8/4/2011	93.0	3.3	0	0
8/29/2011	62.0	0	0	0
9/12/2011	90.0	0	0	2.2
Percentage over 2011 project season	71.7	2.0 A	0.4 B	0.7 AB

For the total project season, percentages followed by different letters (A and B) are significantly different ($P \leq 0.10$).

Table 2. Percentage of *E. coli* O157:H7-positive samples (%) among feedlot surface manure collected in the feedlot pens and leafy greens collected at 200, 400, and 600 feet from the feedlot, at each sampling period—2012 project season.

Sampling period	Leafy Green Type	Feedlot Surface Manure	Leafy Greens		
			200 feet	400 feet	600 feet
6/18/2012	Spinach	83.0	4.4	3.3	1.1
7/2/2012	Spinach	66.0	6.7	3.3	0
7/23/2012	Spinach	47.0	1.1	2.2	0
8/13/2012	Turnips, Mustard	98.0	2.2	1.1	0
8/27/2012	Turnips, Mustard	78.0	5.6	2.2	0
9/10/2012	Turnips	61.0	6.7	12.2	15.6
Percentage over 2012 project season		72.2	4.5 A	4.1 A	2.8 A

For the total project season, percentages followed by different letters (A and B) are significantly different ($P \leq 0.10$).

Air samples

MAS-100 Eco microbial air samplers (EMD Millipore) were used to collect 1000-liter air samples at each of the nine leafy greens plots and at three locations at the north edge of the feedlot pens. In 2011, air samples were collected on five different days in August and September. In 2012, air samples were collected ten different days in June, July, and August. CHROMagar O157 containing 1.0 g/liter sodium pyruvate was used for determination of *E. coli* O157:H7, and CHROMagar ECC containing 1.0 g/liter sodium pyruvate was used for determination of total *E. coli*. On each day, 1000-liter air samples were collected onto each of two plates of CHROMagar O157 and CHROMagar ECC at each site (n = 6 for each bacterial group, at each of 0, 200, 400, and 600 feet from the feedlot). Pen surface conditions and wind speed and direction were recorded.

In 2011, *E. coli* O157:H7 was not recovered from air samples collected at any location; thus, we focused more time on air sampling in 2012 to verify these results. Although *E. coli* O157:H7 was not recovered from air samples at any location in either of 2011 or 2012, total *E. coli* was recovered at the edge of the feedlot and at all three plot distances in both years. Tables 3 and 4 show the average concentrations of total *E. coli* (colony-forming units [CFU] per 1000 liters of air) at each distance on each sampling day in 2011 and 2012, respectively. On some sampling days, total *E. coli* concentrations were significantly higher at the edge of the feedlot than they were at the leafy green plots. On other days, there was no significant difference in the levels of total *E. coli* in air at the edge of the feedlot and at the leafy greens plots at any distance.

Total *E. coli* typically is present on the feedlot surface at 10^6 cells per gram of FSM, and *E. coli* O157:H7 when present in FSM occurs at much lower concentrations in comparison to total *E. coli*. This suggests that the air sampling technique we employed was not adequately sensitive to detect *E. coli* O157:H7 in spite of the high prevalence of the pathogen on the feedlot surface (Tables 1 and 2).

Table 3. Average total *E. coli* concentrations (CFU/1000 liters of air) in air samples collected at the north edge of the feedlot pens and at each of the spinach plots located at 200, 400, and 600 feet north of the feedlot, at each sampling period—2011 project season

Sampling day	Pen surface conditions	Wind direction; wind speed	At Edge of Feedlot	At Spinach Plots		
				200 feet	400 feet	600 feet
8/15/2011	Muddy	East-SE; 10-15 mph, gusts to 20 mph	41.7 A	8.0 B	3.0 B	2.2 B
8/18/2011	Moderately dusty	South-SE; 10-15 mph	4.8 A	2.7 A	2.7 A	0.8 A
8/22/2011	Dusty	South; 5-15 mph	9.8 A	4.0 A	4.0 A	2.2 A
8/26/2011	Very dry and dusty	South; 5-15 mph	41.7 A	5.8 B	2.5 B	1.7 B
9/1/2011	Muddy	South; 10-15 mph, gusts to 25 mph	3.7 A	1.0 A	0.2 A	<1.0 A

Within a row, values followed by different letters (A and B) are significantly different ($P \leq 0.05$).

Table 4. Average total *E. coli* concentrations (CFU/1000 liters of air) in air samples collected at the north edge of the feedlot pens and at each of the leafy green plots located at 200, 400, and 600 feet north of the feedlot, at each sampling period—2012 project season.

