Airborne Transport of Foodborne Pathogens from Bovine Manure to Vegetable Surfaces

Thesis

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By

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Abstract

Contamination of produce is a critical food safety issue and may result from airborne bacteria transmitted during manure application. We conducted a field experiment to determine the distance pathogens can be transferred by air during manure application, and the survival of the pathogens on produce. Romaine lettuce and slicing tomatoes were planted in rows in plots (8 m^2) that were arranged in a completely random design. Liquid dairy manure was spread in a 5-m-wide band next to and perpendicular to the end of the rows. Agar plates, located 24 cm above the ground and at the point nearest the manure spreading (0 m downwind), 15, 30, and 122 m downwind, and 15 m upwind, were left open for 5-15 min after application. Lettuce leaves and tomato fruits were collected before and 15 min after application, then on post application days 1, 3, 5, and 7. Vegetable samples were agitated with PBS, and the resulting solutions were plated. All plates were incubated at 37°C for 36 h, and bacterial colonies (CFU/ml) were counted. The number of airborne bacteria on open agar plates was highest at 0 m downwind. Bacteria decreased between 0 and 30 m downwind ($P<0.05$). Number of bacteria was consistently higher on lettuce leaves than on tomato fruits ($P<0.001$). Counts on lettuceleaves often peaked on Day 5 ($P<0.05$); counts on tomato fruits peaked on Day 7 ($P<0.001$). Low temperature, high relative humidity, and high rainfall may have contributed to high bacteria counts on vegetables ($P<0.001$). From this study's results, we can infer that airborne bacteria transport from liquid manure decreases between 0 and 30 m downwind, that survival of bacteria on vegetables may be related to weather conditions, and that lettuce leaves may capture more airborne bacteria than tomato fruits. Our research provides evidence that

spreading of liquid dairy manure closer than 122 m to a vegetable field may contribute to contamination of field-grown produce.

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Chapter 1: Introduction, Objectives, and Scope

In recent years, many outbreaks of foodborne illness associated with fresh fruits and vegetables have occurred. Increased consumption of fresh produce (resulting in a fourfold increase in fresh fruit and vegetable sales in the United States over the past few years) *(20, 40)*, as well as the lack of cooking and industrial processing involved in produce preparation, have contributed to this phenomenon. Twenty-two percent of all food-related outbreaks in the US between 1990 and 2004 stemmed from eating fresh produce, making produce the second most common source of foodborne illness after seafood *(16)*. It is estimated that every year, 17% of all Americans may contract an illness from fresh fruit and vegetable consumption *(38)*. Outbreaks of diseases caused by coliforms, such as the entohemorrhagic *Escherichia coli* O157:H7, have been linked to consumption of leafy greens, particularly spinach *(6)* and lettuce *(17, 23, 22)*. Tomatoes are also frequent sources of foodborne illness: in 1990, 176 cases of *Salmonella javiana* infection were traced to raw tomato consumption *(19, 54)*, and in 1993, tomatoes were identified as the source of a multistate outbreak of *Salmonella* serotype Montevideo *(19, 7)*.

Produce contamination may result from pre-harvest produce contact with raw livestock manure *(3)*. Livestock, particularly cattle, shed copious numbers of pathogens in their waste. When untreated animal waste is applied to vegetable fields, pathogens may be transferred to fruit and vegetable surfaces *(16, 25, 36)* and cannot be removed by postharvest washing *(15)*. It has been

demonstrated that after *E. coli* O157:H7 is transferred to the surfaces of lettuce leaves, it may become caught in stomata and penetrate though torn or cut leaf edges into the inner leaf tissues *(50, 8, 47)*. Some researchers have provided evidence that the bacterium also enters the plant's inner tissue though the roots *(15, 17, 50)*, although this mechanism remains controversial. Because *E. coli* can persist in many different environments, even under low temperatures and in acidic conditions *(30)*, it is often detected both in the soil and on produce for extended periods of time. In one case, *E. coli* O157:H7 persisted for 154 days in raw-manure-amended soil samples in which lettuce was grown *(25)*. Other studies have demonstrated that *E. coli* O157:H7 may persist in cattle feces for 49 to 56 days at 22°C *(55, 51)*.

Bacteria can be transferred to vegetables not only though soil and water, but though air, via aerosolization. Aerosolization occurs when droplets evaporate completely or close to dryness; bacteria in these droplets become solid particles known as bioaerosols *(11)*. Aerosolization of microorganisms is therefore most relevant where spreading slurry is concerned. Survival of aerosolized bacteria is susceptible to many environmental factors, but the three most significant are relative humidity (RH), temperature, and solar irradiance *(11)*. Studies have demonstrated that survival of bacteria decreases as RH decreases, because many microorganisms become deactivated when they become dehydrated. Survival of bacteria also decreases with increases in temperature and solar irradiance *(11)*.

Many studies have focused on airborne transport of bacteria though aerosolization *(11),* and other experiments describe dust deposition from manure sources *(21, 18)*, but little research has been conducted on how these processes affect produce contamination. Most of the previous studies cited here are concerned with bacteria transfer from soil or water to crop

surfaces. The present research was conducted to explore the process of airborne contamination and how it relates to aerosolization, as well as survival of airborne pathogens on crop surfaces.

Despite the lack of information on produce contamination by airborne microorganisms, it is important to determine clear standards for planting distances from contamination sources and to ensure vegetable growers' awareness of the risk. To this end, two primary aims to this research were established:

Aim 1: To determine a standard safe distance from a contamination source at which to grow produce.

Aim 2: To provide empirical data that can be used to educate Midwestern vegetable farmers about the risk of airborne produce contamination.

GAP (Good Agricultural Practice) metrics for growing both leafy green and tomato have been developed to assess use of adjacent land and proximity of contamination sources. The California Leafy Greens Marketing Agreement (LGMA) recommends that leafy greens be planted at least 122 m downwind from livestock, compost, or sewage treatment facilities *(5)*; unfortunately, scientific data were unavailable to support this recommendation *(4)*. In addition, because weather conditions, farm size, and crop diversity differ between California and the Midwest *(32*, *31)*, it was unknown whether this recommendation, which might be effective for California produce farmers, would be relevant to Midwestern produce farmers.

A 2009 survey conducted to determine Midwestern vegetable farmers' knowledge of GAPs demonstrated that the risks of airborne pathogen transport were not highly regarded *(26)*. Two hundred and nine Midwestern farmers completed the survey. In response to a question about the importance of various causes of pre-harvest contamination, 84.5% of the respondents strongly agreed that raw (completely untreated) manure amendments were primary sources of produce contamination, 84% agreed that poor worker hygiene was a primary source, and 73.4% agreed that irrigation water was a primary source. In contrast, 48.7% agreed that off-site dust was a primary source of pre-harvest contamination, and 60% agreed that proximity to livestock and poultry farms was a risk factor. Clearly, though the vegetable growers who participated in this survey had some idea of the dangers of contamination from off-site dust and from proximity to livestock and poultry farms, they rated the risk from such sources significantly lower than that from more well-documented sources *(26)*. It is hoped that providing more specific data on airborne dispersal of pathogens, as well as survival of pathogens on vegetables, will help determine appropriate recommendations for the Midwest, and provide empirical basis for education aimed at Midwestern produce farmers about the dangers of airborne pathogen transport.

Chapter 2: Research

Abstract

Contamination of produce is a critical food safety issue and may result from airborne bacteria transmitted during manure application. We conducted a field experiment to determine the distance pathogens can be transferred by air during manure application, and the survival of the pathogens on produce. Romaine lettuce and slicing tomatoes were planted in rows in plots (8 m^2) that were arranged in a completely random design. Liquid dairy manure was spread in a 5-m-wide band next to and perpendicular to the end of the rows. Agar plates, located 24 cm above the ground and at the point nearest the manure spreading (0 m downwind), 15, 30, and 122 m downwind, and 15 m upwind, were left open for 5-15 min after application. Lettuce leaves and tomato fruits were collected before and 15 min after application, then on post application days 1, 3, 5, and 7. Vegetable samples were agitated with PBS, and the resulting solutions were plated. All plates were incubated at 37°C for 36 h, and bacterial colonies (CFU/ml) were counted. The number of airborne bacteria on open agar plates was highest at 0 m downwind. Bacteria decreased between 0 and 30 m downwind ($P<0.05$). Number of bacteria was consistently higher on lettuce leaves than on tomato fruits ($P<0.001$). Counts on lettuce leaves often peaked on Day 5 ($P<0.05$); counts on tomato fruits peaked on Day 7 ($P<0.001$). Low temperature, high relative humidity, and high rainfall may have contributed to high bacteria counts on vegetables ($P<0.001$). From this study's results, we can infer that airborne bacteria transport from liquid manure decreases between 0 and 30 m downwind, that survival of

bacteria on vegetables may be related to weather conditions, and that lettuce leaves may capture more airborne bacteria than tomato fruits. Our research provides evidence that spreading of liquid dairy manure closer than 122 m to a vegetable field may contribute to contamination of field-grown produce.

As consumption of fresh produce has increased, incidences of produce-related foodborne illness have also increased. In fact, statistics have shown that 17% of American consumers may become ill every year from eating fresh produce *(38)*. Leafy greens, including lettuce *(17, 23, 22)*, are frequent sources of coliforms such as *Escherichia coli*, which are transferred to lettuce leaf surfaces and may penetrate though torn or cut leaf edges into the inner tissue *(52, 8, 47)*. Tomatoes are often sources of *Salmonella* infection *(19, 54, 7)*.

Many of these pathogens are shed in livestock manure and may persist for extended lengths of time; *E. coli* O157:H7 has been detected in manure-amended soil for 154 days *(25)*, and in untreated cattle manure for 49 to 56 days *(45, 51)*. Contaminated manure may come into contact with vegetables when it is spread on the field *(15, 25, 34)*, washed into irrigation water, or transferred though air by aerosolization, when droplets of liquid manure evaporate *(11)*. Both dispersal and survival of bacteria are affected by temperature, relative humidity (RH), rainfall, and solar irradiance *(11)*.

We designed this study to explore transfer of bacteria though aerosolized manure and persistence of bacteria on vegetables surfaces. We hope that the research will provide data that can be used to develop new food safety standards.

We developed specific hypotheses focusing on the distance of bacteria transfer from a manure application point and bacteria survival over 7 days post-application. We hypothesized that bacteria transfer would decrease between the point of manure application and rows of vegetables 40 m downwind. It was predicted that manure-based pathogens would not be detected 122 m downwind from the point of manure application. This hypothesis was based on evidence from a previous experiment, in which aerosolized microorganisms from a swine operation had decreased 99% at 100 m downwind from the operation *(18)*.

We predicted that number of pathogens on both lettuce leaves and tomato fruits would increase as time passed, drawing on results from a 2009 study in which *E. coli* O157:H7 transfer from soil to lettuce was examined *(34)*. Difference in bacteria counts between lettuce leaves and tomato fruits was expected, because of the difference in surface area between leaves and fruits.

Materials and Methods

Research was conducted at the Ohio Agricultural Research and Development Center (OARDC), Wooster, Ohio, in 2012 and 2013. Transplants of 600 slicing 'BHN602' tomato, 800 romaine 'Parris Island' and 'Green Towers' lettuce, and 400 green leaf 'Tahama' lettuce were propagated from seed at OARDC facilities in Wooster and Celeryville, Ohio. Seeds were planted in Pro-Mix 'Bx' potting soil, containing 75-85% Canadian sphagnum peat moss, perlite, vermiculite, dolomitic and calcitic limestone, macronutrients, micronutrients, and a wetting agent. Transplants were watered daily while in the greenhouse. Sticky traps were used to

monitor pests, and counts of insects were taken every Monday morning, dictating pest management practices.

A field experiment was established at the OARDC, in Horticulture Unit 2, at Wooster. The soil was Wooster silt loam, with 2.7% OM and a pH of 5.6, containing 337 kg/ha of magnesium, 297 kg/ha of potash, 167 kg/ha of phosphorus, and 18 kg/ha of calcium. Previous crops were corn (2010) and soybeans (2011). Fertilizer was 19-19-19 at 90.7 kg/ha. The 20 x 35 m plot used in this research had been tilled with the moldboard plowing method on May 15, 2012 and disked on May 21. Beds were raised and black plastic was laid on May 29. On June 5, the field was planted with romaine lettuce 'Parris Island' and tomato; on June 8, green leaf lettuce 'Tahama' and romaine lettuce 'Green Towers' were also planted. The experimental design was completely random, with four replications. The site was divided into 18 plots; nine plots were planted at random with lettuce and nine with tomato. Each plot contained 4 2-m-wide rows, and each row was planted with either 38-42 lettuce plants or 12 tomato plants (Figure 3.1). The vegetables were watered by drip irrigation. On July 12, 365 ml/ha of Quadris was sprayed on the field; on August 12, the cover sprays were 2.34 L/ha of Bravo and 204 ml/ha of Baythoid.

Liquid bovine manure from a lagoon on the OARDC dairy farm was used in this research. The manure, which contained many species of coliforms, including *E. coli*, and enterococci, was from two-year-old cattle, as well as older cattle, and had been in storage for 4-6 months. 56.1 kL/ha of this manure were sprayed on the west edge of the vegetable field on six days, in a 5-m-wide band. The tractor and manure spreader travelled from south to north. The first application took place on July 2, 2012 and was repeated five times (Table 1), at intervals of two or three weeks. Each application was considered to be a replication and is referred to as

a “run”. Wind speed (m/s) and direction were recorded by the OARDC Weather Station (Table 1), located 274 m southwest of Horticulture Unit 2, at a height of 311 m. Three poles with platforms located 24 cm above the ground were set at five different distances from the point of application: 15 m upwind (as a control), at the point of manure spreading (0 m downwind), and at 15, 30, and 122 m (the distance recommended by the LGMA) downwind. Four medias were placed on each platform: MacConkey (MAC) for coliforms, including *E. coli*, MacConkey agar with an antibiotic (16 µg/ml ampicillin, 8 µg/ml chloramphenicol, and 64 µg/ml streptomycin) added for resistant coliform strains, Enterococcocel (Entero) for enterococci, and Mannitol salt (MSA) for staphylococci. All media had been prepared in the Food Animal Health Research Program (FAHP) lab at the OARDC in Wooster. Eight SKC QuickTake® 30 Impactors were used for air sampling, to examine the effects of high air volume on airborne bacteria transport. 30 L/min of air were captured by these samplers. The air samplers were divided evenly between rows: one was attached to a stake at 15 m upwind, two were placed at 0, 15, and 30 m downwind, and one more was placed at 122 m downwind. Each air sampler contained either a MAC plate or a MACacs plate and was covered with Parafilm® before manure application, to prevent contamination; immediately before application, the Parafilm® was removed and the air samplers were turned on and left running for 5 min at 0 m downwind, 10 min at 15 m upwind and downwind, and 15 min at 30 and 122 m downwind. Fifteen min was the highest length of time that could be set using these air samplers. The plates left in the open air were uncovered immediately before application and left open and untouched for the same lengths of time, at the same distances. After 15 min, all plates were covered, placed in plastic sleeves, and carried to the FAHRP lab.

