Annals of the Entomological Society of America, 110(1), 2017, 83–89
doi: 10.1093/aesa/saw092
Research article

Special Collection: Filth Fly–Microbe Interactions

Filth Fly Transmission of *Escherichia coli* O157:H7 and *Salmonella enterica* to Lettuce, *Lactuca sativa*

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Subject Editor: Dana Nayduch

Received 4 September 2016; Editorial decision 3 November 2017

Abstract

Filth flies have been implicated in the dispersal of human disease pathogens; however, the epidemiological parameters of the transmission of human pathogens from flies to plants are largely undescribed. The capacity of the black blow fly, *Phormia regina* Meigen, to acquire and subsequently deposit bacteria onto baby lettuce leaves was compared with that of the house fly, *Musca domestica* (L.). Adult *P. regina* and *M. domestica* were exposed to green fluorescent protein-tagged *Escherichia coli* O157:H7- or *Salmonella enterica*-inoculated manure and then allowed access to the lettuce plants. Bacteria on the plants and flies were assessed by plating and polymerase chain reaction. Although blow flies acquired significantly more *E. coli* O157:H7 than house flies, there was no significant difference between the deposition of bacteria on lettuce by the two fly species. In contrast, there was no significant difference in the acquisition of *S. enterica* by the two fly species. However, blow flies deposited more *S. enterica* onto lettuce than house flies. To more accurately assess transmission parameters, flies were given timed exposure and inoculation periods of 10 and 30 s. Blow flies acquired more *E. coli* O157:H7 than *S. enterica* in the both time periods. Flies exposed to manure for 30 s were then tested for deposition by forcing the flies to walk on lettuce leaves. Blow flies deposited comparable amounts of *E. coli* O157:H7 and *S. enterica*. Although house flies have historically been implicated in the transmission of human pathogens to food, the data presented suggest that blow flies are more efficient vectors of *E. coli* O157:H7 and *S. enterica* to leafy greens than house flies.

Key words: Diptera, mechanical vector, vector competence

Filth flies are known vectors of several enteric pathogens. In recent years, *Escherichia coli* O157:H7 and *Salmonella enterica* have risen to high levels of importance in food safety because of numerous outbreaks. Flies carrying *E. coli* O157:H7 were found at a primary school where children were infected by the bacteria (Moriya et al. 1999). *Escherichia coli* O157:H7 was detected in flies at a nearby dairy farm, showing that the flies may be potential mechanical vectors of the bacterium (Buma et al. 1999). *Escherichia coli* can be acquired by house flies, Musca domestica (L.), from a broth culture and subsequently inoculated to agar by walking, regurgitation, and excretion (Kobayashi et al. 1999). The bacteria were visualized by electron microscopy on the mouthparts of the fly, as the authors suggested that they could possibly proliferate there. In addition, Kobayashi et al. (1999) determined that *E. coli* O157:H7 moved into the gut of the fly via ingestion, but was excreted rapidly. Under laboratory conditions, house flies acquired green fluorescent protein (GFP)-tagged *E. coli* O157:H7 from contaminated manure and transferred it to spinach plants by regurgitation (Talley et al. 2009). These studies indicate that filth flies can acquire *E. coli* O157:H7 and deposit the bacteria on various surfaces.

Salmonella typhimurium has long been associated with house flies. Early evidence that typhoid fever in soldiers in the late 1800s was associated with flies was collected when Hewitt (1910) documented fly travel from human fecal matter to food and back repeatedly, resulting in food contamination. Ostrolenk and Welch (1942) showed that house flies were capable of mechanically transmitting *Salmonella* spp. to surfaces and could carry viable *Salmonella* spp. in their gut, which could then be regurgitated onto various surfaces. A study in Japan isolated *S. enterica* from a fly on a farm (Matayoshi and Kudaka 2010). *Salmonella* spp. were commonly isolated from flies captured at cattle farms, at barbeque parties, and in urban settings in Australia (Vriesekoop and Shaw 2010). This study suggests that *Salmonella* spp. can be ubiquitous in some environments and relatively accessible to flies.

House flies are mechanical vectors of *E. coli* O157:H7 to plants and animals; however, the impact of this transmission within the environment has not been fully determined. In addition, the transmission of *E. coli* O157:H7 by calliphorid flies has not been assessed. The presence of such filth flies among leafy greens is not well

understood; however, their attraction to leafy greens may lead to contamination. Talley et al. (2009) documented the presence of calliphorid and muscid flies in lettuce fields adjacent to cattle pastures in the Salinas Valley in California. Fly defecation spots were observed on the lettuce leaves of one heavily infested field. A small subsample of flies was tested for *E. coli* O157:H7, and 11 of the 18 subsamples were positive as tested by polymerase chain reaction (PCR) (Talley et al. 2009). The filth flies in the lettuce may have come from cow pastures contaminated with *E. coli* O157:H7 or from on-farm compost piles, nearby poultry houses, or areas of human habitation. In a controlled study, house flies artificially exposed to *E. coli* O157:H7 transmitted the bacteria to calves (Ahmad et al. 2007), and fecal testing showed that the infection lasted 20 d (the entire time of the experiment) for 62% of the calves. A survey of a cattle operation showed that about 2.9% of all flies tested were carrying *E. coli* O157:H7 throughout summer (Alam and Zurek 2004).

