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Transfer of generic *Escherichia coli* and attenuated *Salmonella enterica* Typhimurium from the soil to the surface of in-shell pecans during harvest

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ABSTRACT

During harvest pecan nuts are at risk of contamination with foodborne pathogens from extended contact with the ground. The objective of this study was to determine the potential transfer of *Escherichia coli* and *Salmonella* from the ground to in-shell pecans during the harvesting process. Plots (2 m^2) were sprayed with 1 L of a rifampicin (rif) resistant strain of either E. *coli* TVS 353 or an attenuated *Salmonella* Typhimurium inoculum at a low (~4 log CFU/ml), mid (~6 log CFU/ml) or high (~8 log CFU/ml) concentrations. The following day, nuts were mechanically harvested and samples from each plot were collected at 1 min, 4 h, and 24 h. Samples were enumerated for *Salmonella* and *E. coli* on tryptic soy agar supplemented with rif. The *Salmonella* levels in the soil from the inoculated plots were 2.0 ± 0.3, 4.1 ± 0.1, and 6.4 ± 0.2 log CFU/g for the low, mid, and high inocula, respectively. The *E. coli* levels in the soil from the inoculated plots were 1.5 ± 0.4, 3.7 ± 0.3, and 5.8 ± 0.1 log CFU/g for the low, mid, and high inocula, respectively. There was a significant difference in the average daily rainfall among the three trials. Trial 3 received 23.8 ± 9.2 cm, while trials 1 and 2 received much less (0.1 ± 0.1 0.0 ± 0.0 cm, respectively). Inoculation concentration and trial were significant ($P<0.05$) factors that influenced the transfer of *E. coli* and *Salmonella* to pecans. For the high inoculum treatment, bacterial transfer to pecans ranged from 0.7 ± 0.3 to 4.1 ± 0.2 for *E. coli* and 1.3 ± 0.7 to 4.3 ± 0.4 log CFU/g for *Salmonella*. For the medium inoculum treatment, transfer ranged from <0.3 to 1.5 ± 0.1 for *E. coli* and <0.3 to 1.9 ± 0.2 log CFU/g for *Salmonella*. For the low treatment, transfer ranged from <0.3 to 0.4 ± 0.2 and <0.3 to 0.5 ± 0.1 log CFU/g for *E. coli* and *Salmonella,* respectively. These results show the need for implementing agricultural practices that prevent potential transfer of foodborne pathogens onto the surface of in-shell pecans during harvest.

1. Introduction

In the past, tree nuts have been linked to foodborne pathogen outbreaks in the United States, the most common pathogens

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implicated with these outbreaks are *Salmonella* and *Escherichia coli* O157:H7 [1–3]. While pecans have not been implicated with any foodborne outbreaks in the United States, pecans have been recalled due to pathogen contamination [4]. Likewise, *Salmonella* and Shiga toxin-producing *E. coli* (STEC) have been isolated from in-shell pecans harvested in North America [5–7]. Due to the manner in which they are harvested, pathogen contamination of in-shell pecans is likely to occur during harvest [8]. It is a common practice for livestock, such as cattle to graze in a pecan orchard [6]. This practice provides benefits, such as weed management, secondary land use and income, and soil fertility [9], to the pecan production but presents a risk in terms of pathogen contamination.

Pecans, like other tree nuts are generally harvested mechanically by shaking the trees to dislodge the mature nuts which come into direct contact with the ground of the orchard, where they are subsequently gathered using sweepers. The process presents a potential point of contamination as the pecans are in contact with the ground for what could be up to 24–48 h depending on the operation [10]. The contact of almonds with the ground during harvest was a potential source of contamination for a previous outbreak of *Salmonella* associated with almonds in 2000–2001 [11]. *Salmonella* is capable of surviving in the soil of almond and pecan orchard soils for over 120 days [12,13]. Furthermore, *Salmonella* and STEC have been isolated from the soil of pecan orchards presenting a potential route of contamination. Once on the pecan shell, foodborne pathogens have been known to survive for long periods of time with little to no population reduction [13–15]. As pecans are shelled and often consumed raw with generally no kill step, the control for potential foodborne pathogen contamination during harvest and shelling is essential.