At each manure application and for 7 days thereafter, bacterial colonies established on vegetables were quantified. Sampling for contamination of lettuce began on July 2, and that for tomato began on August 21. Samples were collected 4 h before manure spreading (as a control), 15 min after manure spreading, and 1, 3, 5, and 7 days after manure spreading. Lowest temperature (°C) in the 24 h before sampling, highest relative humidity (RH) in the 24 h before sampling, combined rainfall (cm) in the 48 h before sampling, and solar irradiance (W/m^2) at the time of sampling were recorded on each sampling day (Table 2). Two to three leaves were collected from each of four randomly-selected lettuce plants per plot and were pooled, for a total of nine samples of lettuce. One fruit was collected from each of four randomly-selected tomato plants per plot, and these fruits were pooled, for a total of nine samples of tomato. Latex gloves were worn while harvesting to prevent contamination of samples, and gloves were changed between blocks. Lettuce leaves were cut with a knife which was sterilized with 10% sodium hypochlorite (NaClO) between blocks. Leaves and fruits were placed in Ziploc bags and stored in a cooler with an ice pack for 15 minutes prior to processing.

Plant samples were analyzed in the FAHRP lab. Twenty g from each sample of lettuce were selected and placed in Whirl-Pak bags, to which 80 ml of phosphate buffered saline (PBS) had been added. Whirl-Pak bags were agitated for 15 sec in a Microbiology International Pulsifier® sample processor (manufactured in Frederick, MD). Three dilutions, 1:5, 1:50, and 1:500, were created for the MSA and MAC plates, but only a 1:5 dilution was used for the MACacs and the Entero plates. Each tomato sample was weighed, and a volume of PBS which equaled the sample weight was added to the bag. Each bag of tomatoes was placed on a bed of ice in a VWR® DS 500 Orbital Shaker and was agitated for one minute on each side. Three dilutions: 1:1,

1:10, and 1:100, were created for the MSA and MAC plates, but only a 1:1 dilution was used for the MACacs and Entero plates.

For both vegetables, 100 µl of each dilution was placed on each plate and spread using glass beads.

Plates from both the vegetable and the air experiments were incubated for 36 hours at 37°C. Colony-forming units (CFU) on each plate were counted without magnification. From these counts, the number of CFU per ml for the lettuce and per g for the tomato was determined. The logs of these numbers plus one were calculated in order to convert them to smaller, more manageable values. Whenever dilutions which contained between 30 and 300 CFU per ml or g were available, they were chosen for our records, to ensure that our results would be statistically reliable, and because the CFU were separate from each other and easy to count.

Variables were analyzed using Minitab 16. For all counts, a baseline value, which was the number of bacteria present at 15 m upwind or pre-application, was subtracted. The resulting values had non-normal distributions ($p<0.005$), as indicated by an Anderson-Darling Normality Test. Therefore, significance of data was determined using the Mann-Whitney U-test. Distribution of pathogen counts differed according to each bacteria genus; therefore, results for each genus were analyzed separately. Since run interacted with distance and day to produce a significant effect on count, each of the six runs was analyzed separately.

Distance and sampling day were analyzed as both discrete and continuous variables, using both the Mann-Whitney U-test and the Pearson correlation test. Wind speed, temperature, RH,

rainfall, and solar radiance were analyzed as continuous variables using the Pearson correlation test.

Results and Discussion

As expected, the highest counts of coliforms, *E. coli*, enterococci, and staphylococci on open-air plates were detected at 0 m downwind, the end of the vegetable rows closest to the manure. Coliforms and *E. coli* were not detected at 122 m. Number of coliforms was 2.02 log CFU/ml at 0 m and, following the hypothesized pattern, decreased exponentially between 0 and 30 m (Figure 2a); number of coliforms was correlated negatively with distance (Figure 3a). Number of enterococci was 3.38 log CFU/ml at 0 m and decreased between 0 and 30 m (Figure 2d), but increased between 30 and 122 m. Further research is necessary to determine an explanation for this increase. Number of staphylococci was 1.90 log CFU/ml and remained at a similar level between 0 and 15 m. Staphylococci count decreased between 15 and 30 m but could still be detected at 122 m (Figure 2d); number of staphylococci was negatively correlated with distance (Figure 3b).

Run had no effect on numbers of bacteria on open-air plates except for *E. coli*. *E. coli* count was 0.493 log CFU/ml in at 0 m in Run 2 (Figure 2b) and 0.863 log CFU/ml at 0 m in Run 5 (Figure 2c). In both runs, *E. coli* count remained at a similar level between 0 and 15 m and decreased between 15 and 30 m. Distance did not affect *E. coli* count in any other run. We had predicted that distance would affect *E. coli* counts in all runs, as was the case for the other bacteria. It has been documented that results observed in studies performed on different days vary according to weather conditions, such as wind speed and wind direction *(24)*. However, although

staphylococci counts were highest when wind was from the southwest, wind direction did not affect counts of other genera. Moreover, wind speed did not affect counts of any bacteria. It is possible that other weather effects contributed to the unusual *E. coli* results; higher RH in Runs 2 and 5 and rainfall during manure application in Run 5 might have contributed to the increased counts of *E. coli*.

Sample type—open air or air sampler—had no effect on number of bacteria. Such an observation was unexpected, although the small number of plates in air samplers compared to open-air plates might have led to the insignificance of this variable in the Mann-Whitney U-test. Only four MAC and four MACacs plates were set in air samplers, compared to 15 MAC and 15 MACacs plates left in the open air. To examine whether the effect of sample type is truly insignificant, an equal number of air-sampler plates and open-air plates should be tested in the next trial.

Number of bacteria on lettuce leaves was consistently higher than that on tomato fruits. It is possible that tomato fruits, with their round, smooth surfaces, capture pathogens less efficiently than leaves *(40)*, but further research must be conducted to determine if this is the case. To study the effect of distance on bacteria transfer to vegetable surfaces, only counts on vegetable samples taken pre-spreading and 15 min post-spreading were used. We had predicted that for the bacteria on both plates and vegetables, bacterial dispersal and therefore count would decrease as distance increased; a lower number of bacteria would be detected on vegetables at the 40-m mark than at the 0-m mark. However, effects of distance on bacteria transferred to lettuce did not follow a set pattern. In Run 1, coliforms, enterococci, and staphylococci counts were highest at 24 m (Figures 4a-c), whereas *E. coli* counts were highest at

32 m (Figure 4d). Staphylococci counts increased as distance downwind from the manure application point increased (Figure 4c). Results in Run 2 were more inconsistent. Coliform count was highest at 24 m (Figure 4e) and staphylococci count was highest at 0 m (Figure 4g). *E. coli* count was highest at 0 and 16 m and decreased as distance downwind increased (Figure 4h). Enterococci were highest at 32 m and increased as distance downwind increased (Figure 4f). In Run 3, distance had no effect on coliform or enterococci count. Number of resistant *E. coli* was highest at 16 m (Figure 4i). *E. coli* and staphylococci counts were highest at 40 m and increased as distance downwind increased (Figure 4j-k).

Relationships between distance and number of bacteria transported to tomato surfaces were as varied as the relationships between distance and bacteria in lettuce. In Run 1, coliform and resistant coliform counts were highest at 8 m (5a-b), enterococci counts were highest at 0, 16, and 40 m (Figure 5c), and staphylococci counts were highest at 40 m (Figure 5d). In Run 2, coliform counts were highest at 40 m (Figure 5e), enterococci counts were highest at 8 and 24 m (Figure 5f), and staphylococci counts were highest at 32 m (Figure 5g). All three of these genera increased in number as distance downwind from the manure spreading point increased. Results in Run 3 were more consistent: coliform, resistant coliform, and enterococci counts were highest at 16 m (Figure 5h-i, k), while *E. coli* counts were highest at 40 m (Figure 5j). Distance had no effect on number of Staphylococci in Run 3.

Environmental effects may have contributed to these unexpected results. The vegetable plot sloped slightly between 0 and 42 m downwind; as a result, after rainfall, standing water would remain at 24, 32, and 40 m for longer periods of time than at 0 m. We had observed that the leaves of downwind lettuce plants displayed more damage from disease and that downwind

tomato plants bore more rotten fruits than those closer to 0 m. It is possible that the standing water from rainstorms, combined with the moisture generated by the plants during transpiration and the soil during evaporation, created a wet environment in which bacteria could increase in number, thereby increasing chances of disease and rot on the plants. Moreover, such conditions may have allowed for an increase in number of microorganisms which were not from the manure, both on the plants and in the soil; therefore, we are less confident that the bacteria found on samples collected at 24, 32, and 40 m were transferred from the manure. When conducting similar experiments in the future, the effects of terrain, precipitation, and moisture should be observed. Furthermore, bacteria strains from the manure should be isolated and their species and serotypes identified, in order to distinguish them from environmental microflora.

To study the effect of sampling day on bacterial survival, only the first two runs were used, as the only samples taken in Run 3 were pre-spreading and 15 min post-spreading. We had predicted that counts would be highest on Day 7, but bacteria on lettuce surfaces did not always follow this pattern. In Run 1, *E. coli* count was highest on Day 5 (Figure 6a),resistant *E. coli* count was highest on Day 1 (Figure 6b), enterococci count was highest on Days 1 and 5 (Figure 6c), and staphylococci count was highest 15-min post-spreading (Figure 6d). In Run 2, coliform and staphylococci counts were highest on Day 7 (Figure 6e, h), *E. coli* count peaked on Days 3 and 7 (Figure 6f), and enterococci count increased in a logarithmic curve between 15 min and 7 days post-application (Figure 6g). All four of these genera increased in number as time post-application increased.

The relationship between sampling day and counts on tomato surfaces was consistent and followed the expected pattern. In Run 1, counts of all genera were highest on Day 7 and increased as time post-application increased (Figure 7). Enterococci also peaked on Day 1 (Figure 7d), and staphylococci also peaked 15-min post-application (Figure 7e). In Run 2, no bacteria were detected on tomato surfaces.

Many of these effects of sampling day on bacteria survival were closely related to weather conditions. These weather effects were determined after the pathogens had been transferred to the vegetable surfaces, for Runs 1 and 2, Days 1-7. We examined the effects of air temperature, relative humidity (RH), rainfall, and solar irradiance.

We recorded the lowest temperature (°C) in the 24 h before vegetable sampling. All results related to temperature were similar. In Run 1, temperature did not affect number of bacteria except for resistant coliforms, which decreased as temperature increased (Figure 8a). In Run 2, coliform *E. coli*, enterococci, and staphylococci counts decreased with increasing temperature (Figure 8b-e). All four genera of bacteria increased in number on Day 7, when temperature decreased by 4.5°C, from 19.1 to 14.6°C. Low temperature decreased between Day 1 and Day 7, from 23.9°C to 14.6°C which may also have contributed to the steady increase in enterococci between Days 1 and 7 and in *E. coli* between Days 3 and 7. These results are similar to results collected in many previous experiments, in which microorganism viability and population decreased with increasing temperature (*11, 29, 43)*; in one instance, death rate of aerosolized *E. coli* was observed to increase as temperature increased from -18 to 49°C *(13)*. All these observations suggest that airborne bacteria from manure thrive at lower temperatures, and that produce is in increased danger of airborne contamination at night or in colder weather.

We recorded the highest RH in the 24 h before vegetable sampling, to determine counts in an environment ideal for bacteria growth. Results were consistent; in both runs, counts of coliforms, *E. coli*, enterococci, and staphylococci increased as RH increased (Figure 10). Both the increase in number of *E. coli* and of enterococci on Day 5 in Run 1 and the increase in *E. coli* count on Day 3 coincided with an increase in RH by 6%. These results were consistent with previous observations, which have shown that bacteria survival increases as RH increases (*11, 29, 12, 42)*, and that *E. coli* and other coliforms are sensitive to desiccation at RH less than 84% *(37)*. The direct effect of RH on enterococci and staphylococci has not been documented in published literature. The correlation between RH and number of bacteria was not strong (<0.5), but the distance of the weather station from the vegetables might have played a role in the results. The RH of the air 311 m above ground was recorded, and results might have been more accurate if the RH of the vegetable canopy, where transpiration was occurring, had been recorded instead.

We recorded the solar irradiance (W/m^2) at the time of vegetable sampling. In contrast to temperature and RH, the relationships that enterococci and *E. coli* had with solar irradiance were not consistent with previous results. In both runs, both genera of bacteria increased with increasing solar irradiance. The rise in number of *E. coli* and of enterococci on Day 5 in Run 1, as well as the increase in *E. coli* count between Days 5 and 7 in Run 2, occurred during an increase in irradiance by over 70 W/m^2. These results were unexpected, as it has been demonstrated that both enterococci and *E. coli* in water and sewage decrease exponentially with increased solar radiation *(11, 42, 35, 9, 48, 27)*. The direct effect of irradiance on *E. coli* populations on vegetable surfaces has not been documented, but considering that solar radiation is an effective method of vegetable pasteurization *(41)*, it is probable that solar radiation usually has a negative

effect on vegetable *E. coli* populations as well as aquatic populations. However, research has shown that many other factors are involved in the effect of solar irradiance on bacteria, including wavelength, RH, and oxygen *(11, 28, 46, 2)*. None of these factors was explored in this experiment, but if they are examined further, an explanation for this study's unexpected results may be found.

We recorded combined rainfall in the past 48 h before vegetable sampling. Rainfall affected coliform, resistant *E. coli*, and enterococci counts in Run 2. Coliforms and resistant *E. coli* increased as rainfall increased (Figure 12a-b). The 0.247 log CFU/ml increase in coliform count on Day 7 in Lettuce Run 2 coincided a 1.75 cm rainfall in the past 48 h. Previous studies provide evidence for a close relationship between rainfall and coliforms; in one such experiment, bacterial populations increased by 1.5 to 3.0 log CFU/g in lettuce leaves *(14)*. It is probable that rain splash contributed to the higher number of coliforms and resistant *E. coli* found on lettuce surfaces after rain. Research has indicated that rain splash can transfer soil contaminated with manure onto fresh produce surfaces, and that pathogens are transferred from the soil to the vegetables *(31)*. Enterococci decreased as rainfall increased (Figure 12c); the direct effect of rainfall on enterococci has not been documented.