In cases of simple mechanical transmission, the pathogen does not persist in or on the vector for an extended period of time (Levine and Levine 1991). However, Kobayashi et al. (1999) revealed the ability of the bacteria to survive on house fly mouthparts for at least 3 d and suggested that house flies are "bioenhanced" mechanical vectors. Flies have been observed to land on and contaminate spinach and lettuce by either regurgitation or defecation, and house flies have been shown to transfer *E. coli* O157:H7 to spinach (Wasala et al. 2013), but this was possible because of extended plant exposure times (>18 h). To more precisely determine bacterial attachment to and detachment from flies during casual contact, we studied the transmission process of two filth flies and two bacterial pathogens, and tested the hypothesis that all flies transmit all bacteria with equal efficiency. Furthermore, we used the very efficient vector, *Phormia regina* (Meigen), to describe short-term contact and subsequent inoculation of two foodborne bacteria to lettuce.

Materials and Methods

Plants

Lettuce, *Lactuca sativa*, was grown in a temperature-controlled (22–27°C and a photoperiod of 14:10 (L:D) h) greenhouse at Oklahoma State University (OSU). Seeds were sown into 36 square plug trays (Myers Industries Lawn and Garden Group, Akron, OK) containing Miracle-Gro Moisture Control Potting Mix (Scotts Miracle-Gro, Marysville, OH) and transplanted after 2–3 wk into 10.2-cm standard plastic pots (Myers Industries Lawn and Garden Group, Akron, OH) containing Miracle-Gro Potting Mix (Scotts Miracle-Gro) maintained under the same conditions as the greenhouse at OSU. Lettuce plants were used for experimentation at about 1 mo of age (6–10 leaf stages). They were watered every other day with 1:1 N:K fertilizer water (100 ppm). On the day of the experimentation, plants were watered by pouring ~50 ml of $_{dd}H_2O$ water on the soil.

Insects

A black blow fly (*P. regina)* colony was established from feral flies captured in 2010 at a cattle feedlot in Watonga, OK. The flies were housed at the Medical and Veterinary Entomology building on the OSU campus. The colony room was maintained at 23–29°C, ≤40% RH, and a photoperiod of 18:6 (L:D) h. The colony was maintained in two 75 by 75 by 115 cm^3 (length by width by height) BugDorm containment cage (MegaView Science Co., Taichung, Taiwan). Larvae were provided non-processed beef liver of 57- to 85-g portions that were frozen until the week of its use and then transferred to storage at 4 °C for it to thaw. Adults were given tap water, Calf-manna (MannaPro, Chesterfield, MO) and table sugar ad libitum. Beef liver cubes (10–15 g) were set out for oviposition and consumption every week. House flies were maintained in a colony that was started from feral flies collected at a cattle feedlot in Stillwater, OK. Larvae were kept in 11 l tubs filled halfway with perlite. Emerged adults were transferred to 30.5- × 30.5-cm^2 BioQuip metal cages with cloth sleeves. Adults were provided with Calf-manna, sugar, and water with a cloth wick for oviposition. Adult flies were harvested for use in experiments 2 d to 1 wk after eclosion. No distinction was made between the genders of the flies used in experimentation.

Bacteria

Green fluorescent protein (GFP)-tagged *E. coli* O157:H7 ATCC 43888 and *Salmonella enterica* serovar Enteritidis used were obtained from Dr. L. Ma (Oklahoma State University). Before use in each experiment, bacteria from the frozen stock were cultured on Luria Bertani agar plus 1% ampicillin (LB AMP) plates and passaged twice overnight at 37°C. Bacterial colonies were checked for fluorescence, scraped from plates and re-suspended to 10^6 cfu/ml, and determined by direct counts using an Olympus BX2 compound microscope (Tokyo, Japan) with dark-field capability.