The mechanisms of pathogen transfer to the surface of in-shell pecans are uncharacterized but need investigation to understand the risk of contamination during harvest. To the authors' knowledge, there are no studies that evaluate the transfer of bacteria to in-shell pecans during harvest. Therefore, the objective of this study is to evaluate the potential for the transfer of *Salmonella* and *E. coli* onto pecan shells during the harvest process and determine if contamination level and contact time with the ground influence the transfer.

2. Material and methods

2.1. Experimental design and plot layout

This experiment was conducted at the Southeastern Fruit and Tree Nut Research Station in Byron, Georgia. A block of six pecan (*Carya illinoinensis,* cultivar Stuart) trees within an orchard were selected and sectioned off to control access and through traffic. A total of 21 × 2-m^2 plots were placed under the trees. Each plot was surrounded by a 1-m-tall black plastic barrier. Three trials were conducted on separate days: trial 1 ran from 14 to November 16, 2022, trial 2 from 16 to November 18, 2022, and trial 3 from 29 November to December 1, 2022. Weather variables such as daily maximum temperature, daily minimum temperature, daily relative humidity, daily 5 cm soil temperature, and daily rainfall were collected on the day the plots were inoculated to the last sampling event from a weather station located approximately 215 m from the experimental block (Table 1; https://georgiaweather.net/). For each trial 7 plots placed under two of the pecan trees were randomly designated with one of the following treatments: a control, low *E. coli* inoculum, mid *E. coli* inoculum, high *E. coli* inoculum, low *Salmonella* inoculum, mid *Salmonella* inoculum, and high *Salmonella* inoculum treatments, respectively.

2.2. Bacterial cultures and inoculum preparation

A generic *E. coli* strain TVS 353 (irrigation water; [16]) and an attenuated *Salmonella* Typhimurium strain UK-χ3985 (poultry; [17]) were used in the study. Both strains were conditioned to grow in the presence of 80 μg/ml of rifampicin (TCI Chemicals, Tokyo, Japan) as previously described [18]. The following inoculum procedures were performed for both *E. coli* and *Salmonella* separately. Prior to each trial, each strain was streaked from a stock culture onto tryptic soy agar (TSA; Neogen, Lansing, MI) supplemented with 80 μg/ml of rifampicin (TSA-R) and incubated 24 h at 37 °C. Following incubation three isolated colonies were transferred into a centrifuge tube containing 25 ml of tryptic soy broth (TSB; Remel, Lenexa, KS) supplemented with 80 μg/ml of rifampicin and incubated 24 h at 37 °C. The 25 ml culture was centrifuged (Sorvall ST 16R Centrifuge, Thermo Scientific, Waltham, MA) at 4000 rpm for 10 min. The supernatant was discarded, and the pellet was resuspended in 25 ml of 0.1% peptone water (Oxoid, Hampshire, UK) by vortexing. An additional 25 ml of 0.1% peptone was added to the culture for a final 50 ml volume.

The bacterial suspension (~10 log CFU/ml) was diluted to achieve three different inoculum treatments: low, mid, and high. This was achieved by adding 50 ml of the pure culture to 950 ml of sterile deionized water for the high inoculum treatment (~8 log CFU/

Table 1
The average daily weather data for each of the three days for the three trials following pecan plot inoculation with *Escherichia coli* strain TVS 353 or an attenuated *Salmonella* Typhimurium strain.[a]

Trial	Max Temp (°C)	Min Temp (°C)	Relative Humidity (%)	5 cm Soil Temp (°C)	Rainfall (cm)
1	14.1 ± 0.4 ab[b]	5.5 ± 4.1 a	72.7 ± 14.6 a	11.9 ± 1.2 a	0.1 ± 0.1 b
2	13.3 ± 1.0 b	2.3 ± 4.3 a	55.9 ± 9.7 a	9.6 ± 2.2 a	NP[c] b
3	19.9 ± 4.2 a	3.8 ± 4.2 a	65.5 ± 15.9 a	12.9 ± 3.2 a	23.8 ± 9.2 a