Sampling day	Pen surface conditions	Wind direction; wind speed	At Edge of Feedlot	At Leafy Greens Plots		
				200 feet	400 feet	600 feet
6/6/2012	Moderately dusty	South-SE; 10-15 mph	26.5 A	5.7 B	2.2 B	1.7 B
6/7/2012	Moderately dusty	Southeast; Avg. 9 mph, gusts to 18 mph	15.3 A	2.2 B	0.8 B	0.8 B
6/22/2012	Moderately muddy	South-SW; Avg. 11 mph, gusts to 18 mph	9.3 A	3.3 A	0.7 A	0.4 A
6/26/2012	Dry	South-SE; 10-15 mph	4.7 A	1.8 A	1.5 A	0.3 A
6/27/2012	Dry	Southwest; Avg. 20 mph	1.8 A	0.7 A	1.3 A	0.2 A
7/6/2012	Very dusty	Southwest; Avg. 8 mph	4.0 A	4.5 A	2.0 A	3.5 A
7/17/2012	Moderately dusty	South; 5-10 mph	3.4 A	1.5 A	0.5 A	0.8 A
7/25/2012	Moderately dusty; freshly scraped[a]	South-SW; 5-10 mph	0.0 A	0.0 A	0.0 A	0.2 A
8/15/2012	Very dusty	South; 5-10 mph	15.3 A	3.0 B	3.2 B	1.2 B
8/24/2012	Very dry and dusty	South-SE; 10-13 mph	837.2 A	16.7 B	10.0 B	5.3 B

Within a row, values followed by different letters (A and B) are significantly different ($P \leq 0.05$).

Flies

Cattle pest flies were collected and analyzed eight times in 2011 and four times in 2012. In 2011, baited yellow sticky traps were used to collect flies. To assure that pest flies were not being attracted to the leafy green plots by the bait, in 2012 we used the same yellow sticky traps without bait. At each sampling time, two yellow sticky traps were placed at each of the nine leafy green plots and at three locations next to the feedlot pens. The traps were removed to the lab after 24 to 48 hours, and pest flies were identified and counted. The flies were pooled by species, using sterile forceps to remove flies from the traps (up to ten flies per pool). The

presence of *E. coli* O157:H7 was determined in up to ten fly pools per site, following enrichment in 1.5× brilliant green bile broth, IMS, and plating as described by Keen et al. (2006). Project collaborators Lisa Durso, Jim Bono, and Kristina Friesen were directly involved in fly collection and analyses, and were critical to training and instruction of lab personnel on fly identification. Suspect *E. coli* O157:H7 were confirmed by multiplex PCR as described above, and subjected to pulsed-field gel electrophoresis (PFGE) to show linkages with leafy green and FSM isolates.

A total of 752 fly pools were examined in 2011, and 345 fly pools were examined in 2012. For both years, fly pools were composed primarily of house flies (*Musca domestica*; 327 pools), face flies (*Musca autumnalis*; 311 pools), stable flies (*Stomoxys calcitrans*; 159 pools), flesh flies (Sarcophagidae; 169 pools), and blow flies (Calliphoridae; 92 pools). Prevalence of *E. coli* O157:H7 in house flies and face flies was 17.1 and 15.8%, respectively, and did not differ ($P > 0.05$). Prevalence in house flies tended to be higher ($P = 0.09$) than in flesh flies (10.7%), and was higher ($P < 0.05$) than in blow flies (7.6%) and stable flies (0.63%). Over both years, the percentage of *E. coli* O157:H7-positive fly pools was highest ($P < 0.05$) at the edge of the feedlot (18.5%), although the pathogen was found in 10.4, 8.5, and 9.5% of fly pools at 200, 400, and 600 feet from the feedlot, respectively. The percentage of *E. coli* O157:H7-positive pools of flies collected on traps next to the feedlot and at each of the nine leafy green plots in 2011 and 2012 are shown in Tables 5 and 6, respectively. *E. coli* O157:H7 isolates from pest flies had identical PFGE types to isolates found in both FSM and leafy greens.