Manure

Manure was obtained from a local herd of free-range, mixed breed beef cattle that were not treated with any parasiticides or antibiotics. Manure was collected from fresh pats and transported in a bucket back to the laboratory and then stored at 4°C until use 24 h later. The manure was spread to a depth of ~2.5 cm in a large tray and sterilized by autoclaving for 1 h. The manure was separated into foil packets of various sizes (5–50 g) and autoclaved for another 1 h. Sterile autoclaved manure was weighed out in 4-g aliquots into 60-cm sterile petri dishes and mixed with 2 ml of ~2.0 × 10^6 cfu/ml of either *E. coli* O157:H7 or *S. enterica* in peptone (0.01% peptone; Thermo Fisher Scientific, Waltham, MA) or sterile peptone water for the control.

Vector Competence of Flies Transmitting Human Pathogens to Plants

Fly Acquisition

Flies were transported to the laboratory in 50-ml conical tubes, then anesthetized by freezing at −20°C for 2–3 min, and then transferred to inoculated manure. All portions of the experiment involving flies were conducted in a 60 by 60 by 120 cm^3 BugDorm containment cage (MegaView Science Co). In total, 15 flies of either *E. coli*- or *S. enterica*-inoculated manure were used per plate. Further, 10 flies were used per plate for the control manure treatment. For each of the treatments, five replicate plates of identical composition were prepared for each trial. Flies were allowed contact with manure for 2 h and then the plates were placed at −20°C for 2–3 min to anesthetize the flies. Five flies from each treatment were taken from each individual plate and placed into a sterile 15-ml conical tube and placed at −20°C overnight and later tested for GFP bacterial numbers.

Plant Exposure

Anesthetized flies exposed to treated manure were transferred to clear cylindrical cages (25 × 6.5 cm^2), each containing a single 4-wk-old lettuce plant (five flies/plant, five plants). The flies were allowed 18–24 h of contact with the lettuce plants at 26–27°C, and then removed by releasing into a sterile 30.5- × 30.5-cm BugDorm containment cage.

Plants and flies were immediately harvested, and the flies were killed by freezing. The experiment was replicated five times.

Sample Processing

The individual plants were processed in entirety by severing the stem at the soil level, weighing, and crushing with a pestle in a 710-ml Whirl-Pak filter bag (Nasco, Fort Atkinson, WI) in a 1:10 dilution (weight:volume) of peptone water. After complete maceration, 100 µl of the sample was plated in triplicate on LB AMP media. The flies were individually weighed and processed by crushing with a pestle in 207-ml Whirl-Pak filter bags in a 1:100 dilution (weight:volume) of peptone water. After complete maceration, 100 µl of the sample was plated in duplicate on LB AMP media. All plates were incubated at 37°C for 18–24 h and fluorescent colonies enumerated. Three fluorescent colonies were randomly selected and picked from each plate, placed individually into sterile microfuge tubes, and stored in the freezer. In the absence of fluorescent-positive colonies, no colonies were picked. Selected colonies were later tested for identity by end-point PCR. The primers for the *S. enterica* reaction (*invA* F: GTGAAATTATCGCCACGTTCGGGCAA-F; *invA* R: TCATCGC ACCGTCAAAGGAACC-R) were designed to amplify a portion of *invA* (Timmons et al. 2013). The primers for *E. coli* O157:H7 reaction (*rfbE* F: GTGTCCATTTATACGGACATCCATG; *rfbE* R: CCTATAACGTCATGCCAATATTGCC) were designed to amplify a portion of *rfbE* (Timmons et al. 2013).

Timed Bacteria Acquisition by House Flies and Blow Flies

This experiment was performed to test differential acquisition of *E. coli* O157:H7 and *S. enterica* by house flies and blow flies using very short exposure times of 10 and 30 s to test the hypothesis that longer contact with contaminated surfaces results in acquisition of more bacteria.

Fly Preparation

Adult house flies and blow flies were transported to the laboratory in 50-ml conical tubes. The tubes were place on ice to slow down fly movement, and then all flies were transferred into a 60-cm petri dish on ice. Each fly was removed separately, and its wings were cut as close to the thorax as possible without injuring the fly. Two plates of 15 clipped-wing flies (treatment) and one plate of 10 clipped-wing flies (control) were prepared. These plates were allowed to sit at room temperature (25–26°C) until all of the flies were active and walking. Each fly plate was placed back on ice just before beginning the *E. coli* O157:H7, *S. enterica*, or buffered peptone water (BPW) exposure.

Fly Acquisition of Bacteria

Sterile manure was aliquoted (1.25 g) into triplicate sterile 50-ml conical tubes, and then inoculated just before fly exposure with 2.5 ml of 2.0×10^6 cfu/ml of either *E. coli* O157:H7 or *S. enterica* in 0.01% peptone or BPW and vortexed until no chunks of manure were visible (slurry). The tube was vortexed for an additional 10 s immediately before removing 75 µl, which was added to the 3.5 cm petri dish and spread with a sterile plastic spreader until the slurry formed a thin, even coat on the bottom of the dish. A single clipped-wing fly was placed on the slurry and allowed to walk freely for ~10 or 30 s of acquisition time. The only behaviors allowed on the acquisition plate were walking and feeding. Flies that deviated from these two behaviors were not used.