[a] Values represent the mean daily value for each weather measurement across the three days for each trial ± standard deviation across daily values.
[b] Different letters (a and b) represent significant differences between the rows for the different trials. Differences were considered significant at $P \leq 0.05$.
[c] NP = No precipitation.

ml). The pure culture was diluted by adding 5 ml of the pure culture to 45 ml of 0.1% peptone, with a total of two and four dilutions to achieve the mid and low treatment inoculum, respectively. The two diluted 50 ml cultures were added to 950 ml of sterile deionized water for the mid and low inoculum treatments (~6 and ~4 log CFU/ml respectively). Inoculum concentrations were determined by performing standard dilutions in 9 ml 0.1% peptone blanks and plating on TSA-R for both *E. coli* and *Salmonella* inoculums.

2.3. Plot inoculation

Prior to inoculation, the plots were cleared of visible debris (e.g. leaves, branches, pecans) that may have fallen into the plot. Each plot was sprayed with the designated treatment as previously described [19]. Briefly, 1 L of the *E. coli* or *Salmonella* inoculums was sprayed approximately 7–10 cm from the ground to avoid wind drift onto the designated 2-m^2 plot in increasing concentration (low to high) using a 15-L battery powered backpack sprayer (HD Hudson, Lowell, MI). Between the *E. coli* and *Salmonella* inoculums the sprayer was sterilized with 70% ethanol through the system and flushed with sterile deionized water to remove any residue. For the control plot, 1 L of sterile deionized water was sprayed onto the plot. Immediately following inoculation, a composite soil sample (approximately 40 g) was collected with a sterile scoop from three random locations within the plot taking the top 2 cm and combining to make a composite sample. Once the soil samples were collected, plots were covered with black plastic to prevent leaves, branches, or nuts from falling into the plots overnight. The *E. coli* and *Salmonella* bacteria in the composite soil samples were enumerated as previously described [20]. Briefly, 25 g of soil was removed from the composite samples and diluted by adding 100 ml of 0.1% peptone water. The soil samples were homogenized by hand for 30 s, serially diluted, plated onto TSA-R, incubated at 37 °C for 24 h, and enumerated following incubation.

2.4. Pecan harvesting and sample collection

The coverings were removed from the plots prior to the trees being harvested. Each tree was shaken via a mechanical shaker to dislodge the nuts from the tree. To ensure that a sufficient number of nuts fell into each plot for sampling purposes, nuts that fell outside of the plots during harvesting were added using sterile glove to ensure contamination did not occur into plots that did not have a sufficient number for sampling (18 total nuts). Nut samples were collected approximately 1 min, 4 h, and 24 h following harvest. Samples were collected by aseptically transferring two nuts (approximately 20 g) into one bag. Three samples were collected from each treatment at each timepoint except for the control which only had one sample collected at each timepoint.

2.5. Enumeration of bacterial transfer

Samples were enumerated <30 min following collection. Bacterial transfer enumeration followed a modified sampling procedure previously described [21]. Briefly, pecan samples were weighed and a 1:2 w/v dilution was performed by adding an equivalent volume of 0.1% peptone to the weight of the pecans. Samples were rinsed by rubbing the surfaces of the pecans and agitating by hand for 90 s. Standard dilutions were performed in 9 ml 0.1% peptone blanks and samples were plated on to TSA-R. Plates were incubated at 37 °C for 24 h and enumerated the following day. To obtain the limit of detection (<0.3 log CFU/g), 250 μl of the 1:2 diluent was plated onto four TSA-R plates and the counts were combined between the four plates. No growth was observed on any of the control plates at the limit of detection.

2.6. Statistical analysis

The experiment was conducted in triplicate with three separate trials performed and analyzed individually. A generalized linear model (GLM) was performed with a Chi square effects test to determine factors that significantly influence bacterial transfer. Inoculation treatment (low, mid, and high), trial, timepoint (1 min, 4 h, and 24 h post-harvest) and organism (*Salmonella* and *E. coli*) were all factors in the linear model. Further analysis was performed on significant factors with an analysis of variance table and performing a Tukey's honestly significant difference test ($\alpha = 0.05$). An analysis of variance table and Tukey's honestly significant difference test ($\alpha = 0.05$) were performed on the weather data that was collected. All statistical analyses were performed using JMP® Pro 15 statistical software (version 15.2.0, SAS Institute Inc., Cary, NC).