Relationships between sampling day, weather conditions, and survival of bacteria on tomato fruits were more consistent than those for lettuce leaves. In Run 1, temperature was correlated positively with numbers of all genera of bacteria (Figure 9), an observation which appears to contradict the results from our lettuce tests and from previous experiments. However, we have observed that after prolonged sunlight, fruit surfaces are warmer to the touch than leaf surfaces; it is possible that fruits absorb more solar heat than leaves do. RH and

rainfall were correlated positively with numbers of all genera of bacteria (Figures 11, 13) and solar irradiance was correlated negatively with numbers of all genera of bacteria (Figure 15). Considering the difference in surface area between lettuce and tomato, it would not be surprising if the tomato leaves were capturing most of the manure-based bacteria. 0.53 cm of rain between Days 5 and 7 in Tomato Run 1 may have washed bacteria from the leaves to the fruits, and an 8% increase in RH at the same time could have contributed further to an increase in pathogen populations. The rain may have washed the bacteria off the fruits as well, but we have observed that the tomato calyx holds rainwater for extended periods of time. Although calyces were removed before agitating tomatoes with PBS, bacteria which inhabited the standing water on the calyces could have been left behind on the fruits. Further research will have to be conducted on the capture efficiency of tomato leaves and fruits, the heat absorption of leaves and fruits, and how bacteria on tomato leaves vary with precipitation and RH.

	Lettuce			Tomato		
	July 2	July 23	Aug. 15	Aug. 21	Sept. 4	Sept. 20
Pre-application	1.35, SW	1.92, SW	1.27, NW	0.00	1.11, SW	2.65, SW
Application	1.81, NW	3.80, SW	2.02, NE	2.21, NW	1.88, SW	2.23, SW
15-min post-application	1.65, SW	4.19, SW	1.92, NE	1.75, NE	1.30, SW	2.21, SW

Table 1: Dates of manure application and corresponding wind speeds (m/s) and directions 4 h before application, during application, and 15 m after application. Wind speed and direction were determined by the OARDC weather station.

	Temperature	**Relative humidity**	**Rainfall**	**Solar irradiance**
Lettuce Run 1	°C	%	cm	W/m^2
July 3	19.0	95.8	0.00	176
July 5	20.9	92.6	1.30	191
July 7	20.8	98.0	0.76	267
July 9	14.6	96.8	0.00	291
Lettuce Run 2				
July 24	23.9	85.9	0.00	187
July 26	22.3	91.2	0.00	147
July 28	19.1	96.5	1.75	182
July 30	14.6	93.8	0.23	258
Tomato Run 1				
Aug, 22	9.89	98.0	0.31	274
Aug. 24	13.0	97.7	0.00	265
Aug. 26	14.4	90.9	0.00	256
Aug. 28	19.8	98.0	0.53	219
Tomato Run 2				
Sept. 5	18.4	98.8	2.13	224
Sept. 7	16.7	99.2	0.03	159
Sept. 9	9.89	96.8	1.91	154
Sept. 11	6.72	98.6	0.00	254

Table 2: Weather conditions on Days 1, 3, 5, and 7 after the first two manure applications for lettuce and tomato. All weather statistics were determined by the OARDC weather station.

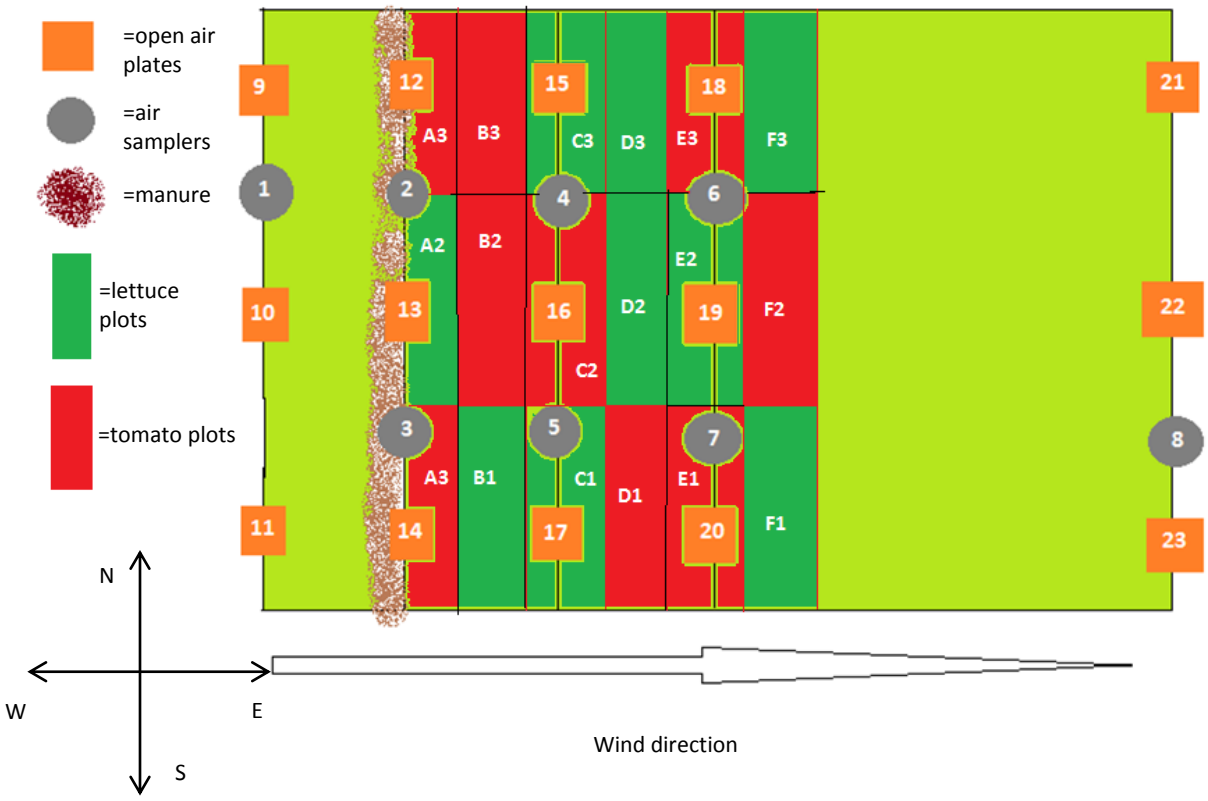

Figure 1: Diagram of Horticulture Experimental Unit 2, laid out with vegetable plots, open plates, and air samplers. Number labels were for lab personnel.

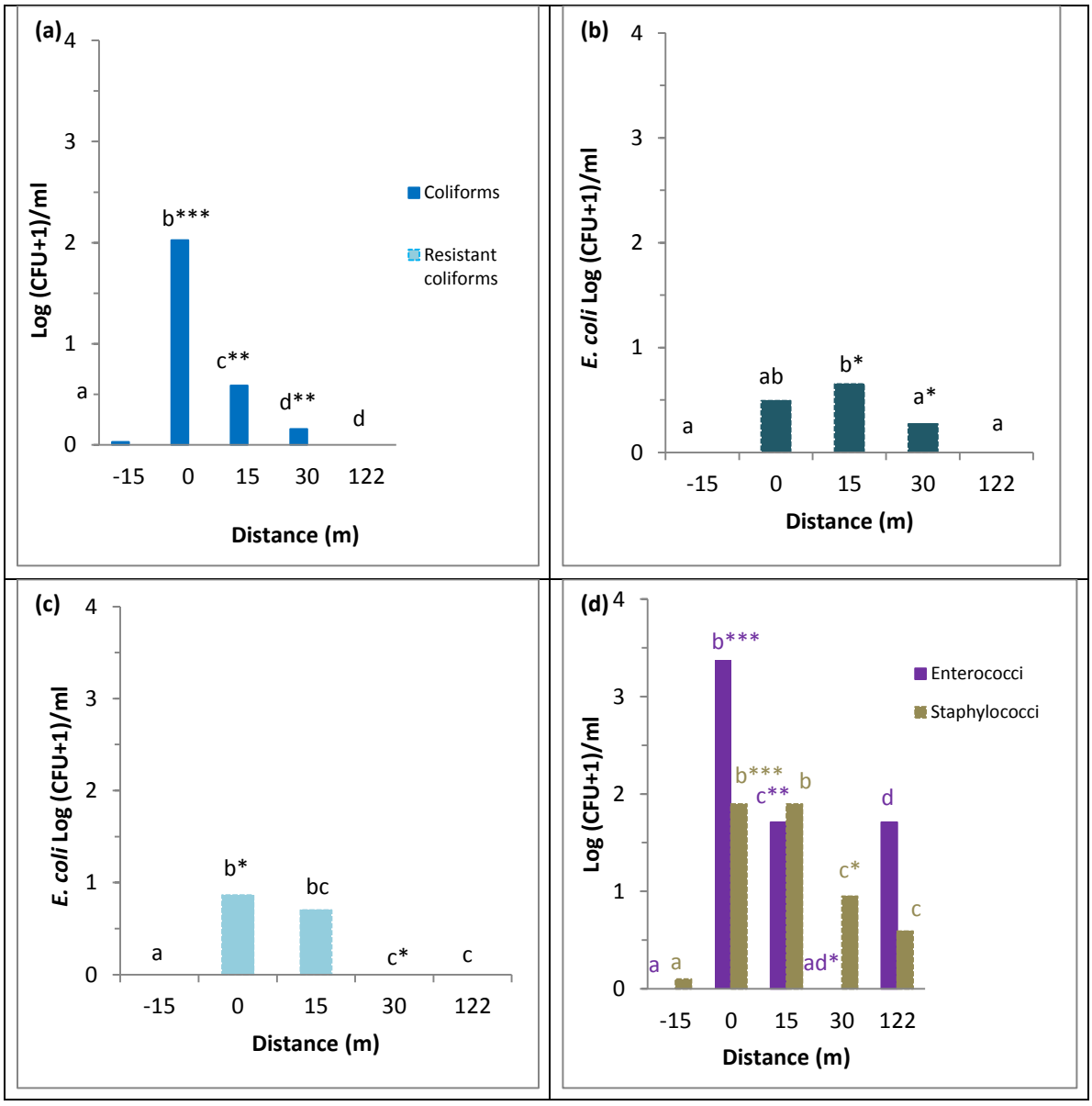

Figure 2: The relationship between distance downwind from the point of manure application and number of bacteria dispersed onto media plates. Different letters represent statistically significant values (Mann-Whitney U-test). Asterisks represent p-values (*<.05, **<.01, ***<.001). (a) Coliforms and resistant coliforms (b) *E. coli*, Run 2 (c) *E. coli*, Run 5. (d) Enterococci and Staphylococci

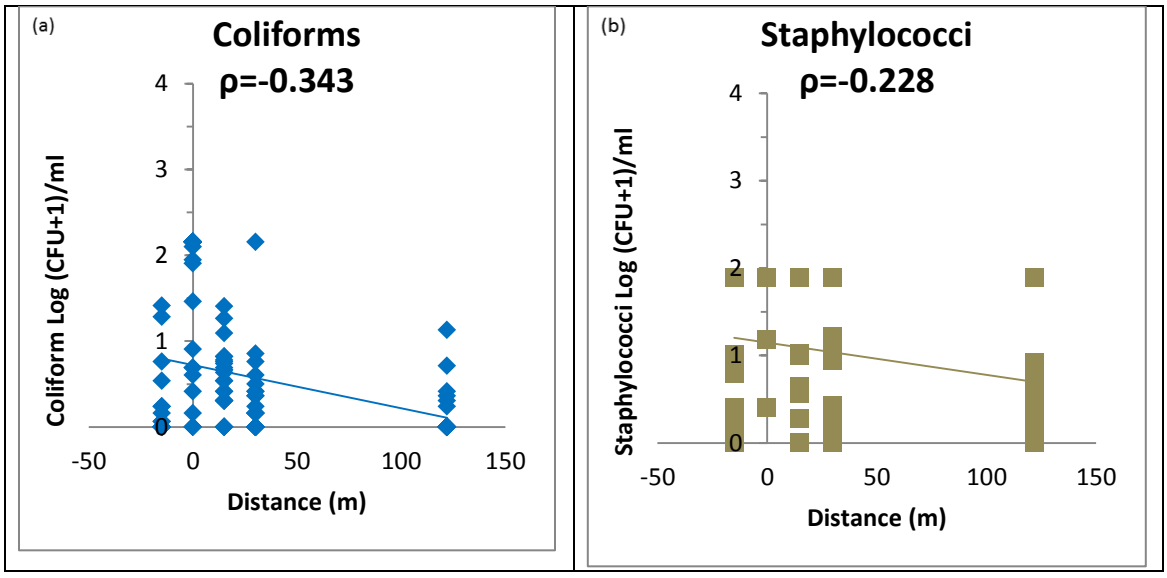

Figure 3: Correlations between distance downwind from the point of manure application and number of bacteria dispersed onto media plates. ρ=Pearson correlation coefficient. (a) Coliforms (b) Staphylococci