Both house flies and blow flies were tested. Five flies of each species were exposed to the control slurry and 10 each to either the *E. coli* O157:H7 or the *S. enterica* slurry for each replication. After that, the fly was removed and placed into a 5-ml screw cap tube with 1 ml of sterile peptone and homogenized with a sterile Hard Tissue Omni Tip using an Omni Tissue Homogenizer (Omni International, Kennesaw, GA). The homogenate was diluted 10-fold in peptone water and 100 µl was plated on LB AMP media. The plates were incubated at 37°C for 18–24 h and fluorescent colonies were enumerated. The experiment was replicated three times.

Timed Acquisition and Deposition of Bacteria to Lettuce Disks by Blow Flies

To better understand how short, casual contact with a plant surface could impact detachment of *E coli* and *S. enterica* cells from fly cuticle, bacteria-exposed blow flies were placed on two lettuce disks in quick succession (two 30-s exposures). Blow flies were selected because they are larger than house flies and easier to control in the de-winged state. Blow fly inoculation of bacteria to lettuce was tested with both *E. coli* O157:H7 and *S. enterica* prepared as in previous experiments.

Romaine lettuce heads were obtained from a local grocery store. Lettuce disks were prepared immediately before use by punching the leaf blade with the bottom of a sterile 3.5-cm petri dish. Lettuce disks were arranged adaxial side up in the dish bottom. Bacterial slurries were prepared as before to 10^6 cfu/ml in manure. Five individual de-winged blow flies were exposed to bacteria for 10 or 30 s as previously described, and then immediately processed by homogenation in 1-ml BPW and plated as described above to determine the initial bacterial load acquired by each fly. Immediately following this group, 10 test flies were individually exposed to the bacterial slurry and transferred immediately onto lettuce disks. Flies were precisely placed on Disk 1 using soft forceps for 30-s inoculation access period (IAP), then gently removed and placed on Disk 2 for a second 30-s IAP. Immediately after the second 30-s IAP, the flies were placed in capped conical tubes containing 1-ml BPW. Individual flies were then homogenized as previously described and plated in the duplicate. Lettuce disks ($n = 10$) were also exposed to control flies (exposed to manure without bacteria) and processed as described. Resulting fluorescing colonies were enumerated, and a proportion of recovered colonies was tested by PCR, as previously described, to confirm identity as either *E. coli* or *S. enterica*. This experiment was replicated three times for each bacterial species (total $n = 30$).

Statistical Analysis

Data were not normally distributed, and non-parametric Wilcoxon rank sum tests were used with pre-planned comparisons, with an alpha level of 0.05 used to determine significance (SAS 2008; SAS Institute).

Results and Discussion

Transmission of *E coli* O157:H7 and *S. enterica* to Whole Plants

Both house flies and blow flies could acquire both *E. coli* O157:H7 and *S. enterica* from contaminated manure sources and transfer them to caged lettuce plants, but with different levels of efficiency. Blow flies and house flies acquired more *E. coli* O157:H7 cells than *S. enterica* cells, but not significantly so. *Escherichia coli* cell numbers recovered from house fly and blow fly bodies were twice that of recovered S. enterica cells, but extremely high variation contributed to the lack of biological significance. This same pattern was reflected in the recovery of *E. coli* and *S. enterica* from the fly-exposed lettuce