3. Results

3.1. Weather data for each trial

There was no significant difference ($P>0.05$) between the daily average minimum temperature, daily average relative humidity, and the daily average 5 cm soil temperature (Table 1). The average maximum temperature for trial 2 was significantly lower ($P \leq 0.05$) than the average maximum temperature for trial 3 which was 13.3 ± 1.0 and 19.9 ± 4.2 °C respectively. The greatest difference was observed in the daily average rainfall among the three trials. The soil was visibly saturated following rain events regardless of canopy cover and plastic cover, especially for trial 3 where more rainfall was observed. There was significantly more ($P \leq 0.05$) daily rainfall in trial three (23.8 ± 9.2 cm) compared to trials 1 and 2 (0.1 ± 0.1 cm and no precipitation, respectively).

3.2. Transfer of E. coli and salmonella onto pecans during harvesting

Inoculation level and trial period were both significant factors ($P \leq 0.05$) affecting the transfer of bacteria to the surface of inshell pecans during harvest. Organism (*Salmonella* and *E. coli*) and timepoint were not significant factors ($P > 0.05$) in the model. The *Salmonella* soil inoculation levels were 2.0 ± 0.3, 4.1 ± 0.1, and 6.4 ± 0.1 log CFU/g for the low, mid, and high inoculation treatments, respectively across all three trials combined. The *E. coli* soil populations were 1.5 ± 0.4, 3.7 ± 0.3, and 5.8 ± 0.1 log CFU/g for the low, mid, and high inoculation treatment, respectively across all three trials combined. Since trial was a significant factor, the bacterial counts on the pecans were differentiated by trial and will be presented separately for comparison.

For the low *Salmonella* inoculated plots, four of the samples were below the LOD (<0.3 log CFU/g) while the bacterial counts transferred to the pecans ranged from 0.3 ± 0.1 to 0.5 ± 0.2 log CFU/g for the remaining pecan samples (Table 2). No significant differences ($P > 0.05$) in bacterial counts on the pecans were observed between the different trials for the low *Salmonella* inoculation treatment. For the mid *Salmonella* inoculation treatment, only one sample was below the LOD, the transfer ranged from 0.3 ± 0.1 to 1.9 ± 0.3 log CFU/g for the remaining pecan samples. Trial 1 had significantly higher ($P \leq 0.05$) bacterial counts compared to trials 2 and 3 at the 4 h (1.9 ± 0.3, 0.8 ± 0.5, and 0.3 ± 0.1 log CFU/g respectively) and 24 h (1.6 ± 0.3, 0.6 ± 0.3, and <0.3 log CFU/g respectively) timepoints, while there was no significant difference in bacterial counts between the trials at the 1 min timepoint for the mid inoculation treatment. For the high *Salmonella* inoculation treatment, the bacterial counts ranged from 1.3 ± 0.9 to 4.3 ± 0.5 log CFU/g. There were significant differences in bacterial counts between the trials at all three timepoints for the high inoculation treatment. Pecan samples from trials 1 and 2 had higher *Salmonella* transfer compared to samples collected in trial 3 for all three timepoints in high inoculation treatment. The *Salmonella* bacterial counts were significantly higher ($P \leq 0.05$) on pecans in the high inoculated treatment compared to the mid and low inoculation treatments for all three time periods (Fig. 1A).

