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The Association between Weather and Contamination on Crops Prior to Harvest: A Mixed Models Analysis

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The Association between Weather and Contamination on Crops Prior to Harvest: A Mixed Models Analysis

By

Michelle Ward

Bachelor of Arts
Princeton University
2010

Thesis Committee Chair: Juan Leon, PhD, MPH

An abstract of
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Abstract

The Association between Weather and Contamination on Crops Prior to Harvest:
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By Michelle Ward

Foodborne illness associated with produce is costly in terms of human health and economic losses. The need to improve food safety is increasing as demands on the global food supply are also increasing. However, the environmental conditions that influence the inoculation, proliferation, and diffusion of fecal contamination on produce are not well understood. Therefore, we aimed to assess an association between temperature or precipitation and fecal contamination on produce prior to harvest. Between 2000 and 2002, we assayed for three fecal indicators (APC, coliforms, or *Enterococcus*) on 10 different types of produce collected from 15 fields that were clustered in the southern U.S. Weather data was obtained from a single NOAA weather station within 100km of the fields. We used a mixed models approach to analyze the relationship between indicator concentrations on crops and weather over a week-long lag period prior to sample collection. Average daily temperature was significantly associated with indicator growth for five days prior to sample collection. On the other hand, daily total precipitation had a significant association with indicator concentrations for only one or two days prior to collection. Our results indicated that there is a significant association between weather and fecal indicator proliferation on crops in the field. Indicator concentrations may have increased as the temperature increased towards the optimal growing temperature for the bacteria. Precipitation may be creating moist conditions conducive to bacterial growth, spreading contamination onto the field, or washing contamination off of the plant. Therefore, new food safety policies that are weather dependent may be necessary to improve the safety of the global food supply.

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LITERATURE REVIEW

Why Study Produce Contamination?

Improving the safety of food is critical to preventing gastrointestinal illness because an estimated 48 million cases and 3,000 deaths due to foodborne illness occur annually in the United States [1]. Foodborne illness in the U.S. also results in an annual loss of $14 billion to the U.S. economy [2, 3], a loss that is acutely felt in already strained agricultural communities. Undercooked meat, eggs, and unpasteurized milk have long been known to be the culprits of foodborne outbreaks, but in the last few decades, produce has become an important vehicle for foodborne pathogens, as contaminated produce is responsible for 1,200,000 foodborne illnesses in the U.S. and $1.4 billion dollars in losses to the U.S. economy [2]. The reason for this is threefold: 1) we now have better methods of monitoring and identifying foodborne illnesses so more cases are reported (reviewed in [4]); 2) as a country, Americans purchase and consume more fresh produce, largely in response to 'healthy eating' campaigns; and 3) the change in distribution of food around the world has led to more extensive, world-wide outbreaks (reviewed in [5]). As produce becomes a more prominent cause of foodborne illness, we need to better understand what drives pathogen evolution and fecundity in order to improve the safety of our food supply.

What is Produce Contamination?

Produce contamination refers to the presence of animal or human fecal matter on fruits and vegetables; the fecal matter may contain viruses, bacteria, and parasites, some of which may be pathogens. The term 'pathogen' is defined as an organism that can cause

disease. There is generally a range of non-pathogenic and pathogenic organisms within a family. For example, *Escherichia coli* is one of the most prevalent types of bacteria, and there are many types of *E. coli* that are present in the typical human environment that cause no adverse effects. However, an outbreak with the pathogenic *E. coli* O104:H4 in 2011 was one of the worst foodborne outbreaks in recent memory, with 3,842 people infected [6]; the outbreak was linked to contaminated sprouts [7]. Some of the most common pathogens associated with contaminated produce include viruses (e.g. norovirus), bacteria (e.g. *E. coli*, *Salmonella,* and *Shigella*), and parasites (e.g. *Cyclospora cayetanensis*), but several other organisms have also caused produce-associated outbreaks [2].

Where Contamination Occurs

Unfortunately, produce contamination can occur anywhere in the production process from growth, to harvest, to transport, to food preparation and consumption; this is known as the farm-to-fork continuum. To list a few examples of the myriad ways produce contamination may occur: animals may come into contact with crops and defecate in the field (reviewed in [8]); a farm worker may be ill and inadvertently contaminate the produce while handling; the equipment to clean and sort produce may become contaminated [9]; bacteria may reproduce during long transports (reviewed in [10]); and various routes of contamination may occur during food preparation in the kitchen (reviewed in [4]). While precautions are implemented at various stages during produce production, they are less effective if contamination is present at the first stage: the farm.