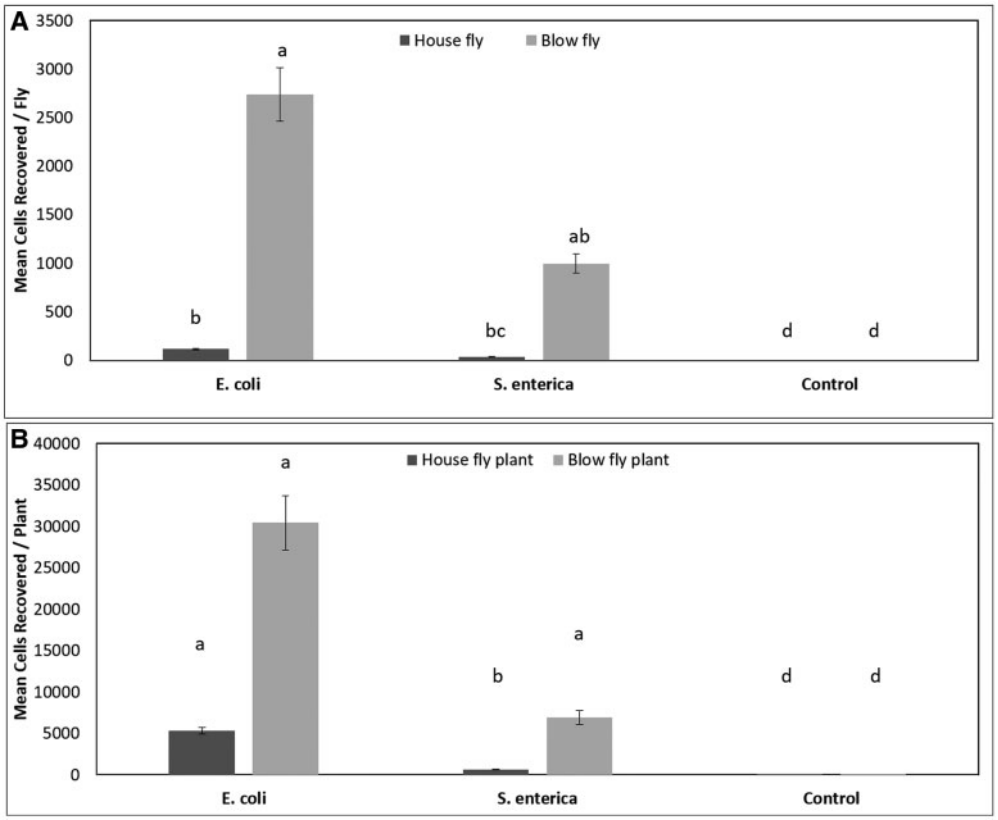

Fig. 1. (**A**) Mean number of *E. coli* O157:H7 and *S. enterica* cells recovered from individual blow flies, *Phormia regina*, and house flies, *Musca domestica*, after exposure to plants. (**B**) Mean number of *E. coli* O157:H7 and S. *enterica* cells recovered from individual plants exposed to bacteria-exposed flies (five flies per plant). Note that the scales of the Y axis are not the same. Error bars are standard error of the mean. Columns with different letters are significantly different at $P \leq 0.05$, determined by the Wilcoxon rank sum test.

plants (Fig. 1B). Blow flies and house flies deposited more *E. coli* cells onto lettuce than S. *enterica* cells, but with house flies, the difference was significant at $\alpha = 0.05$.

Interestingly, when comparing fly species for transmission efficiency, blow flies acquired significantly more *E. coli* cells and *S. enterica* cells than house flies (Fig. 1A). This did not translate into matching inoculation numbers: blow flies did transfer more *E. coli* cells to lettuce than house flies, but again, extremely high variation masked any possible significance. Lower variation observed in the recovered *S. enterica* cells resulted in significantly more cells transferred to lettuce by blow flies than by house flies (Fig. 1B).

Limitations in the experimental design may, in part, explain the high level of variation observed for recovered cells of both *E. coli* O157:H7 and *S. enterica* cells from individual flies and plants. The flies in the vector competence experiment were placed in large petri dishes in cohorts of 10 adults. During the 2-h exposure to the contaminated manure, several behaviors were observed that may have affected bacterial acquisition including feeding on the manure, walking on the manure, flying across the dish, avoiding the manure by walking only on the plastic walls/floor of the petri dish, landing on top of each other, grooming, regurgitating, defecating, copulating, or simply standing still. All of these activities alter the extent of contact with, and therefore the acquisition of, the target bacteria. These are all normal fly behaviors (West 1951) and presumably would contribute to the same natural variation in bacterial uptake by feral flies from the environment. This is supported by the acquisition and transmission percentages reported in Table 1. Not all flies tested positive for the pathogen they were exposed to and not all plants exposed to the flies tested positive. This was particularly evident for house flies where transmission efficiency for five flies per plant was only 60% and 36% (Table 1), and this result coincides with similar results obtained for house flies transmitting *E. coli* O157:H7 to whole spinach plants in which only 35% of the plants exposed to flies that acquired bacteria from contaminated manure were positive (Talley et al. 2009).

Table 1. Recovery of *E. coli* O157:H7 and *S. enterica* from individual house flies, blow flies, and test lettuce plants

Fly	% Positive flies		% Positive plants	
	E. coli O157:H7	*S. enterica*	*E. coli* O157:H7	*S. enterica*
House flies	72% (18/25)	52% (13/25)	60% (15/25)	36% (9/265)
Blow flies	88% (22/25)	80% (20/25)	64% (16/25)	72% (18/25)

Proportions in parentheses following percentages are the number of flies that tested positive by PCR over the number tested.