With the low *E. coli* inoculation treatment, five of the samples were below the LOD (<0.3 log CFU/g), while the bacterial counts ranged from 0.3 ± 0.1 to 0.4 ± 0.2 log CFU/g for the remaining pecan samples (Table 3). No significant differences ($P > 0.05$) in bacterial counts were observed between the different trials for the low *E. coli* inoculation treatment. For the mid *E. coli* inoculation treatment, only one sample was below the LOD, and the transfer rate ranged from 0.3 ± 0.1 to 1.5 ± 0.2 log CFU/g. Trial 1 had significantly higher ($P \leq 0.05$) bacterial counts (1.5 ± 0.2 log CFU/g) compared to trials 2 and 3 (0.5 ± 0.2 and 0.6 ± 0.3 log CFU/g, respectively) at the 1 min timepoint, but at the 4 h timepoint there was no significant difference between the trials, and at the 24 h timepoint trials 1 and 2 had significantly higher bacterial counts (1.2 ± 0.3 and 1.0 ± 0.3 log CFU/g, respectively) compared to trial 3 (0.3 ± 0.1 log CFU/g) for the mid *E. coli* inoculation treatment. With the high *E. coli* inoculation treatment, the bacterial counts ranged from 0.7 ± 0.4 to 4.1 ± 0.2 log CFU/g. Trial 1 had significantly higher bacterial counts (4.1 ± 0.2 log CFU/g) compared to trails 2 and 3 (2.2 ± 0.1 and 2.5 ± 1.0 log CFU/g, respectively) at the 1 min timepoint, and trials 1 and 2 had significantly higher *E. coli* bacterial counts compared to trial 3 at the 4 h (3.6 ± 0.4, 2.8 ± 0.7, and 0.9 ± 0.3 log CFU/g, respectively) and at the 24 h (3.4 ± 0.4, 3.5 ± 0.3, and 0.7 ± 0.4 log CFU/g, respectively) timepoints for the high inoculation treatment. The *E. coli* bacterial counts were significantly higher ($P \leq 0.05$) on pecans with the high inoculation treatment compared to the mid and low inoculation treatments for all three time periods and trials (Fig. 1B).

4. Discussion

These results show that population levels contribute to the transfer of foodborne bacteria like *E. coli* and *Salmonella* onto the surface of in-shell pecans during harvesting procedures. Time in contact with the inoculated surface was not a significant factor and did not influence bacterial counts suggesting that most transfer occurs during harvest when the pecans came in contact with the ground. Previous research has found in-shell pecans positive for foodborne pathogens including *Salmonella* and STEC [5,6]. The results from the current study found bacterial population transfers was as high as 4.3 ± 0.5 and 4.1 ± 0.2 log CFU/g for both *Salmonella* and *E. coli* respectively in little as 1 min after harvest. In a study that evaluated the quantity of *Salmonella* on in-shell pecans in the US market, the mean populations were 2.4 MPN/100 g [5]. Contamination of orchards and harvesting environments may occur from a number of different sources such as from various species of wildlife, animal intrusion, livestock grazing, or flooding [6,22,23]. In a study conducted by Diaz et al., 11.1 and 24.4% of the pecans collected were positive for STEC and *Salmonella* respectively in orchards that were grazed by livestock, while 6.7 and 0.0% of pecans were positive for STEC and *Salmonella* when collected from orchards that had no

Table 2
The transfer of an attenuated *Salmonella* Typhimurium strain to in-shell pecans from soil inoculated with various concentrations of the bacteria and sampled at different time intervals following harvesting for each of the three trials.[a]

	1 min			4 h			24 h		
Trial	Low	Mid	High	Low	Mid	High	Low	Mid	High
1	<0.3 a	1.8 ± 0.8 a	4.3 ± 0.5 a[b]	0.3 ± 0.1 a	1.9 ± 0.3 a	3.5 ± 0.2 a	0.5 ± 0.2 a	1.6 ± 0.3 a	3.0 ± 0.5 a
2	<0.3 a	0.8 ± 0.5 a	2.8 ± 0.1 ab	0.3 ± 0.1 a	0.8 ± 0.5 b	2.6 ± 0.3 b	<0.3 a	0.6 ± 0.3 b	3.7 ± 0.5 a
3	0.5 ± 0.4 a	0.7 ± 0.8 a	1.7 ± 1.2 b	<0.3 a	0.3 ± 0.1 b	1.7 ± 0.3 c	0.3 ± 0.1 a	<0.3 b	1.3 ± 0.9 b

[a] Values represent the mean population (log CFU/g ± standard deviation) transferred from triplicate samples (n = 3) for each trial and timepoint. Samples represented as <0.3 were below the limit of detection.