Contamination on the Farm

We must take steps along the entire farm-to-fork continuum to ensure safety, but reducing contamination on the farm, the first possible source of contamination, can greatly improve the safety of food and reduce the negative economic impact associated with outbreaks. Produce contaminated on the farm can be exceedingly difficult to identify because it is often distributed over a large geographic area. Because the produce can pass through several hands and stages before it is consumed, the exact source of contamination is sometimes impossible to identify (reviewed in [5]). Furthermore, when produce from a farm is blamed for an outbreak, a resulting ban on that particular type of produce from the specific country can cause a devastating blow to the local economy, whether or not the source of the outbreak was correctly identified [11].

Protecting produce at the source – the farm – is crucial to the safety of the nation's food supply. However, this task can prove quite difficult as environmental factors, such as wildlife and weather, are often beyond our immediate control. That being said, we can better understand how the environment, particularly climate, affects produce and pathogens so that we can adapt our growing practices to conform to the climate.

Indicators of Contamination

As described, contamination is detrimental to produce safety because of the potential presence of human pathogens, but monitoring the presence of pathogens directly is often not the most effective way to assess produce safety. Obtaining data on pathogens is often time consuming and expensive, largely because pathogens on produce are

relatively rare (reviewed in [12]), and thus difficult to identify in the vast quantities of fruits and vegetables produced around the world. In order to directly study pathogens on produce, a study would likely need to be so large that it would be cost prohibitive (reviewed in [13]). Since most of the pathogens of concern are spread through feces, monitoring the presence of fecal contamination can indicate whether there is the potential for a pathogen to be present. Fecal contamination is monitored through fecal indicator organisms, or 'indicators', which are non-pathogenic organisms commonly found in feces (reviewed in [13]). Indicators are more prevalent than pathogens, and they are generally easier, quicker, and cheaper to identify. However, just because the indicator is present does not mean that the pathogen is present, and vice versa, but, currently, this is the best system available for monitoring the likelihood of contamination on a large scale (reviewed in [13]). Climate studies often assess fecal indicators instead of pathogens [14].

Three common fecal indicators used to assess food quality are *Enterococcus*, aerobic plate count (APC), and coliforms. Enterococci are categorized as "all streptococci of fecal origin that produce group D antigen" (reviewed in [15]). Many of the bacteria in the enterococci group are heat and cold-tolerant and grow well on plants (reviewed in [15]). *E. faecalis* and *E. faecium* are the most common enterococci found in food. Enterococci are commonly found in a range of animal intestines, so the presence of Enterococci may indicate the presence of fecal contamination. However, Enterococci can also survive and reproduce in the environment, so the presence of Enterococci does not necessarily indicate contamination (reviewed in [15]). The presence of fecal indicators does not completely correlate with the presence of pathogens, but testing for

multiple indicators can improve the reliability of the correlation between indicators and fecal contamination.

To assess the general sanitary conditions of a food production environment, scientists often measure the aerobic plate count (APC). The APC is a measure of general aerobic bacteria. The APC is the resulting number of colonies; more colonies indicate more bacteria on the sample. While this test does not diagnose the origin or type of bacteria, it indicates the presence of bacteria, which implies that contamination may be present or that the environmental conditions are suited for bacterial growth. Similar to *Enterococcus*, the lack of a high APC does not mean that pathogens are not present, but a high APC is a red flag for contamination and the food quality should be assessed (reviewed in [16]).

Scientists also frequently test for the presence of coliforms to monitor food safety and quality. Coliforms are a general type of *Enterobacteriaceae*; they can be any gram-negative aerobic bacteria that ferments lactose into gas and acid at temperatures of 44.5-45.5°C (reviewed in [17]). Coliforms can live in animal intestines, but they are not confined to the intestinal tract. These indicators can survive and reproduce in the environment, and many of them are resistant to freezing temperatures but may not survive in hot conditions (reviewed in [17]). While coliforms measurements are frequently used to assess food safety, they are not perfect indicators. As with nearly all fecal indicators, the presence of coliforms does not mean that pathogens or even contamination are present, and their absence does not indicate the absence of contamination (reviewed in [17]). Despite their limitations, indicators are currently the

best alternative to measuring pathogens directly in order to evaluate food safety and fecal contamination.