Although not measured in this study, distinct volatile production by *E. coli* O157:H7 and *S. enterica* may also have contributed to differential attraction by the two fly species to each bacterial source. *Escherichia coli* O157:H7 has a distinct volatile organic compound (VOC) profile compared with that of other bacteria (Tait et al. 2014). Indole, which is attractive to flies, particularly blow flies orienting to carrion (Liu et al. 2016), was detected in higher quantities for *E. coli* O157:H7 compared with two other bacterial species by

gas chromatography–mass spectrometry (Tait et al. 2014). Although *S. enterica* (*typhimurium*) also gives off indole, the VOC signature is much broader (Sohrabi et al. 2014). It is possible that other volatilized compounds reduced the attractiveness of *S. enterica*-contaminated manure. This could have resulted in more or less contact with the treated manure during the acquisition period.

Timed Acquisition of *E. coli* and *S. enterica* by Flies

To reduce the level of variation in bacterial acquisition observed in the first experiment, we gave flies very short (10 and 30 s) exposure times to manure slurries containing bacteria and enumerated the number of cells picked up by both fly species. As hypothesized, longer contact with contaminated manure resulted in greater recovery of bacterial cells. Both house flies and blow flies acquired more *E. coli* and *S. enterica* cells when exposed for 30 s compared with 10 s (Table 2). Comparing the two flies, blow flies were more efficient at acquisition of *E. coli* O157:H7 than house flies, picking up 90% to 32% more cells at 10 s and 30 s, respectively ($P = 0.05$ and 0.09, respectively). In contrast, house flies were significantly more efficient at acquiring *S. enterica* than blow flies, picking up significantly more cells at 10 s and 30 s ($P = 0.0001$ and 0.0001, respectively). Target bacteria were never recovered from either the control house flies or blow flies exposed to uncontaminated manure.

Table 2. Comparison of bacterial acquisition by house flies and blow flies exposed to *E. coli* O157:H7- and *S. enterica*-contaminated manure for 10 and 30 s

Fly	Exposure time (s)	*E. coli*	*S. enterica*	Control	*P* value
House flies	10	3342.5 (657.3)	6450.0 (1115.5)	0.0 (0.0)	0.0007
	30	7120.0 (1794.0)	7180.0 (1191.8)	0.0 (0.0)	0.6957
Blow flies	10	6325.6 (1132.3)	508.3 (257.0)	0.0 (0.0)	0.0407
	30	9696.8 (1925.4)	818.3 (146.2)	0.0 (0.0)	0.9684

Numbers in parentheses are standard errors of the mean. *P* values were determined using the Wilcoxon rank sum test and were considered significant at $P \leq 0.05$.

In these experiments, we have shown differential attachment of two bacterial species to flies. On plant surfaces, differential attachment of these two pathogens is also observed, but it is the *Salmonella* spp. that attach more readily to tomatoes (Zhang et al. 2014) and alfalfa sprouts (Barak et al. 2002) than *E. coli* O157:H7. The opposite was observed on banana leaves that have a very high surface wax content: *E. coli* O157:H7 attachment was significantly higher compared with that of *S. enterica* (Chua and Dykes 2013). Similar to some plant surfaces, insect cuticle is hydrophobic because of the cuticular waxes secreted onto the exoskeletal surface. This outer wax layer is composed of mainly non-polar components. Hydrocarbons compose a large part of the cuticular lipids present on the surface of insects (Nelson and Blomquist 1995). Like plants, flies differ in their external cuticular waxes (Golebiowski et al. 2015).