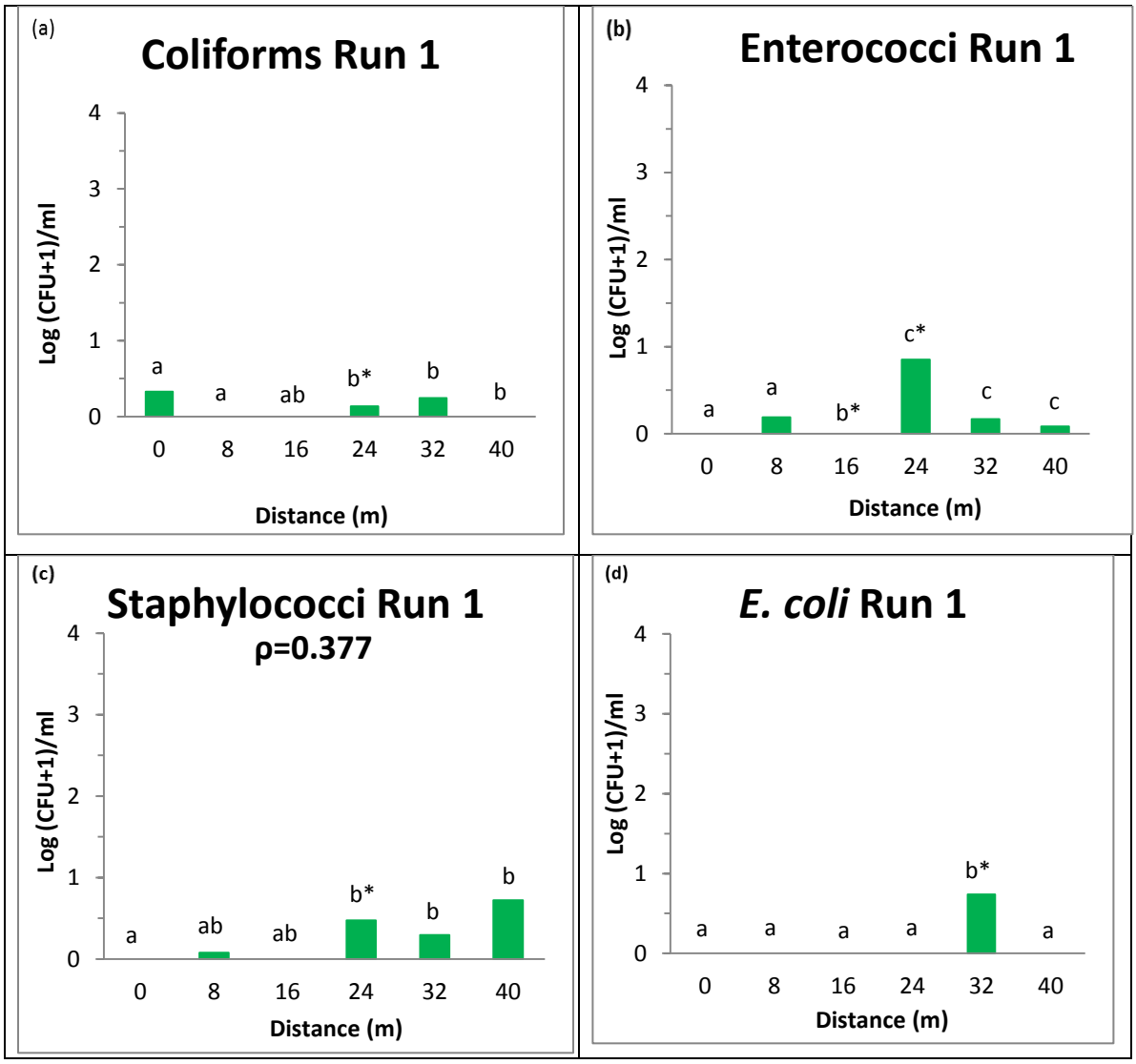

Figure 4: The relationship between distance downwind from the point of manure application and number of bacteria dispersed onto lettuce surfaces. Different letters represent statistically significant values (Mann-Whitney U-test). Asterisks represent p-values (*<.05, **<.01, ***<.001). ρ=Pearson correlation coefficient. (a) Coliforms (b) Enterococci (c) Staphylococci (d) *E. coli* (e) Coliforms (f) Enterococci (g) Staphylococci (h) *E. coli* (i) Resistant *E. coli* (j) *E. coli* (k) Staphylococci

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Figure 4 continued.

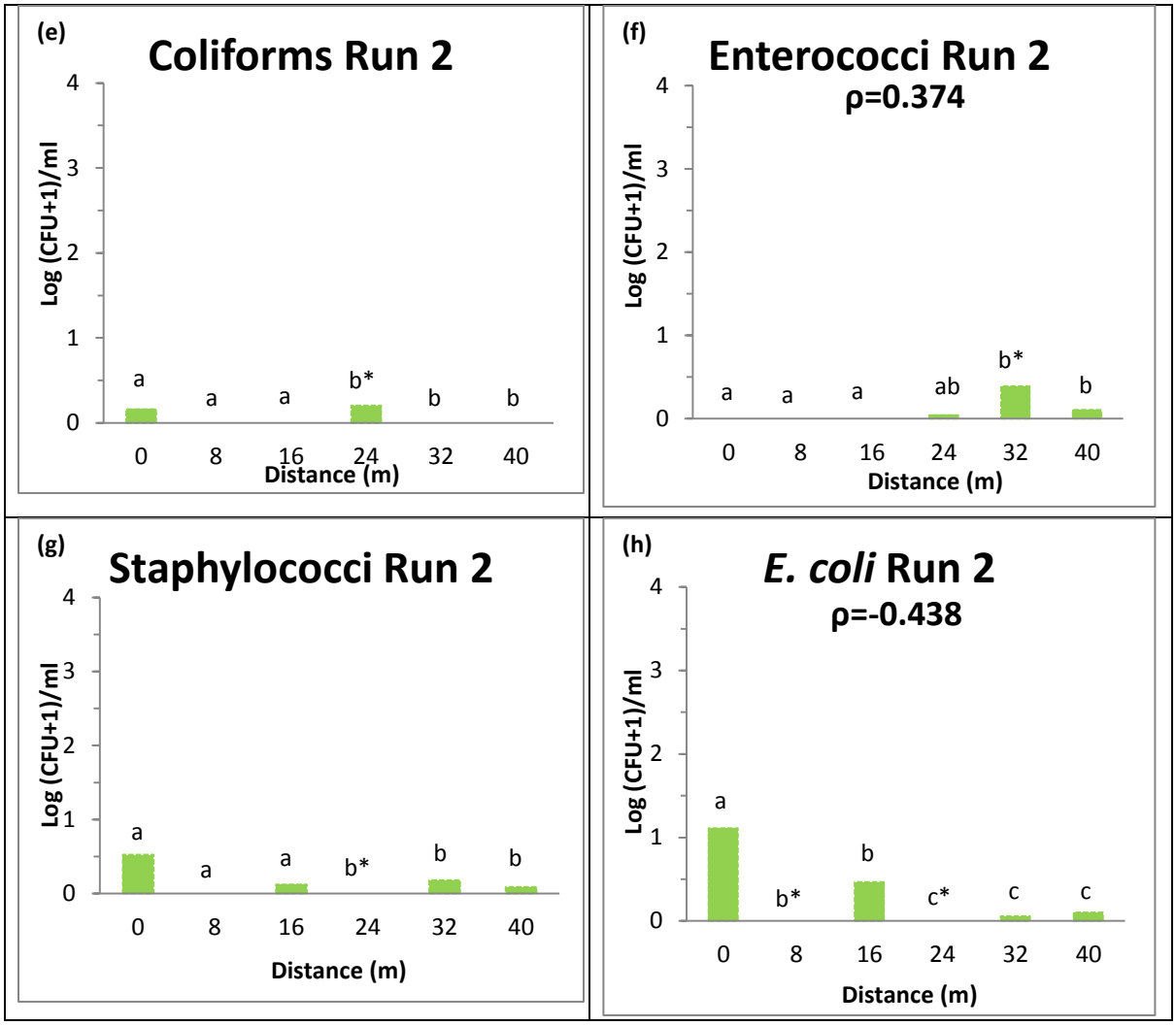

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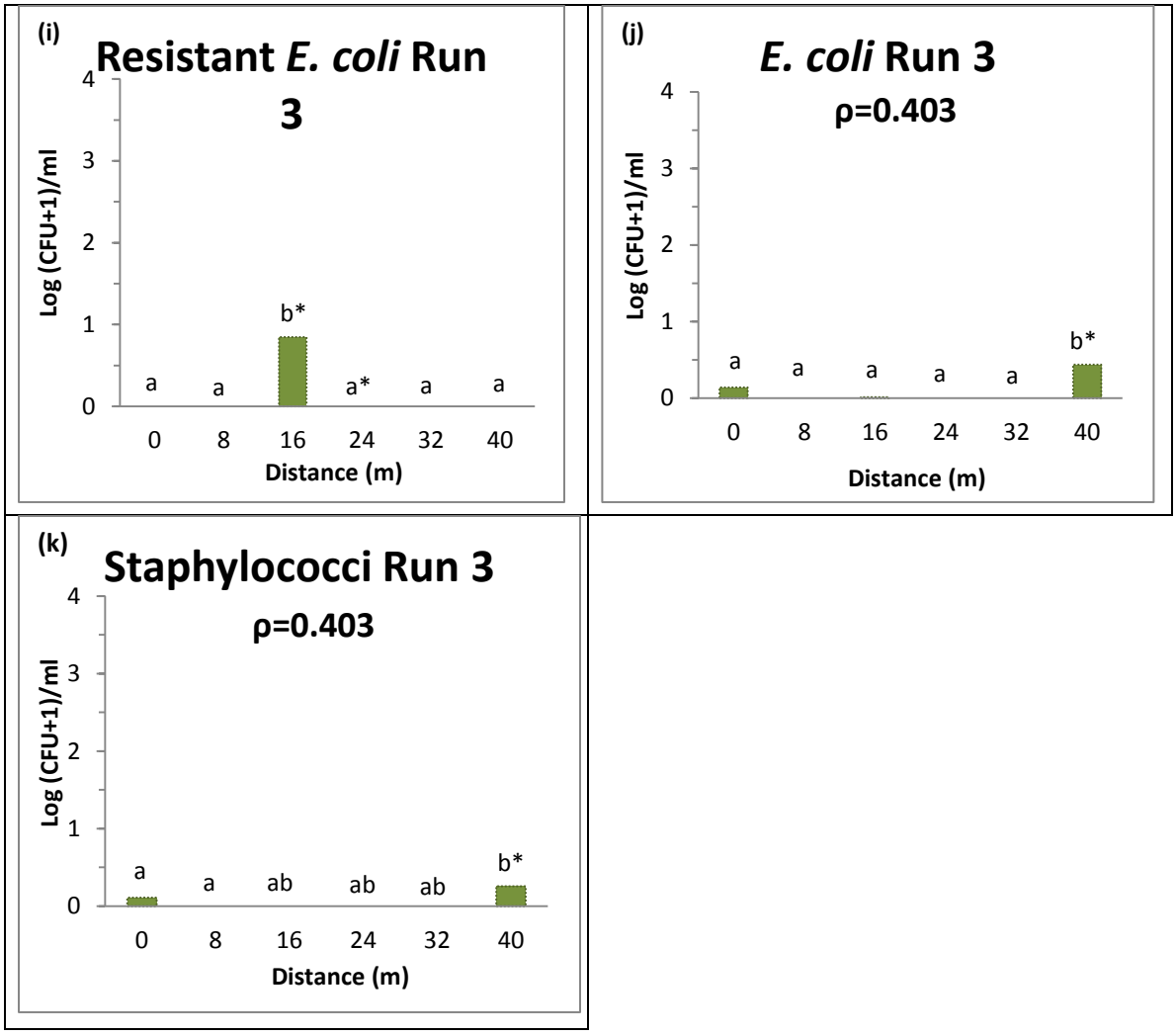
Figure 4 continued.



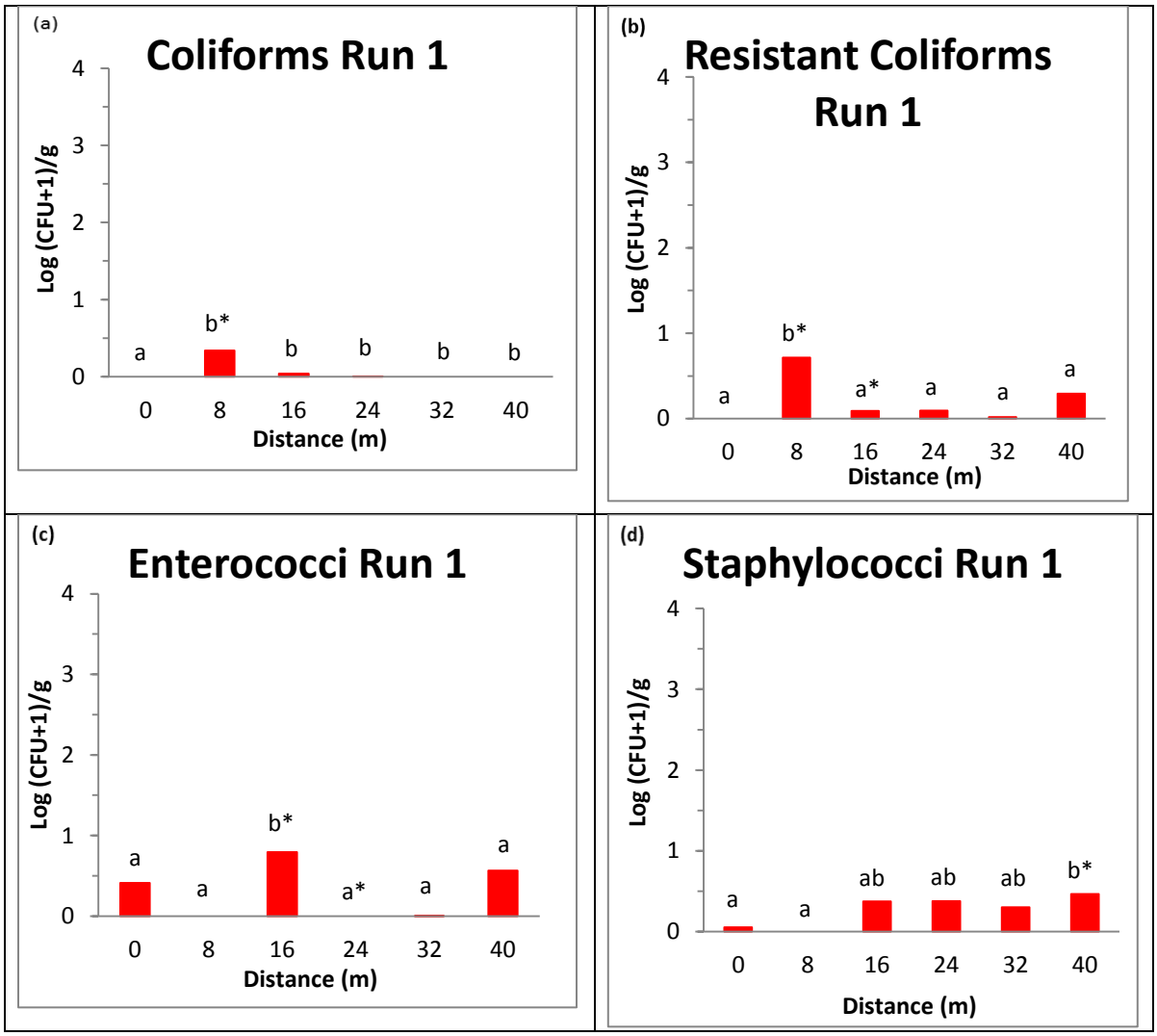


Figure 5: The relationship between distance downwind from the point of manure application and number of bacteria dispersed onto tomato surfaces. Different letters represent statistically significant values (Mann-Whitney U-test). Asterisks represent p-values (*<.05, **<.01, ***<.001). ρ=Pearson correlation coefficient. (a) Coliforms (b) Resistant coliforms (c) Enterococci (d) Staphylococci (e) Coliforms (f) Enterococci (g) Staphylococci (h) Coliforms (i) Resistant coliforms (j) *E. coli* (k) Enterococci