Climate Effects on Plants and Pathogens

Both plants and their pathogens have evolved to adapt to each other and to the environment, but with changing climate, the plant-pathogen balance can be disturbed. Changing climate factors, such as warmer temperatures, which affect plant pathogens, may also affect human pathogens that reside on plants. The global climate has changed significantly in the past one hundred years, and it is predicted to continue to change. However, the changes will not be uniform across the planet, or even across individual countries or regions. While models can estimate the changes that will occur, they are still only predictions. Regardless of the accuracy of the models, change appears inevitable. A few or the predicted changes include: overall higher temperatures, warmer winters, major temperature swings, redistribution of rainfall, increases in drought and flooding, and increases in carbon dioxide levels in the air (reviewed in [18, 19]).

Based on previous research, the three variables most likely to affect the plant-pathogen dynamic include temperature, rainfall, and carbon dioxide levels (reviewed in [18]). Increases in temperature stimulate plant growth (reviewed in [20]), which invariably leads to more places for pathogens and indicators to shelter, but warm moist conditions also stimulate bacterial growth (reviewed in [21]). Warmer temperatures also encourage bacterial evolution (reviewed in [22]), and warmer winters may enable certain organisms and viruses to better survive the winter months (reviewed in [21]). In the human population, diarrheal illnesses tend to increase in the week following warmer-

than-average temperatures [23] (reviewed in [24, 25]). This phenomenon may or may not occur on the farm, but increases of foodborne illness in humans may indirectly lead to more contamination on the farm.

With warmer temperature comes increased evaporation, which can lead to both depletion of water stores and greater rainfall (reviewed in [25]). Drought can inhibit plant defenses against pathogens, and farmers may be pushed to use water that is less safe than what they would normally use to water their crops (reviewed in [24]). Some areas may experience increased rainfall or fewer, but more intense, days of rain. Both scenarios can contribute to flooding as water stores overflow or the dry ground is unable to absorb the sudden heavy rainfall. Flooding plays a crucial role in transmission of pathogens to produce because heavy rainfall on farms can push animal waste into the fields (reviewed in [26]). Outside of the farm, flooding can overwhelm sewer systems, thereby spreading waste and pathogens directly onto fields or into water stores used to irrigate fields (reviewed in [4, 26, 27]).

Increasing carbon dioxide levels can also stimulate plant growth (reviewed in [28]), but this can further deplete water supplies and nitrogen in the soil, thereby discouraging plant growth (reviewed in [18, 29]). Increasing carbon dioxide levels appears to have pathogen and plant-specific effects and may encourage pathogen growth and persistence [30]. Because the dataset used in this research does not contain information on carbon dioxide levels, we will instead focus on temperature and precipitation.

Climate Models

The role of environmental factors on contamination of produce is not well understood. Rather than examining comprehensive weather effects, several of the existing mathematical and modeling studies examine the relationship between one particular pathogen and one particular weather variable. One such example is a study by Lake et al. that utilizes a two-way ANOVA test to examine the effects of carbon dioxide on the plant pathogen *Erysiphe cichoracearum* on the plant *Arabidopsis thaliana* [30]. More complex models including time and space are necessary for a deeper understanding of how weather affects contamination, and some researchers have begun developing more sophisticated models. To assess the effects of location on disease and weather, Curriero et al. utilized spatial clustering models and Monte Carlo simulations to analyze the relationship between waterborne disease and extreme rainfall. They defined extreme rainfall as those events in the top 10% of rainfall events in a given time period [31]. In another study, Curreiero et al. examined the length of time for which temperature affects human mortality by using a log-linear regression analysis for time-series data [32]. A study by Kriss et al. focused on the time frame in which environmental variables affect wheat disease [33]. Weather events or variables, as well as the time frame of particular weather events, likely play a role in the survival and growth of bacteria on produce.