[b] Different letters represent significant differences between the rows for trials 1, 2, and 3 (a, b, and c). Differences were considered significant at $P \leq 0.05$.

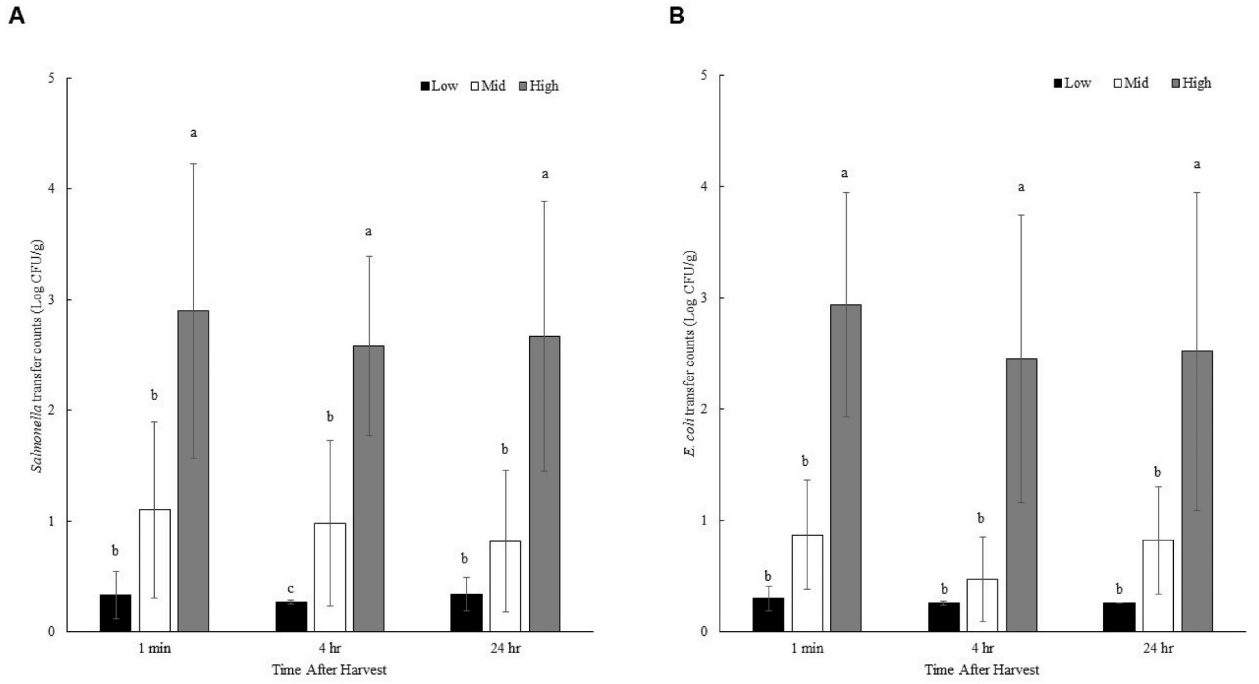

Fig. 1. The average (n = 9) transfer of *Salmonella* (A) and *E. coli* (B) counts (log CFU/g ± standard deviation) on to pecans from high (grey bars), mid (white bars) and low (black bars) inoculated plots at 1 min, 4 h, and 24 h after harvest. Letters (a, b, and c) represent significant differences between plot inoculation levels at each timepoint ($P \leq 0.05$).

Table 3
The transfer of *Escherichia coli* strain TVS 353 to in-shell pecans from soil inoculated with various concentrations of the bacteria and sampled at different time intervals following harvesting for each of the three trials.[a]