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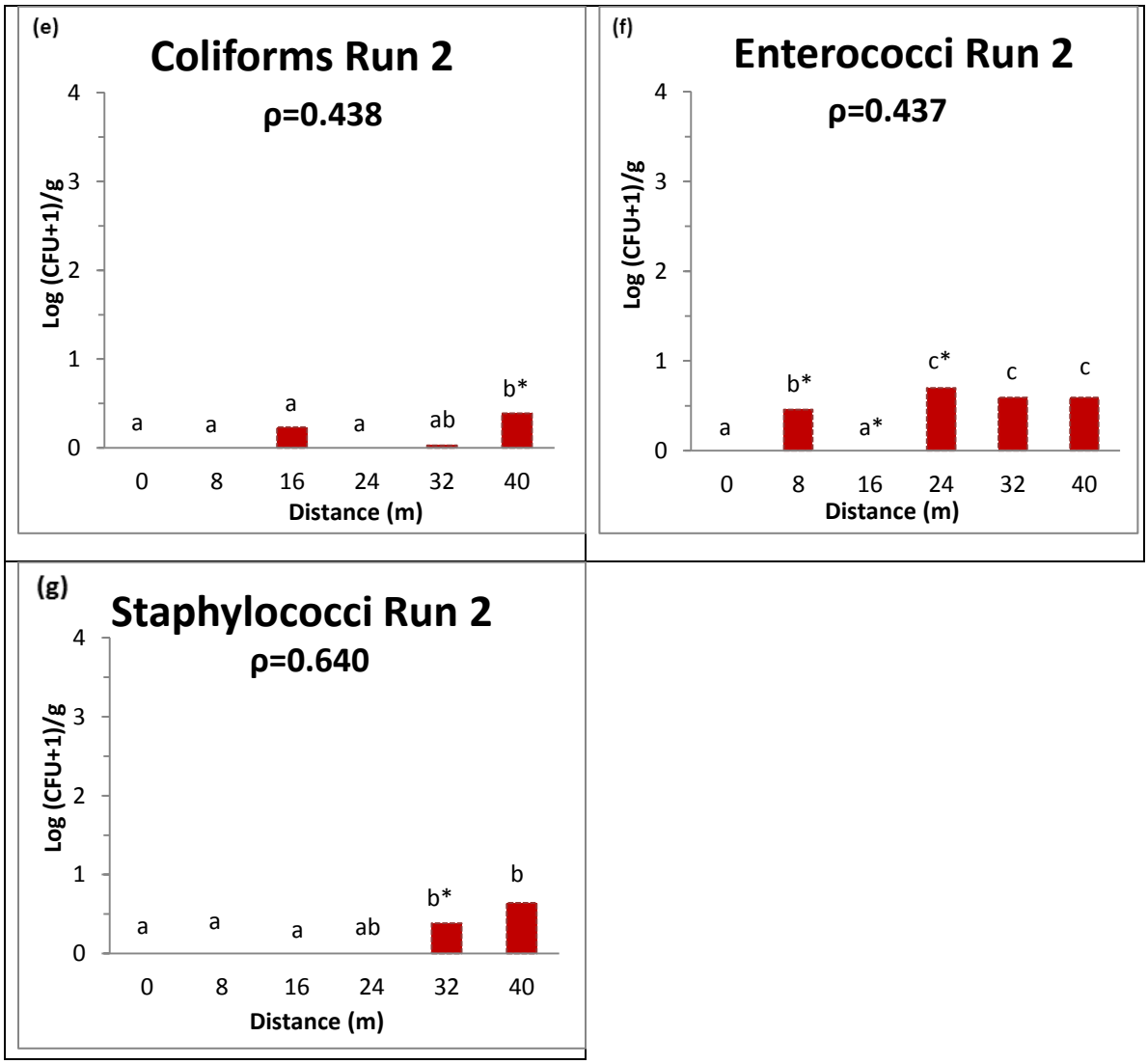
Figure 5 continued.



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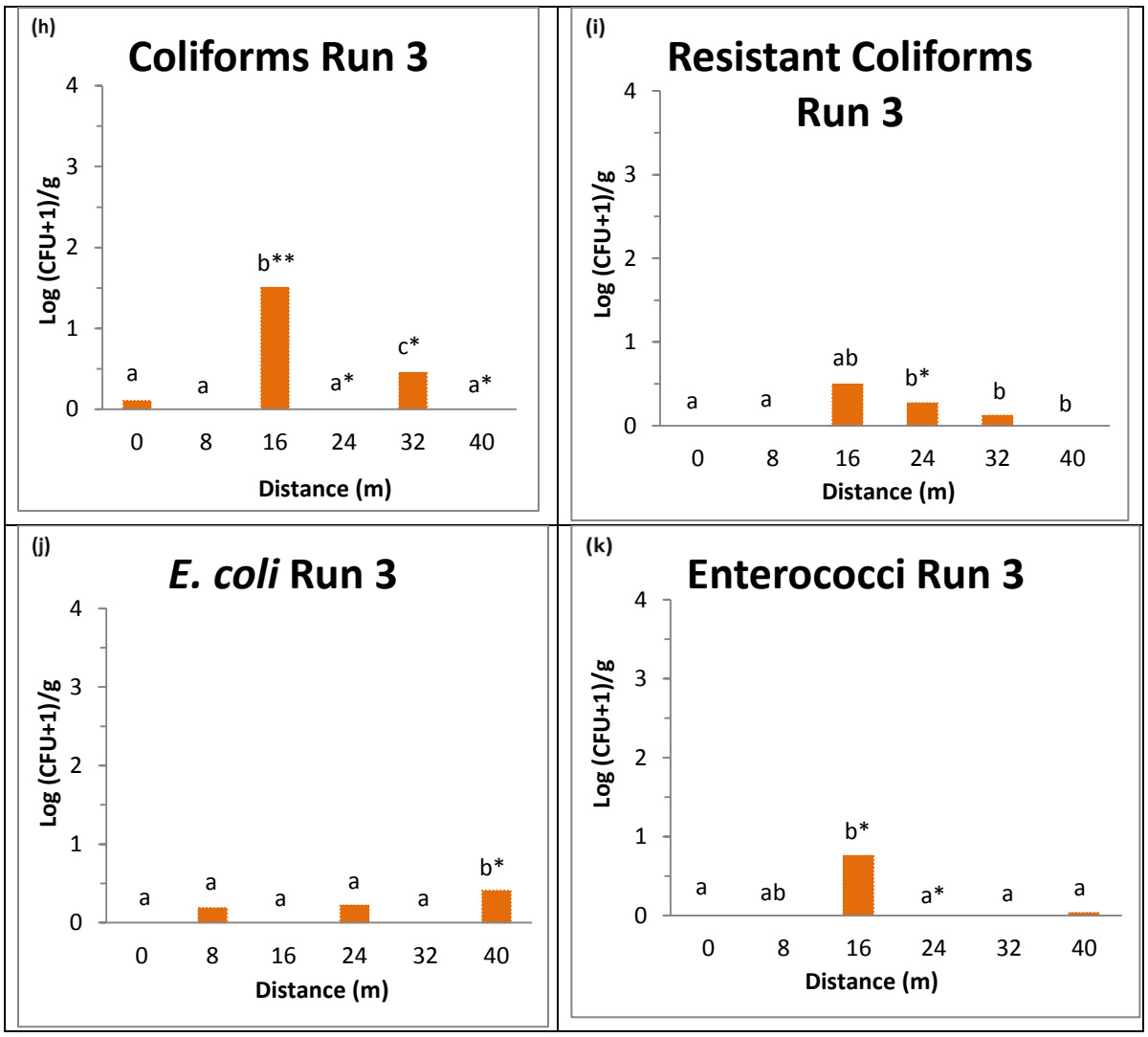


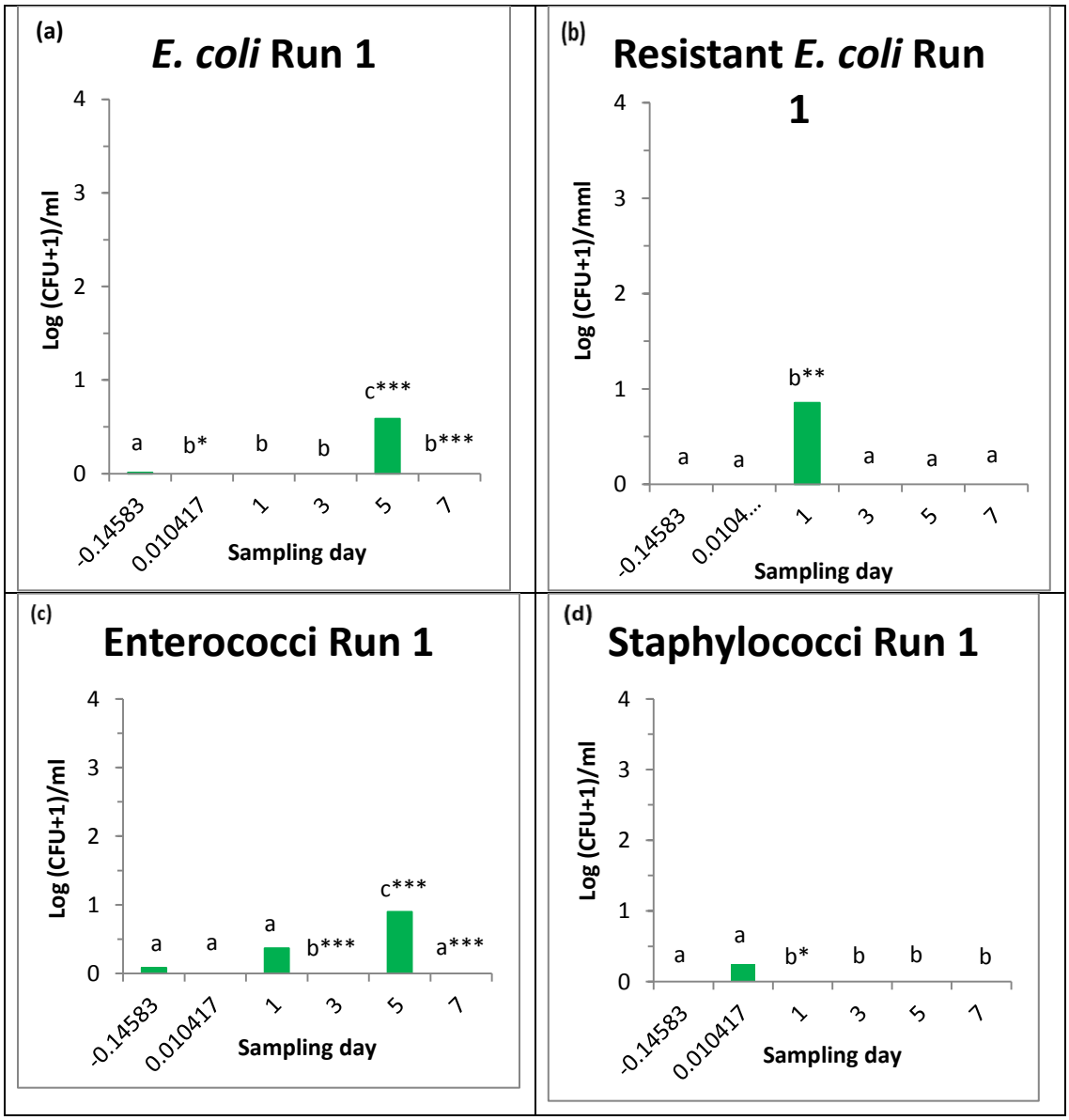
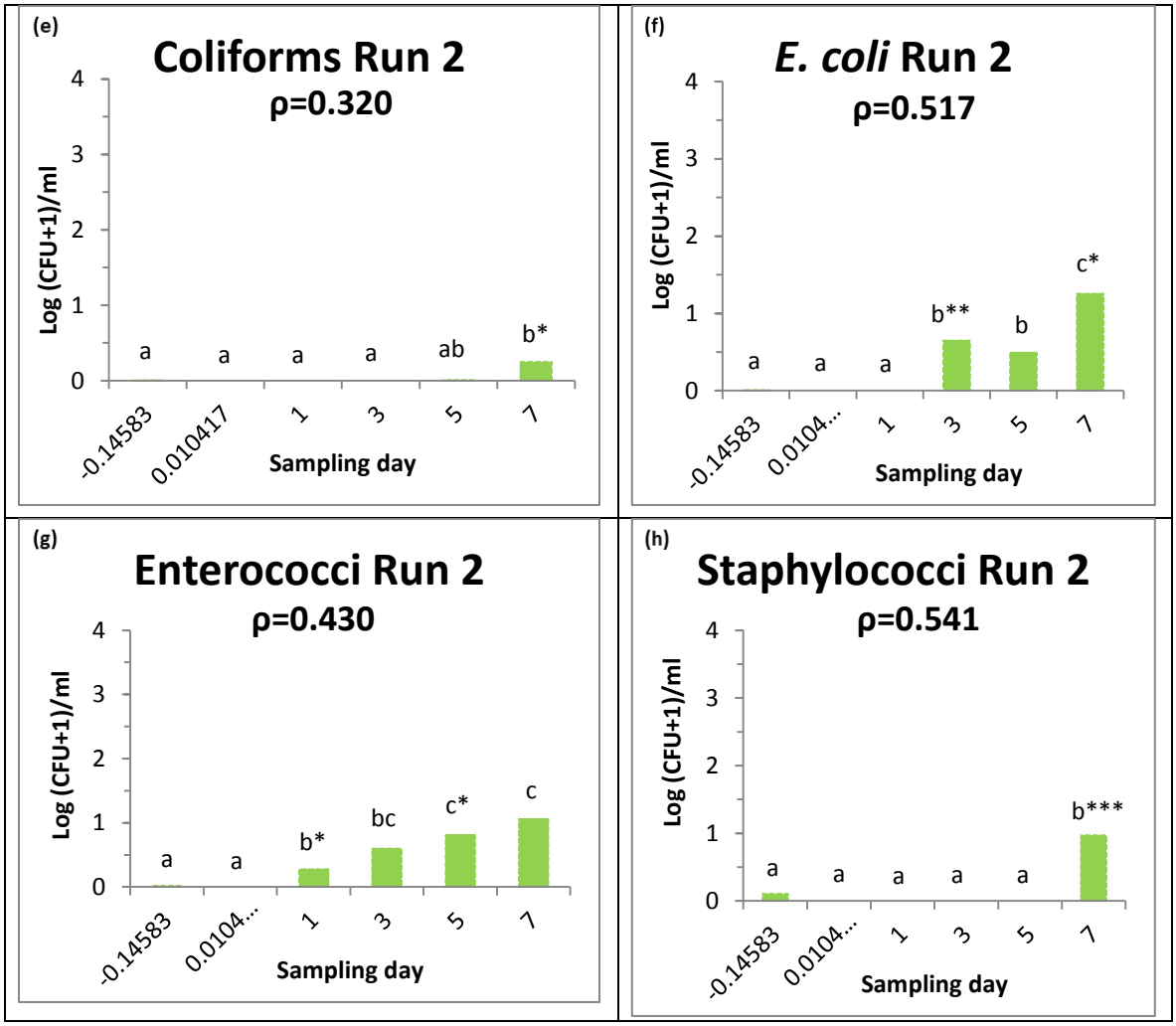


Figure 6: The relationship between sampling day post manure application and number of bacteria that survived on lettuce surfaces. Different letters represent statistically significant values (Mann-Whitney U-test). Asterisks represent p-values (*<.05, **<.01, ***<.001). ρ=Pearson correlation coefficient. (a) Coliforms (b) Resistant *E. coli* (c) Enterococci (d) Staphylococci (e) Coliforms (f) *E. coli* (g) Enterococci (h) Staphylococci

Continued

Figure 6 continued.