Clean Greens I study

The proposed research will be based on data collected in a study called Clean Greens I (CGI), which sought to identify risks for contamination on farms near the U.S.-Mexico border [34]. Samples of produce, farm workers' hands, and farm equipment

were collected and tested for bacteria associated with fecal contamination, such as coliforms, *Enterococcus*, and APC. The epidemiological results indicated that farm workers' hands, the method of packing produce, and the equipment in the packing sheds posed a high risk for contamination [9, 34]; the concentration of coliforms and APC increased throughout the packing process [9]. Some of the contamination may also have been introduced to or propagated on the farm through environmental variables and may be affected by the season [34]. The CGI study also tested for the presence of pathogens (*Salmonella, Listeria monocytogenes, Shigella,* and *E. coli* O157:H7) because pathogens are of more public health concern than fecal indicators. However, pathogens are quite rare, and only 3 samples out of 466 in the CGI study were found to be contaminated with *Listeria monocytogenes* [35]; these results support the use of fecal indicators in studying contamination on produce.

Goals and Aims

To better protect the food supply for an increasing global population and the economy of agriculture communities, we need to better understand the process by which produce on the farm becomes contaminated and how short-term climate influences contamination. Thus, the goal of this research is to quantify the relationship between weather and fecal indicators on farms along the U.S.-Mexico border in order to inform future food safety research. To attain this goal, we aim to 1) assess the quality of climate data from local weather stations to inform creation of a climate database; 2) create and validate the model of the relationship between local climate and produce contamination; 3) based on the model created, identify the climate risk factors most associated with

bacteria prevalence; and 4) identify the climate time frame prior to harvest that has the greatest influence on bacteria prevalence.

Significance

A mathematical model describing the relationship between weather and fecal indicators on produce will help to identify some of the factors that affect the safety of produce while on the farm. Improving produce safety is vital to reducing the disease burden and negative economic impacts due to foodborne illness. Specifically, this model will indicate the particular weather variables and weather timeframe that influences produce contamination. Understanding how weather affects produce contamination can lead to policies and the development of good agricultural practices that will help farmers plan safer ways to grow and harvest their crops based on environmental conditions. Furthermore, this model will serve as a starting point for other scientists to build on the research of climate and foodborne illnesses in other crops, in other regions, for longer periods of time, with more detailed weather information, and with pathogens instead of only indicators. Researching the causes and facilitators of produce contamination is necessary to reduce produce destruction, devastation for local agricultural communities, and illness or death in consumers of fresh produce.

INTRODUCTION

Foodborne outbreaks have long been associated with consumption of undercooked meat, raw eggs, and unpasteurized milk [36], but in the last few decades, produce has become an important vehicle for foodborne pathogens [2, 34] (reviewed in [5]). Annually, in the United States, contaminated produce causes an estimated 1.2 million cases of foodborne illness and $1.4 billion in economic loses [2]. The increase in produce-related outbreaks from <1% of outbreaks in the 1970s to 6% in the 1990s [37] is due in part to better surveillance and detection methods, an increase in the consumption of fresh produce, and changes in the industrialization and distribution of food around the world (reviewed in [4, 5, 38]). Outbreaks are often the result of fecal material contacting produce, such as when animal waste containing pathogenic microorganisms contaminates a field, or when farm workers handle produce after insufficient hand washing [9] (reviewed in [4, 8]). Contamination may occur at any place along the production chain, from the field to the point of preparation by the consumer, but reducing contamination on the farm, the first possible source of contamination, can improve food safety (reviewed in [5]).

Once foodborne pathogens are introduced in the field, their survival, proliferation and diffusion are dependent, in part, on weather conditions, but these relationships are complex and poorly understood (reviewed in [18, 24, 38, 39]). The association between weather and *plant* pathogens, however, has been more thoroughly studied [40], and evidence drawn from plant systems can serve as a guide for how improved understanding of these relationships can enhance risk management approaches. For instance, plant pathogens can proliferate between growing seasons during warm winters, and knowing the plant-specific temperature threshold necessary to inactivate pathogens can aid in

management decisions [41] (reviewed in [22]). As an example, temperatures of <-10°C reduce the survival of the stem rust causing fungus *Puccinia* graminis subsp. *graminicola* on perennial ryegrass and tall fescue by at least 75%, while temperatures of -3°C to -6°C failed to inactivate the fungus [41]. Understanding these thresholds enables growers to implement costly stem rust management practices only when the risk of pathogen over-winter survival is highest (winter temperatures > -10^{o}C). As another example, the spread of *Dothistroma* needle blight is positively associated with sustained precipitation during warm temperatures [42], and moist conditions can also encourage plant-associated bacterial proliferation, with implications for the cultivation of a variety of crops [40].