Table 5. Percentage of *E. coli* O157:H7-positive fly pools (%) of flies (all species) collected at the edge of the feedlot pens and at each of the spinach plots located at 200, 400, and 600 feet from the feedlot, at each sampling period—2011 project season

Sampling period	At Edge of Feedlot	At Spinach Plots		
		200 feet	400 feet	600 feet
6/22/2011	0.0	11.8	0.0	13.3
6/29/2011	10.0	14.3	16.0	10.7
7/13/2011	15.0	20.8	7.7	8.7
7/27/2011	5.0	6.7	3.4	0.0
8/12/2011	30.0	3.6	10.0	13.3
8/17/2011	62.5	30.8	8.3	18.8
9/1/2011	16.7	3.3	7.7	12.0
9/7/2011	24.1	15.4	4.3	16.7
Percentage over 2011 project season	22.7	11.7	7.6	11.3

Table 6. Percentage of presumptive *E. coli* O157:H7-positive fly pools (%) of flies (5 pest fly species) collected at the edge of the feedlot pens and at each of the leafy greens plots located at 200, 400, and 600 feet from the feedlot, at each sampling period—2012 project season.

Sampling period	At Edge of Feedlot	At Leafy Greens Plots		
		200 feet	400 feet	600 feet
7/18/2012	3.3	4.5	12.5	0.0
8/8/2012	36.8	15.8	18.8	11.1
9/5/2012	0.0	3.7	3.6	5.9
9/19/2012	13.6	8.0	12.0	4.0
Percentage over 2012 project season	10.9	7.5	10.6	4.5

Outcomes and Accomplishments

The overall goal of the project was to evaluate the impact of proximity to a beef cattle feedlot on the *E. coli* O157:H7 contamination of a leafy green produce crop, with special attention to the potential for bioaerosol transmission of *E. coli* O157:H7 (Objectives 1 and 2) and transmission of *E. coli* O157:H7 by cattle pest flies (Objective 3). This was accomplished by conducting a comprehensive study over two seasons that included intensive sampling of leafy greens, feedlot surface manure, air, and cattle pest flies. The study was designed in the context of the current LGMA guidelines, that recommend a distance of 400 feet between concentrated animal feeding operations and leafy green crops, by planting the leafy green plots 200, 400, and 600 feet from the feedlot. The recovery of *E. coli* O157:H7 from spinach and the other leafy greens of the same PFGE types as isolates recovered in feedlot surface manure demonstrated the dissemination of this pathogen from the feedlot to the crop. In addition, the recovery of the pathogen from leafy greens planted at all three plot distances indicates the risk for planting these crops near cattle feedlots. Bioaerosol or airborne transport of *E. coli* O157:H7 was not observed, as the air sampling technique we used likely was not adequately sensitive to detect the pathogen. However, airborne transport of total *E. coli* was verified, which indicates that airborne transport of *E. coli* O157:H7 also can occur. Total *E. coli* was detected in air samples at all three plot distances, including 600 feet, although decreases in concentrations were observed as distance from the feedlot increased. Results obtained from the cattle pest fly analyses provide quantitative data regarding the occurrence of *E. coli* O157:H7-positive flies in a leafy green crop planted near a cattle feedlot and information about the various fly species that can harbor this pathogen. To our knowledge, this work provides the first report of the carriage of *E. coli* O157:H7 by face flies and Sarcophagid flies. *E. coli* O157:H7-positive pest flies were found at all three plot distances tested.

Summary of Findings and Recommendations

The major findings of this work were the occurrence of *E. coli* O157:H7 both in leafy greens and pest flies at distances up to 600 feet from the cattle feedlot. Although the pathogen was not detected in air samples at 600 feet, the detection of total *E. coli* in air samples collected at this

distance indicates the risk for airborne transport of *E. coli* O157:H7. These findings suggest that the current leafy green field distance guidelines of 400 feet may not be adequate to limit the occurrence of *E. coli* O157:H7 in crops planted near concentrated animal feeding operations. Additional research will be needed to determine a safer set-back distance that will further reduce contamination risk.

While this study provided detailed data regarding the dissemination of *E. coli* O157:H7 to leafy greens from a feedlot, the findings did not fully confirm the specific role for either of bioaerosol transport and pest flies in this dissemination. Further work is suggested to clarify the significance of these two potential modes of pathogen transmission to the microbial safety of produce, which may suggest other potential means to reduce the risk for crop contamination.

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APPENDICES

Publications and Presentations (required):

Publications

Berry, E. D., J. E. Wells, L. M. Durso, J. L. Bono, K. M. Friesen, B. L. Woodbury, T. V. Suslow, G. Lopez-Velasco, and P. D. Millner. Effect of proximity to a cattle feedlot on the occurrence of *Escherichia coli* O157:H7 in leafy greens, bioaerosols, and pest flies. Journal of Food Protection. *In preparation*.