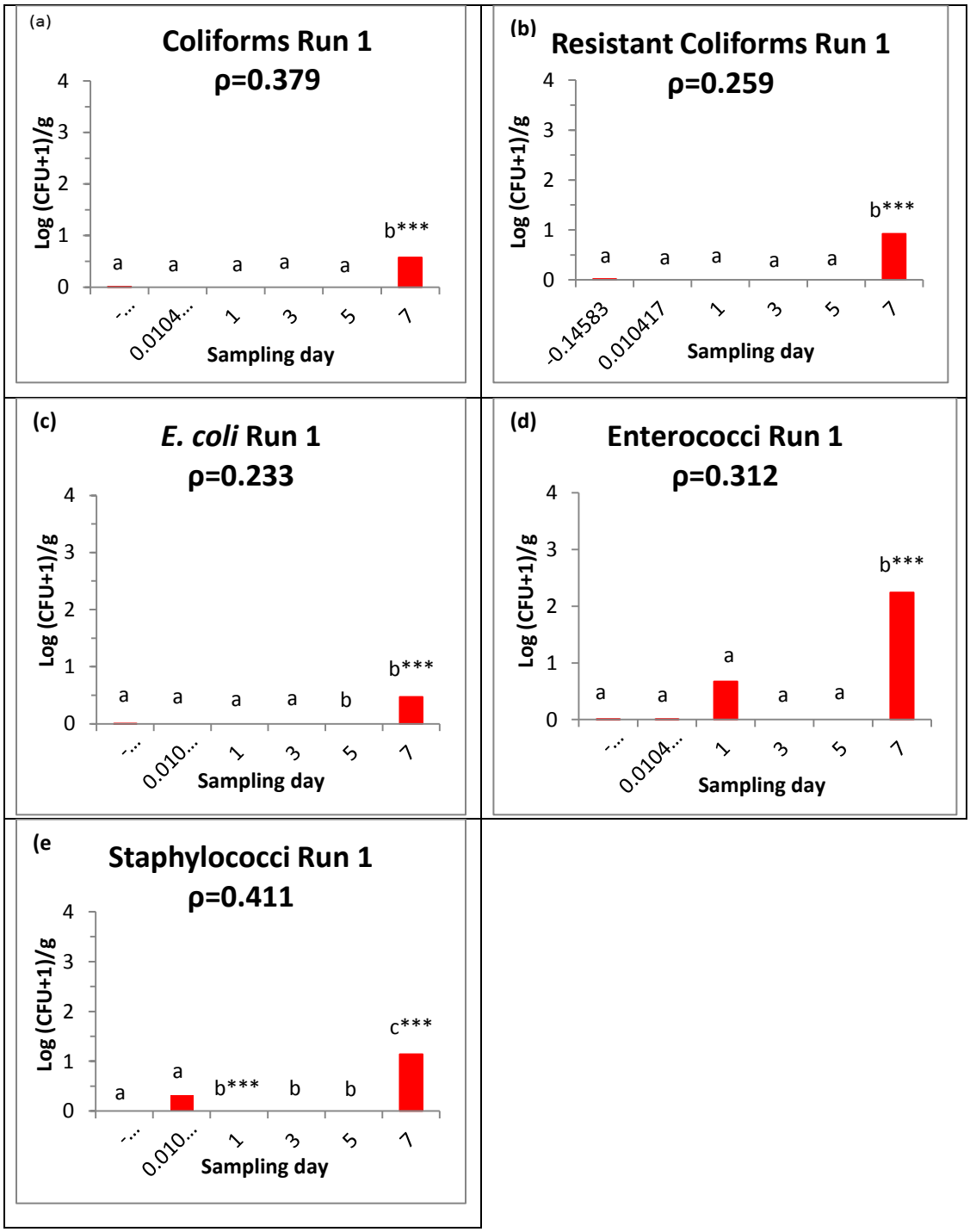


Figure 7: The relationship between sampling day post application and number of bacteria that survived on tomato surfaces in Run 1. Different letters represent statistically significant values (Mann-Whitney U-test). Asterisks represent p-values (*<.05, **<.01, ***<.001). ρ=Pearson correlation coefficient. (a) Coliforms (b) Resistant coliforms (c) *E. coli* (d) Enterococci (e) Staphylococci

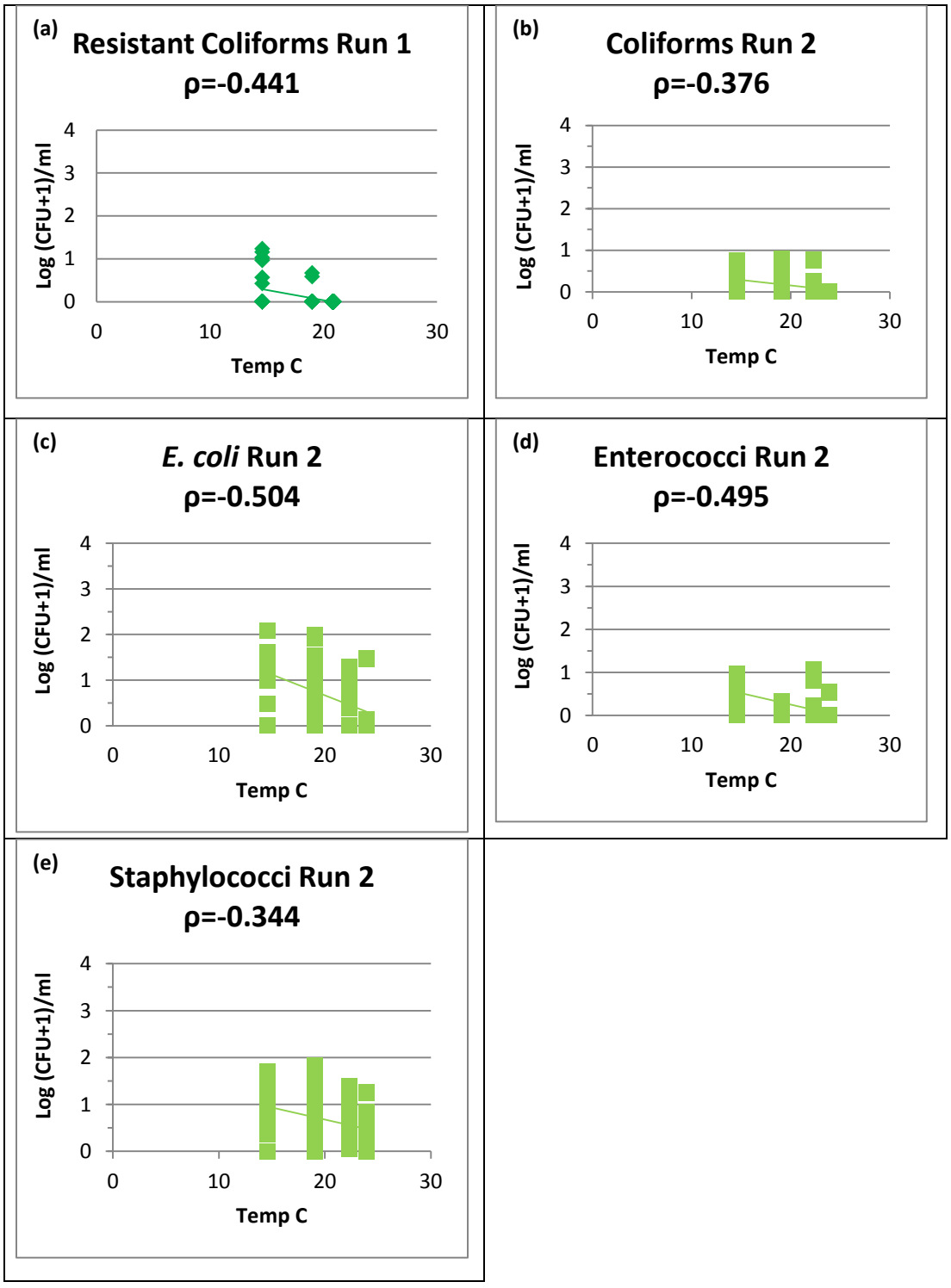


Figure 8: Correlations between low air temperature in the 24 hours before sampling and number of bacteria that survived on lettuce surfaces. Diamonds=Run 1, squares=Run 2. ρ=Pearson correlation coefficient. (a) Resistant coliforms (b) Coliforms (c) *E. coli* (d) Enterococci (e) Staphylococci

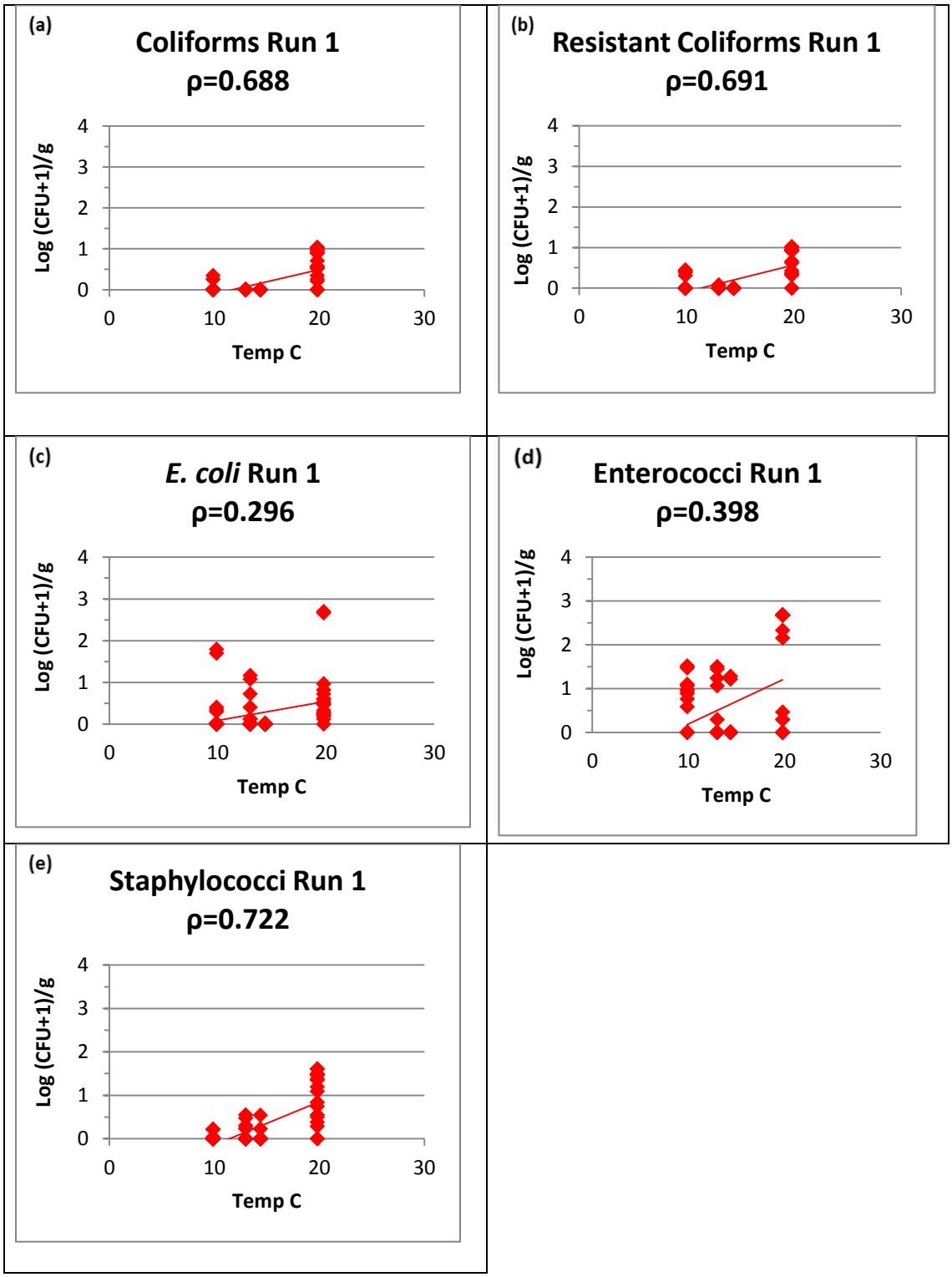


Figure 9: Correlations between low air temperature in the 24 hours before sampling and number of bacteria that survived on tomato surfaces in Run 1. ρ=Pearson correlation coefficient. (a) Coliforms (b) Resistant coliforms (c) *E. coli* (d) Enterococci (e) Staphylococci