Likewise, in human pathogen systems, experimental studies have shown that warm temperatures can also stimulate pathogen proliferation; for instance, *Salmonella* concentrations were 14-fold higher on inoculated cilantro plants incubated at 30°C compared to those incubated at 24° [43]. In another example, the fall harvest, compared to the winter or spring harvest, was associated with an increased concentration and prevalence of fecal indicators on produce samples, but the mechanisms underlying these patterns were not explored [34]. Thus, while evidence for associations between weather (temperature and precipitation) and plant pathogens is robust, research is sparse on the association between weather and foodborne pathogens on crops (reviewed in [19, 21]).

At the same time, several epidemiological studies have examined the relationship between weather and general gastrointestinal illness due to foodborne pathogens in humans. For instance, an 11-year study across 5 cities in Australia found that cases of foodborne illness from *Salmonella* were positively associated with warm temperatures during the previous month, leading the authors to speculate that temperature affected

Salmonella proliferation prior to food preparation [44]. Rainfall has also been positively associated with acute all-cause gastrointestinal illness, such as in a Wisconsin study where pediatric visits to the emergency department for acute gastrointestinal illness were found to be positively associated with rainfall 4 days prior to the visit [45]. Excessive rainfall can flood sewer systems, which may contaminate drinking or irrigation water, or the rain may wash animal waste onto crop fields, thereby contaminating fresh produce (reviewed in [4, 26, 27]).

Despite the epidemiological evidence for a link between weather and gastrointestinal illness, the mechanisms that link foodborne pathogens on crops to weather conditions have not been explored. Foodborne pathogens on produce are rare and can be cost-prohibitive to study (reviewed in [12]). Therefore we [9, 12, 34, 35, 46] and many others [47-53] use microbiological indicators of fecal contamination to characterize contamination in fields, irrigation waters, packing facilities and on produce, despite their limitations [12]. Here, we analyze the degree to which precipitation and temperature, and variability in these factors, are associated with three bacterial fecal indicators (aerobic plate count [APC], coliforms, and *Enterococcus*) on produce sampled at, and prior to, harvest from fields in the southern United States between 2000 and 2002 [9, 34], and we discuss the implications for food safety, particularly in the context of a changing climate.

METHODS

Produce and Indicator Data

Produce fecal indicator data were drawn from the Clean Greens I study (CGI), carried out between 2000-2002 by collaborators at Emory University and North Carolina State University (NCSU) on 15 fields in the southern United States; the specific location of sample sites was kept confidential to protect the identity of the farmers. Produce sampling was not evenly distributed over time, field, or crop type (Table 1). At each field visit, 400 – 600g samples from produce on the field (i.e., pre-harvest) were collected and shipped on ice overnight to NCSU and analyzed using standard bacterial assays for aerobic plate count [APC], *Enterococcus*, and coliforms within 24 hours of collection as described in detail elsewhere [9, 35]. The final dataset consists of 191 produce samples assayed for the three indicators, consisting of ten crop types (arugula, cabbage, cantaloupe, celery, cilantro, collards, dill, mustard greens [greens], parsley, and spinach).

Weather Data

Weather data was acquired from NOAA station ID 722506, adjacent to the sampled fields (Figure 1), and was selected based on its proximity to the fields, as well as the quality and completeness of the available data records when compared to alternative stations available via the National Climatic Data Center [54]. Daily minimum and maximum temperature and hourly precipitation were obtained for the station from October 1, 2000 through May 28, 2002, the last day of produce sample collection. Daily average temperature (°C) and daily total precipitation (millimeters) were calculated and joined with the produce indicator data.

Statistical Analysis

We analyzed the relationship between weather variables and each fecal indicator: APC, coliforms, and *Enterococcus*. ANOVA was used to evaluate variation in the mean concentration of indicators across produce type and field. Because the data were collected over different crops and across fields, the sampling field and produce type were treated as groups in a multi-level analysis with cross-level (additive) random intercepts estimated for field and produce types. Indicator data were $\log_{10}$ transformed where appropriate. To evaluate the effects of weather over time on bacterial proliferation, temperature and precipitation values at each day within 0 to 6 days prior to harvest were included in models, as were the average temperature and precipitation values for 1 week prior to harvest. While no previous studies on fresh produce have identified appropriate lag times, lags up to one-week were selected to capture the diminishing influence of weather given the short lifetimes of key indicator organisms.