Presentations

Berry, E. D. *et al. Escherichia coli* O157:H7 in bioaerosols from cattle production areas: evaluation of proximity and airborne transport on leafy green crop contamination. Poster presentation at the 2nd Annual CPS Produce Research Symposium, June 2011, Orlando, FL.

An overview of the objectives and approach of the CPS-funded project "*Escherichia coli* O157:H7 in bioaerosols from cattle production areas: evaluation of proximity and airborne transport on leafy green crop contamination" were presented in a poster at the Western Food Safety Summit, May 2012, Salinas, CA.

Berry, E. D. *et al*. *Escherichia coli* O157:H7 in bioaerosols from cattle production areas: evaluation of proximity and airborne transport on leafy green crop contamination. A summary of Year One results were presented in a poster and a presentation at the 3rd Annual CPS Produce Research Symposium, June 2012, Davis, CA.

Berry, E. D. *et al*. *Escherichia coli* O157:H7 in bioaerosols from cattle production areas: evaluation of proximity and airborne transport on leafy green crop contamination. A summary of final results will presented in a presentation at the 4th Annual CPS Produce Research Symposium, June 2013, Rochester, NY.

Berry, E. D., J. E. Wells, L. M. Durso, J. L. Bono, K. M. Friesen, B. L. Woodbury, T. V. Suslow, G. Lopez-Velasco, and P. D. Millner. Effect of proximity to a cattle feedlot on the occurrence of *Escherichia coli* O157:H7-positive pest flies in a leafy green crop. Annual Meeting of the International Association for Food Protection, July 28-31, 2013, Charlotte, NC. *Submitted.*

Budget Summary (required)

The funds requested were adequate to support the research. Salary and benefits of $76,038 were granted and expended; $202,962 for Materials and Supplies was granted and $201,112 has been expended; $2,048 in travel was granted and $1,114.70 has been expended; and $1,200 for publication costs was granted but is not yet expended. Indirect costs were $14,112. A total of approximately $4,000 remains to support travel for the Principal Investigator to attend the 2013 CPS Produce Research Symposium in Rochester, NY, and for manuscript publication costs.

Tables and Figures (optional)

Elaine Berry, USDA, ARS, USMARC
Escherichia coli O157:H7 in bioaerosols from cattle production areas: evaluation of proximity and airborne transport on leafy green crop contamination

Suggestions to CPS (optional):

No suggestions

Title: Effect of Proximity to a Cattle Feedlot on the Occurrence of *Escherichia coli* O157:H7-Positive Pest Flies in a Leafy Green Crop

Authors: Elaine D. Berry, James E. Wells, Lisa M. Durso, James L. Bono, Kristina M. Friesen, Bryan L. Woodbury, Trevor V. Suslow, Gabriela Lopez-Velasco, and Patricia D. Millner

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Format: Poster

Category: Produce

Introduction: *Escherichia coli* O157:H7 outbreaks linked to produce have focused attention on cattle as contamination sources. Cattle pest flies can harbor this pathogen, and may disseminate it to produce. The Leafy Greens Marketing Agreement proposes an interim guidance distance of 400 feet between concentrated animal feeding operations and leafy green crops to reduce contamination risk.

Purpose: The objective was to determine the occurrence of *E. coli* O157:H7-positive pest flies in leafy greens planted at different distances from a cattle feedlot.

Methods: Spinach was planted to nine plots sited at 200, 400, and 600 feet from a cattle feedlot (3 plots each distance). Sticky traps were used to capture flies at the spinach plots and the feedlot in June through September. Flies were identified, counted, and pooled by species (up to ten flies per pool). *E. coli* O157:H7 was determined by immunomagnetic separation and plating in up to ten fly pools per site.

Results: Prevalence of *E. coli* O157:H7 in house flies (*Musca domestica)* and face flies (*Musca autumnalis)* was 17.1 and 15.8%, respectively, and did not differ ($P > 0.05$). Prevalence in house flies tended to be higher ($P = 0.09$) than in flesh flies (Sarcophagidae; 10.7%) and was higher ($P < 0.05$) than in blow flies (Calliphoridae; 7.6%) and stable flies (*Stomoxys calcitrans;* 0.63%*).* Overall, the percentage of O157:H7-positive fly pools was highest ($P < 0.05$) at the edge of the feedlot (18.5%), although the pathogen was found in 10.4, 8.5, and 9.5% of fly pools at 200, 400, and 600 feet from the feedlot, respectively.

Significance: Current leafy green field distance guidelines may not be adequate to limit the occurrence of pest flies in crops planted near cattle feedlots. Further work is needed to clarify the risk for transmission of *E. coli* O157:H7 to leafy greens by flies.