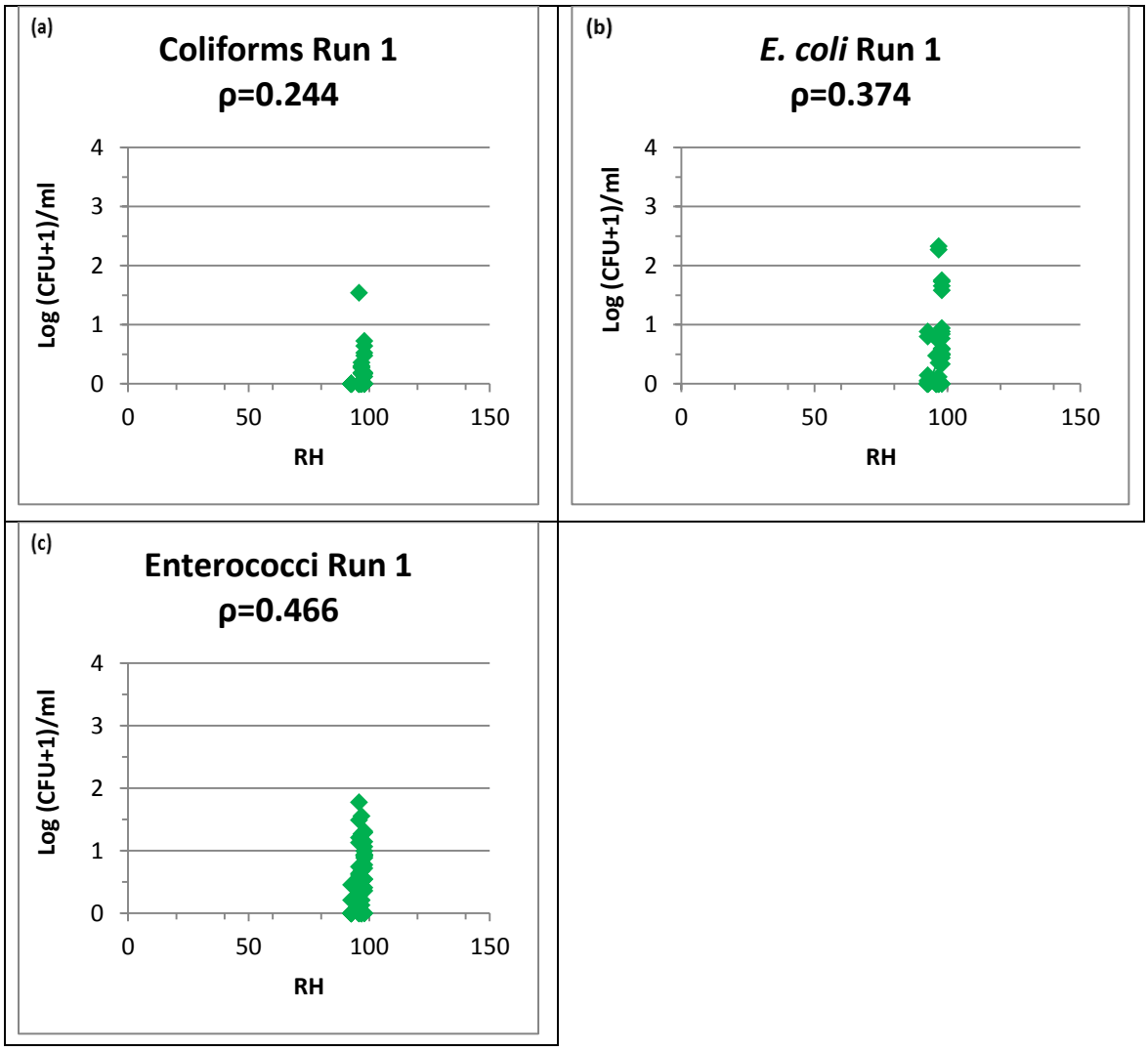


Figure 10: Correlations between high relative humidity (RH) in the 24 hours before sampling and number of bacteria that survived on lettuce surfaces. Diamonds=Run 1, squares=Run 2. ρ=Pearson correlation coefficient. (a) Coliforms (b) *E. coli* (c) Enterococci (d) Coliforms (e) *E. coli* (f) Staphylococci

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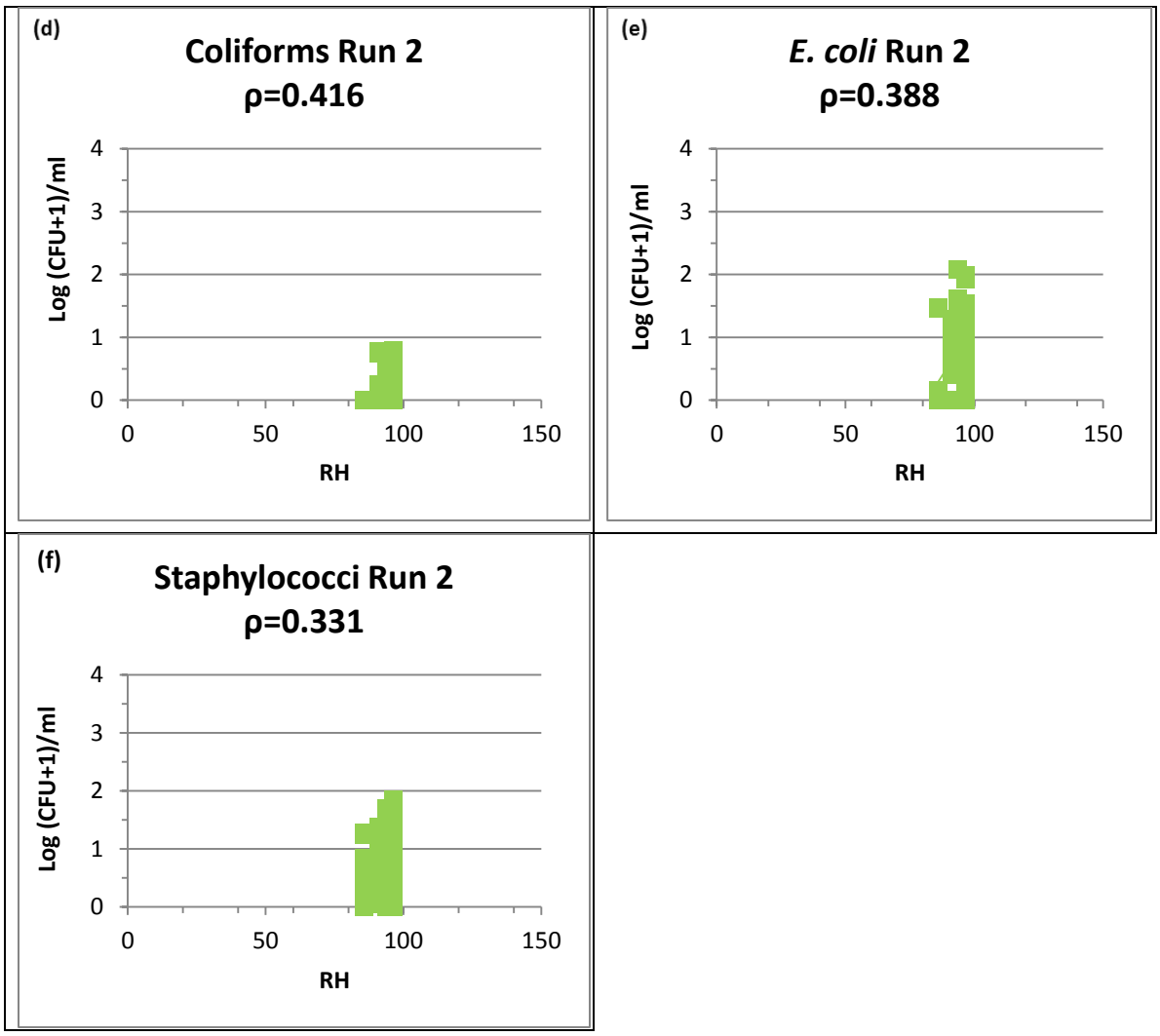
Figure 10 continued.



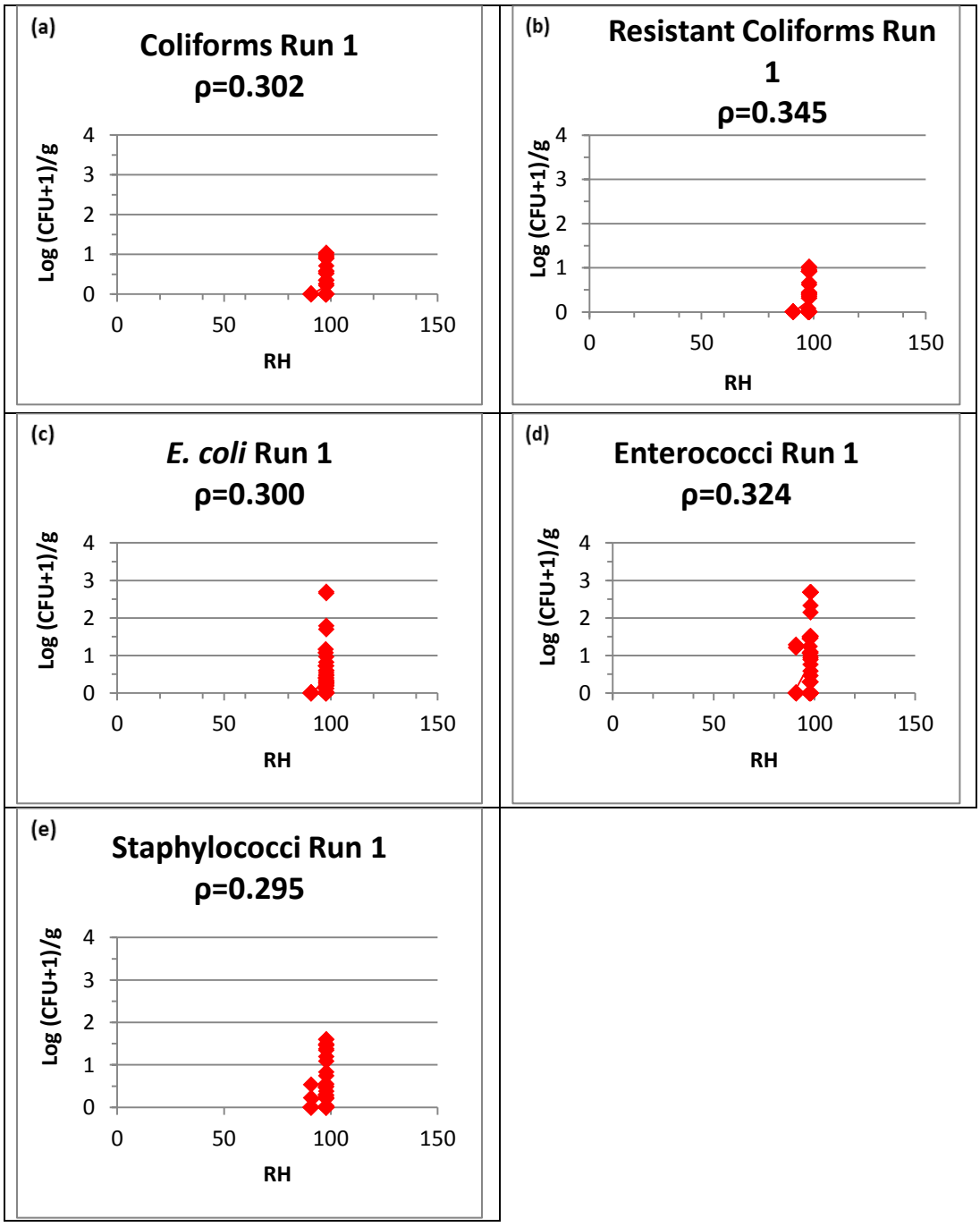


Figure 11: Correlations between high relative humidity (RH) in the 24 hours before sampling and number of bacteria that survived on tomato surfaces in Run 1. ρ=Pearson correlation coefficient. (a) Coliforms (b) Resistant coliforms (c) *E. coli* (d) Enterococci (e) Staphylococci

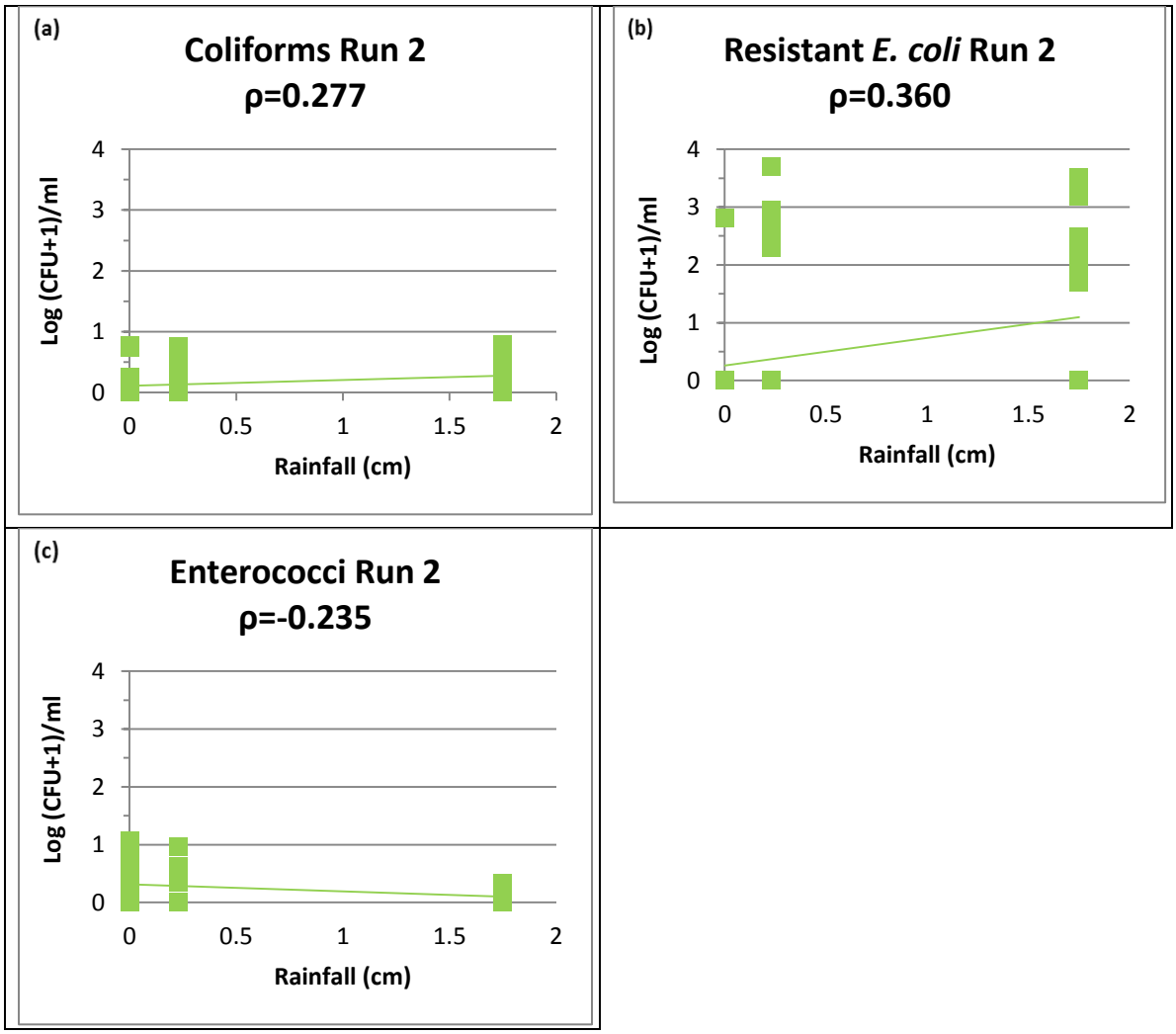


Figure 12: Correlations between combined rainfall in the past 48 hours and number of bacteria that survived on lettuce surfaces in Run 2. ρ=Pearson correlation coefficient. (a) Coliforms (b) Resistant *E. coli* (c) Enterococci