Each fecal indicator concentration was considered as the response variable in a univariate analysis of average temperature or daily precipitation, lagged 0 - 6 days prior to sample collection (or the average of 0 – 6 days). Given field, *j*, produce type, *k*, and individual sample, *i*, we fitted the following hierarchical model:

$$\Pi_{ijk} = \gamma_0 + \mu_{0j} + \mu_{0k} + \gamma_1 * \bar{E}_{ijk} + e_{ijk}$$

where the γ and μ coefficients represent fixed and random effects, respectively; Π_{ijk} is the $\log_{10}$ transformed fecal indicator (APC, coliforms, *Enterococcus*) assayed on produce type *k* from field *j* for sample *i*; $\bar{E}$ is the weather variable (temperature or precipitation); and e represents the deviation of the individual sample from the group means of the field and produce type. We assumed that $\mu_{0j} \sim N(0,\tau_0)$ and $\mu_{0k} \sim N(0, \tau_0)$, where τ is the

variability among group means. Next, average daily temperature and total daily precipitation were both included in a multivariate model for each indicator to evaluate the effect on the fixed slope as compared to the univariate model.

A second set of models were then created to evaluate the effects of precipitation or temperature for a range of different weather values, such as heavy rainfall or high temperatures. Because extreme weather events could not be included due to the limited time period examined (and thus the difficulty designating extreme values), a quadratic temperature or quadratic precipitation variable was added to the first set of linear mixed models to evaluate how weather may affect indicator levels as temperature or rainfall increases. For all models, covariance parameters were estimated using residual restricted maximum likelihood, and all statistical analyses were carried out in SAS 9.3 [55].

No human research was conducted during this study, and we were not required to gain IRB approval.

RESULTS

The indicator concentrations ($\log_{10}$[CFU/g]) of the 191 samples of produce collected between November 2000 and May 2002 are shown by crop type and field in Table 2. There was significant variation in mean indicator concentrations among crop type and field for each indicator (Table 2); we conducted hierarchical analysis to account for this variation in our models. Cantaloupe and mustard greens had the highest mean concentrations of APC and *Enterococcus*, while arugula had the highest mean concentration of coliforms. Field 13 had the highest mean concentrations of APC and *Enterococcus*, and field 15 had the highest mean concentration of coliforms (Table 2). The average daily temperature for the study period was 20.1°C (SE 0.28), and the median daily precipitation was 0.4 mm (IQR=1.6 mm).

We first assessed the significance of the association between precipitation and indicator concentrations for individual lags. Precipitation was significantly positively associated with indicator levels on various lag days: for $\log_{10}$ APC, lag day 1 ($p<0.05$) and lag day 2 ($p<0.06$) (borderline significance); for $\log_{10}$ *Enterococcus*, lag day 3 ($p<0.05$) and lag day 4 ($p<0.05$); and for the weekly average of $\log_{10}$ coliforms ($p<0.05$) and for the weekly average of $\log_{10}$ *Enterococcus* ($p<0.05$). There was generally a positive association between precipitation and $\log_{10}$ APC starting as a significant relationship at lag day 1, and a waning, statistically insignificant association throughout the week (Figure 2A). Throughout the week prior to sample collection, there was a positive yet fairly uniform association between precipitation and $\log_{10}$ coliform (not significant) and *Enterococcus* levels (significant on lag days 3 and 4, $p<0.05$; Figure 2B, 2C). Precipitation on lag day 6 exhibited the strongest, but insignificant association with

indicator levels, for reasons detailed in the Discussion (Figure 2A, 2B, 2C). There was no significant variation in the effect of precipitation on indicator levels between crop types or between fields for any lag day (results not shown).