	1 min			4 h			24 h		
Trial	Low	Mid	High	Low	Mid	High	Low	Mid	High
1	0.4 ± 0.2 a	1.5 ± 0.2 a[b]	4.1 ± 0.2 a	<0.3 a	0.8 ± 0.6 a	3.6 ± 0.4 a	<0.3 a	1.2 ± 0.3 a	3.4 ± 0.4 a
2	<0.3 a	0.5 ± 0.2 b	2.2 ± 0.1 b	0.3 ± 0.1 a	0.4 ± 0.2 a	2.8 ± 0.7 a	<0.3 a	1.0 ± 0.3 a	3.5 ± 0.3 a
3	0.3 ± 0.1 a	0.6 ± 0.3 b	2.5 ± 1.0 b	<0.3 a	<0.3 a	0.9 ± 0.3 b	<0.3 a	0.3 ± 0.1 b	0.7 ± 0.4 b

[a] Values represent the mean population (log CFU/g ± standard deviation) transferred from triplicate samples (n = 3) for each trial and timepoint. Samples represented as <0.3 were below the limit of detection.

[b] Different letters represent significant differences between the rows for the different trials 1, 2, and 3 (a, b, and c). Differences were considered significant at $P \leq 0.05$.

livestock grazing [6].

Based on the results of this study reducing food-borne pathogens to as low a level as possible in pecan orchards could therefore reduce the risk of transfer of pathogens to in-shell pecans during harvest. Pecans sampled from the low inoculated plots were below the limit of detection (<0.3 log CFU/g) in 4 and 6 of the 9 sampling periods for *Salmonella* and *E. coli* respectively. The most transfer that occurred from the low plots was 0.5 ± 0.4 and 0.4 ± 0.2 log CFU/g for *Salmonella* and *E. coli* respectively. Limiting factors that may introduce potential sources of food-borne pathogen contamination could reduce the risk of pathogen transfer events from occurring. Programs such as the USDA Good Agricultural Practices & Good Handling Practices audit program requires that untreated manure (manure that has not gone through composting or a similar treatment process) needs to be applied at a minimum of 120 days prior to harvest [24]. Along the same lines, if animals are grazing in orchards, then removing them at least 4 months prior to harvesting pecans should be considered to reduce risk. While pecans are exempt from the FSMA Produce Safety Rule, the Produce Safety Alliance has provided the recommendation of at least 60 days between flooding and replanting of harvestable food crops [25]. However, if contaminated, pathogens such as *Salmonella* and STEC may survive for long periods of time on the surface of in-shell pecans and pecan kernels [13–15]. If this is the case, then measures must be taken to ensure that pecan kernels do not become contaminated during the shelling process. Previous research has determined hot water and steam treatments to be effective mitigation strategy to reduce microbial contamination on in-shell pecans while maintaining kernel quality [26,27].

Due to various weather effects between the trials, *Salmonella* and *E. coli* transfer could potentially be influenced by environmental factors. Weather factors including relative humidity, air temperature, and dew point have been found to influence the survival of *Salmonella* and *E. coli* in agricultural soil and water [17,28]. While precipitation was a factor that influenced the likelihood of detecting

Salmonella in fresh produce farms [23]. Environmental factors such as low relative humidity have been shown to reduce *Salmonella* populations on the surface of in-shell almonds [29]. Differences in transfer of both *Salmonella* and *E. coli* were observed between trials likely due to heavy rainfall. While environmental factors were determined to potentially influence the transfer of pathogens to the surface of pecan shells, further research is needed to further evaluate these and other factors such as soil moisture and shell moisture. Many factors including pecan cultivar, geographic location, and weather, and availability of harvesting equipment will dictate when pecans are harvested. These factors present a wide range of potential environmental conditions and potential exposure times faced during the harvesting period.

5. Conclusion

The transfer of *Salmonella* and *E. coli* to in-shell pecans during harvest presents a risk for potential product contamination. Proper good agricultural practices are necessary to maintain or reduce the risk of contamination of foodborne pathogens. While bacterial transfer is possible, there are control procedures available including sterilizing pecan shells following harvest that will assist in preventing the contamination of pecan kernels during the subsequent shelling process.

Author contribution statement

Cameron A. Bardsley: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Kaicie Chasteen: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

David Shapiro-Ilan, Clive H. Bock: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Brendan A. Niemira: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Govindaraj Dev Kumar: Contributed reagents, materials, analysis tools or data.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors confirm that the work is original and has not been previously published or under consideration or review elsewhere. Likewise, all authors have approved the submission of this work.

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