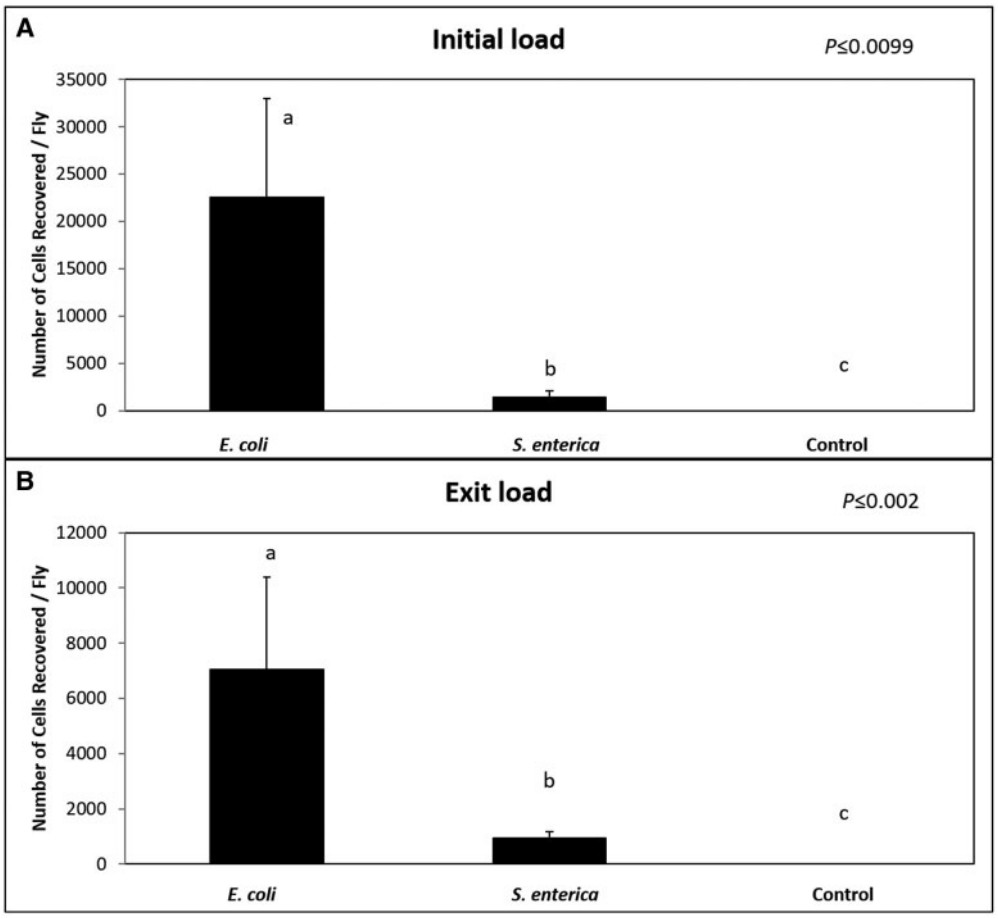

Fig. 2. Mean number of *E. coli* O157:H7 and *S. enterica* cells recovered from single blow flies, *Phormia regina*, exposed to bacterial slurries (**A**) before (initial load) and (**B**) after (exit load) two consecutive 30-s exposure periods on lettuce disks. Error bars are standard error of the mean. Columns with different letters are significantly different at $P \leq 0.05$, determined by using the Wilcoxon rank sum test.