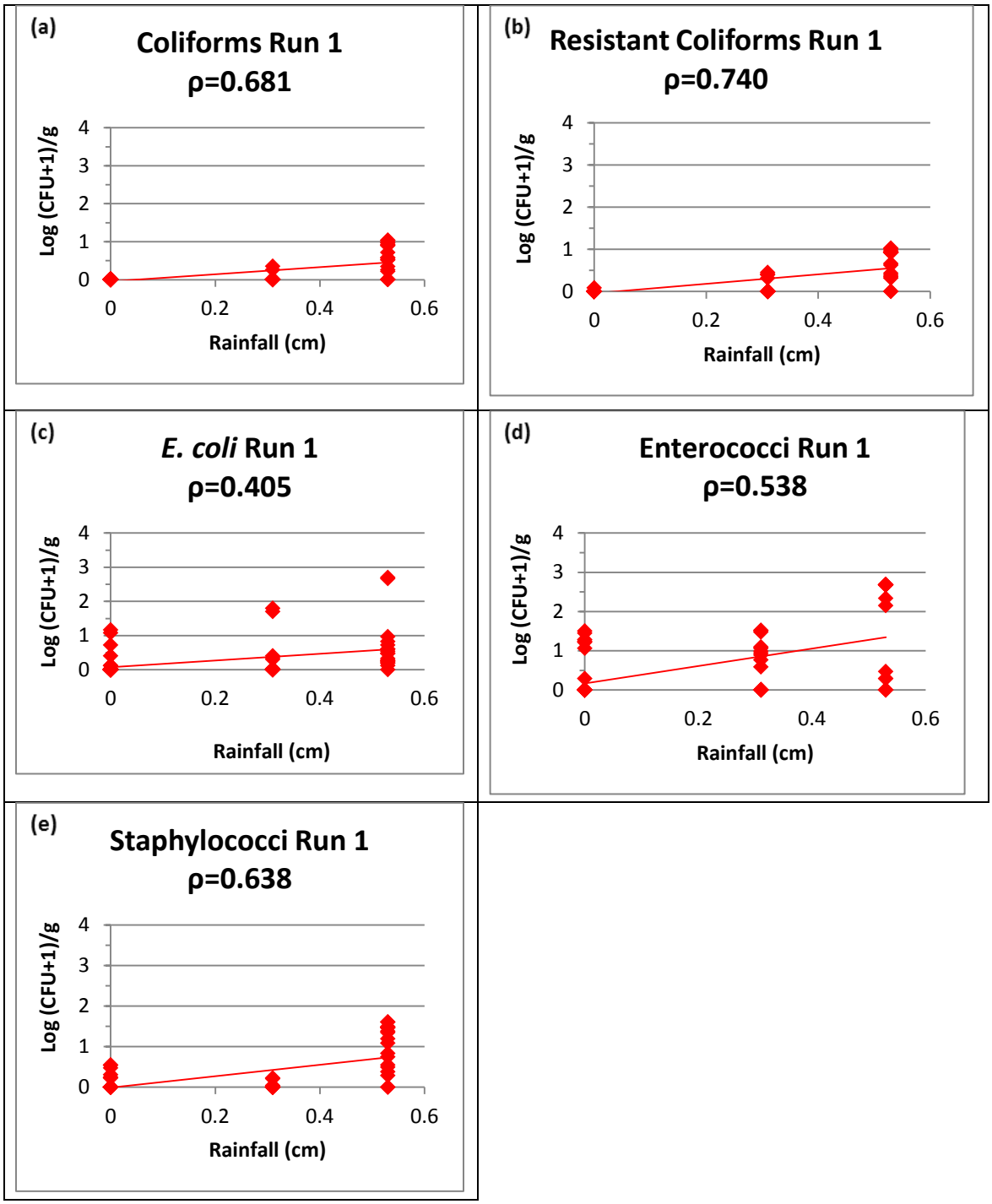


Figure 13: Correlations between combined rainfall in the past 48 hours and number of bacteria that survived on tomato surfaces in Run 1. ρ=Pearson correlation coefficient. (a) Coliforms (b) Resistant coliforms (c) *E. coli* (d) Enterococci (e) Staphylococci

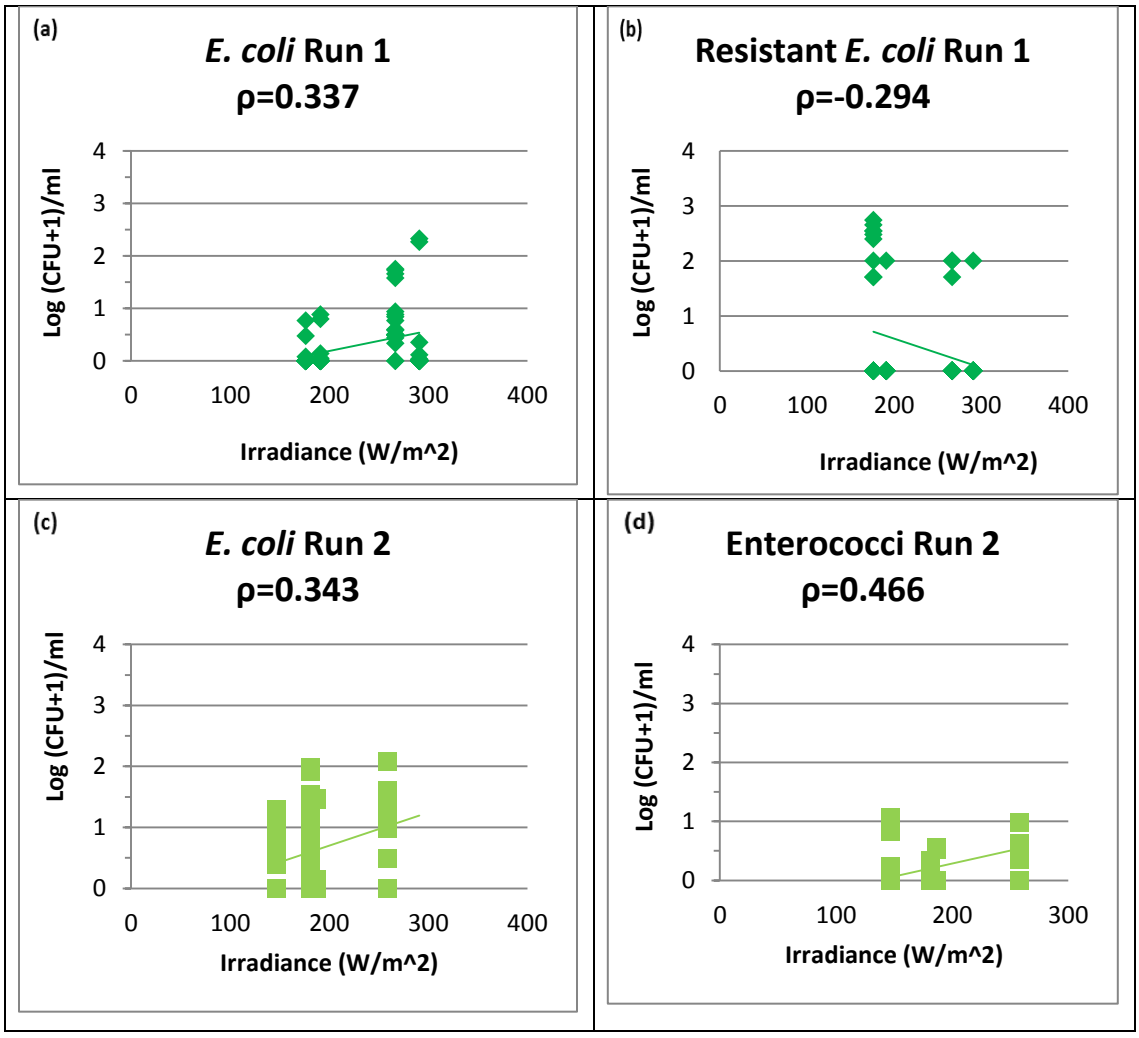


Figure 14: Correlations between solar irradiance during sampling and number of bacteria that survived on lettuce surfaces. Diamonds=Run 1, squares=Run 2. ρ=Pearson correlation coefficient. (a) *E. coli* (b) Resistant *E. coli* (c) *E. coli* (d) Enterococci

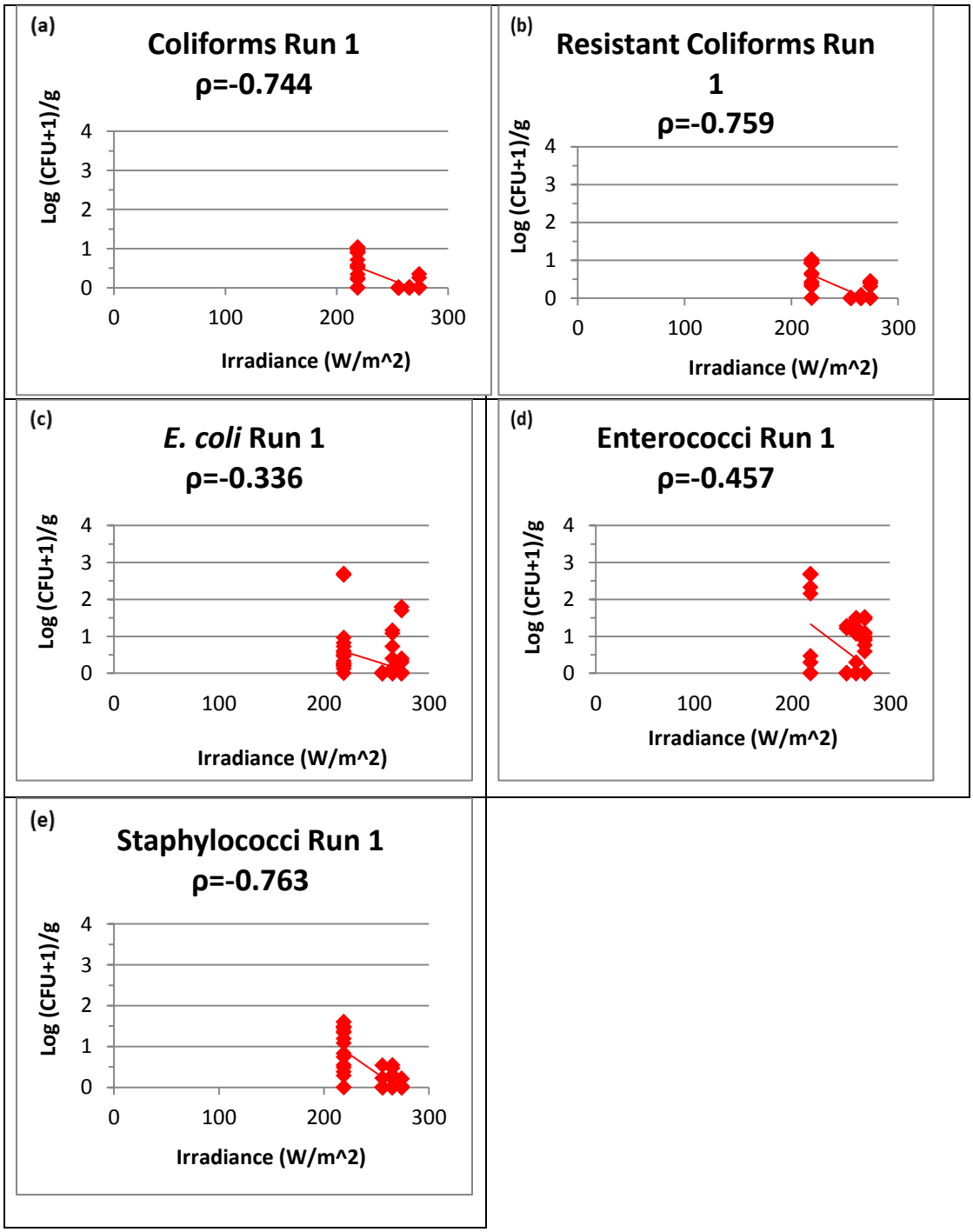


Figure 15: Correlations between solar irradiance during sampling and numbers of bacteria that survived on tomato surfaces in Run 1. ρ=Pearson correlation coefficient. (a) Coliforms (b) Resistant coliforms (c) *E. coli* (d) Enterococci (e) Staphylococci

Conclusion

Airborne transfer of bacteria to open-air plates decreases with increasing distance downwind from a liquid manure source. Since coliforms and *E. coli* were undetected on open-air plates at 30 m and 122 m downwind from the application point, there is evidence that 122 m downwind from a liquid manure source is a safe distance at which to plant produce, and it is possible that 30 m downwind is a safe distance as well. However, further research should be conducted to determine if produce may be safely planted at 30 m downwind from a contamination source, or at any distance downwind between 30 and 122 m. Moreover, these results only provide evidence for cases involving liquid dairy manure from a lagoon. Potential safe distances at which to plant produce should be determined for dry dairy manure, completely untreated dairy manure, and composted dairy manure, as well as for various treatments of manure from other animals such as beef cattle, swine, and poultry. Finally, enterococci and staphylococci decreased in number with increasing distance, but could still be detected at 122 m; it is necessary to further examine the dispersal patterns and distances of these two genera of bacteria.

Airborne transfer of bacteria to vegetable surfaces appears to increase as distance downwind from manure application increases. This observation does not fit with the observations of bacteria on open-air plates, which decreased as distance increased. It is hypothesized that a moister environment downwind, with the effect of standing water added to transpiration and soil evaporation, contributed to an increase in number of bacteria.

Bacteria from liquid dairy manure may survive on lettuce leaf surfaces for at least seven days. There is evidence that variations in bacteria populations are closely correlated with weather conditions. As established in the literature, number of bacteria increased with increasing RH and decreased with increasing temperature. Number of coliforms increased with increasing rainfall, as shown in previous experiments. However, contrary to results obtained in previous studies, number of bacteria increased with increasing solar irradiance. In measuring solar irradiance, only sunlight (W/m^2) was measured; other factors affecting solar irradiance, such as wavelength, relative humidity, or oxygen, should be explored in the future to determine an explanation for our present results.

Tomato fruits do not appear to capture bacteria from dairy manure. Any bacteria detected on tomato fruits appeared after rainfall, and the number of bacteria found on tomato fruits was consistently lower than that on lettuce leaves. It is possible that tomato leaves capture most of the bacteria and that rainfall washes bacteria from the leaves onto the fruits, but further research must be conducted to provide evidence for this possibility. In general, bacteria detected on tomato fruits followed weather patterns consistent with previous results: number of bacteria increased with increasing RH and rainfall and decreases with increasing solar irradiance. However, contrary to results obtained in previous studies, number of bacteria increased with increasing temperature. It is predicted that this observation was related to fruits absorbing more heat than leaves, but further research should be conducted to test this theory.

Overall, our results indicate that airborne contamination of field-grown produce is highly dependent on environmental conditions. It may be worthwhile to develop flexible food safety standards, which can be adapted to a variety of environmental circumstances.

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