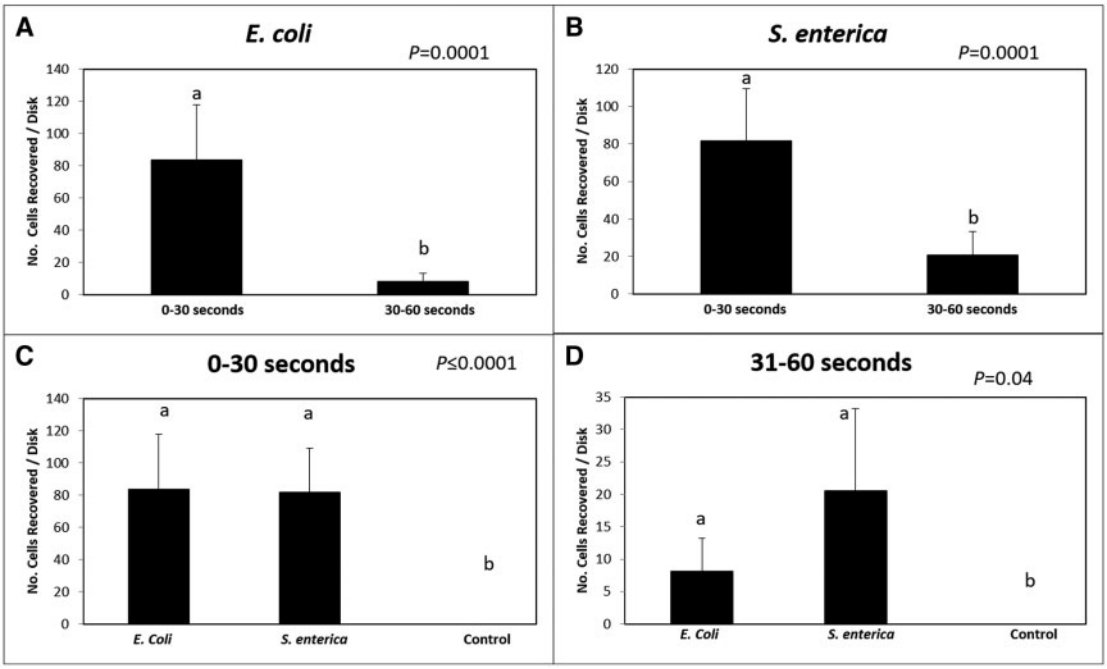

Fig. 3. Mean number of cells recovered from lettuce disks exposed to bacteria-exposed blow flies, *Phormia regina*. (**A**) Mean number of *E. coli* O157:H7 cells deposited on lettuce disks during the first 30-s exposure and the second 30-s exposure to single inoculative blow flies. (**B**) Mean number of *S. enterica* cells deposited on lettuce disk during the first 30-s exposure and the second 30-s exposure to single inoculative blow flies. (**C**) Comparison of *E. coli* O157:H7 and *S. enterica* cells deposited on lettuce disks during the first 30-s exposure. (**D**) Comparison of *E. coli* O157:H7 and *S. enterica* cells deposited on lettuce disks during the second 30-s exposure. Error bars are standard error of the mean. Columns with different letters are significantly different at $P \leq 0.05$, determined using the Wilcoxon rank sum test.

Blow fly cuticle is hard and shiny, whereas house fly cuticle is dull and more pliable. Analysis of the cuticular waxes of these two flies revealed the presence of 9-tricosene and oleoyl glycerol in blow flies, but none in house fly cuticle. There may be other compounds present on the cuticular surface, including sex pheromones. House flies and blow flies both have sex pheromones on the surface; therefore, males and females have different epicuticular hydrocarbon profiles (Pechal et al. 2014). These differences may cause the bacteria to differentially interact with the surface of male and female flies. The two species also have different hydrocarbon profiles (Nelson et al. 1981, Stoffolono et al. 1997, Pechal et al. 2014), possibly leading to the differences observed in acquisition and deposition between house flies and blow flies.

Timed Acquisition and Inoculation of *E. coli* and *S. enterica* to Lettuce Disks by Blow Flies

On the lettuce disks, flies were observed to be either only standing or briefly walking. In some instances, grooming behavior was observed, but rarely was either regurgitation or defecation noted. The initial load of bacteria acquired by the blow flies was significantly higher for *E. coli* than for *S. enterica* ($P = 0.01$; Fig. 2). Moreover, blow flies, on average, picked up >20,000 *E. coli* cells compared with 1,500 *S. enterica* cells. When flies were tested for their exit load (number of cells recovered after exposure to the two lettuce disks), they had fewer *E. coli* than *S. enterica* cells, but not significantly different ($P = 0.23$ and 0.49, respectively). For E. coli, the number of cells recovered from blow flies dropped from 22,000 (initial load) to 7,000 (exit load), whereas for S. entrica, the number dropped from 1,500 to 1,000 cells. The cells lost between the initial load and exit load cannot be accounted for by the number of cells recovered from the lettuce disks. Figure 3 showed the mean number of cells recovered from Disk 1 and Disk 2 for both *E. coli* and *S. enterica*. On average, only 80 cells were recovered from lettuce Disk 1 for both bacteria, and <20 cells were recovered from Disk 2, considerably short of the thousands of cells "missing" between the initial and exit loads of the flies. What is more interesting, however, is the difference in the numbers of deposited bacteria on Disk 1 and Disk 2. There was a 10× reduction in the number of *E. coli* cells ($P = 0.0001$) and a 4× reduction in the number of *S. enterica* cells ($P = 0.0001$) deposited on Disk 2. This suggests the following two possibilities: either fewer cells were present on the fly cuticle (tarsi, abdomen, and labellum) after exposure to Disk 1 or bacteria detached less readily from the cuticle after the first lettuce disk exposure.

Kobayashi's hypothesis of "bioenhanced" mechanical transmission is supported by these data. Simple mechanical transmission would predict equivalent levels of bacterial acquisition and inoculation, but blow flies could pick up more *E. coli* O157:H7 than house flies, suggesting that the interaction between blow flies and *E. coli* O157:H7 is more specific than that between house flies and *E. coli* O157:H7. In addition, the interaction between *S. enterica* and blow flies and that between *S. enterica* and house flies are different. Additional evidence points to blow flies as being more competent vectors of *E. coli* O157:H7 than *S. enterica* in a laboratory setting. They could acquire more *E. coli* O157:H7 than *S. enterica*. Blow flies are highly efficient vectors of *E. coli* O157:H7 (this study) and have been shown to occur in very high numbers in leafy greens where aphid populations are elevated (Talley et al. 2009). How these results will translate to blow flies' or house flies' differential transmission efficiency in a field setting remains to be determined, but they already support the findings of Maldonado and Centeno's (2003) danger-index that presents the larger calliphorid flies as a greater threat for pathogen transmission than the smaller *M. domestica*.

Acknowledgments

We thank Leah Vest, Kelsie Brooks, and Kasi Hamlin for technical assistance. This project was funded by United States Department of Agriculture National Integrated Food Safety Initiative # 2009-51110-05856 and by the Oklahoma Agricultural Experiment Station Project # OKL-2936.

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