In evaluating the association between temperature and indicator levels, temperature on lag days 0, 2, 3, 4, 5, and the weekly average, were significantly associated with the $\log_{10}$ APC (Figure 3A). While not significantly different from the slopes on other lag days, the magnitude of the effect (i.e. the fixed slope) was strongest on lag days 3 and 4, with $\log_{10}$ APC increasing by 0.15 CFU/g for every degree rise in temperature, which was similar to the week-long average temperature effects (0.14 CFU/g). Temperature was only significantly associated with $\log_{10}$ coliform on lag day 2. The $\log_{10}$ coliform increased 0.12 CFU/g for every degree increase in temperature, but the effect appeared to wane after 2 days. Temperature was significantly associated with $\log_{10}$ *Enterococcus* on lag days 0 – 5, as well as with the weekly average. For lag days 0 – 5, the effect was fairly consistent and ranged from 0.23 CFU/g to 0.29 CFU/g increase in the $\log_{10}$ *Enterococcus* for every degree rise in temperature. The week-long temperature average had the strongest association with *Enterococcus*; $\log_{10}$ *Enterococcus* increased 0.35 CFU/g for every degree increase in temperature (Figure 3C). Modeling precipitation and temperature together did not significantly alter the results of the univariate models (results not shown).

To evaluate how a range of precipitation values or temperatures may affect indicator concentrations, we added a quadratic term of either weekly average precipitation or temperature to the weekly average basic models from Figure 2 and 3. For the quadratic precipitation models, the $\log_{10}$ APC and $\log_{10}$ *Enterococcus* concentrations

increased after weekly average precipitation reached approximately 3 mm (Figure 4A, 4C), while the $\log_{10}$ coliforms concentrations increased consistently until weekly average precipitation reached approximately 10mm (Figure 4B). For the quadratic temperature models, the $\log_{10}$ APC and $\log_{10}$ coliforms increased once the weekly average temperature reached approximately 15°C and 18°C, respectively (Figures 5A, 5B). Log_{10} *Enterococcus* concentrations increased consistently for all observed temperatures (starting at approximately 10°C; Figure 5C).

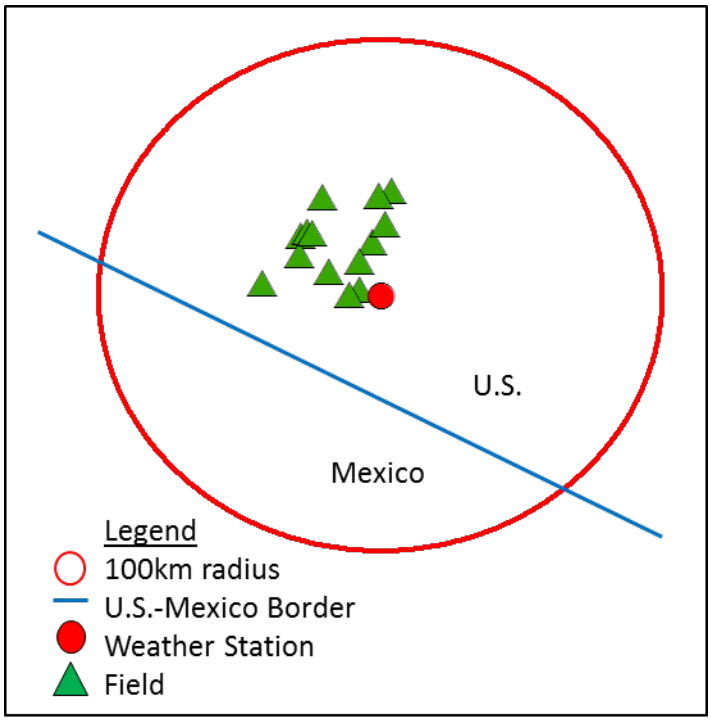

Figure 1. Relative locations of sample collection, weather station and U.S.-Mexico border. Note: the specific location of sample sites was kept confidential as described in the text.

Table 1. Sample distribution according to month and crop

Date	Total Samples	Unique Dates	Fields	Sample number by crop type									
				Arugula	*Cabbage*	*Cantaloupe*	*Celery*	*Cilantro*	*Collards*	*Dill*	*Greens*	*Parsley*	*Spinach*
Nov. 2000	12	2	2					9					3
Jan. 2001	12	3	2					6				3	3
Feb. 2001	12	3	2	6									6
Mar. 2001	12	3	3	3				6					3
Apr. 2002	6	1	2					6					
Feb. 2002	20	4	3				12	4			4		
Mar. 2002	39	5	5					3			21	6	3
Apr. 2002	42	7	7		12				6	6	6	18	
May 2002	36	6	5			36							
Total	191	34	15*	9	12	36	12	34	6	6	31	27	18

*There are 15 total fields, but some fields produced more than 1 type of crop and produced in more than 1 month.

Table 2. Mean and standard error (SE) for indicator concentrations for crop and field

Crop	N	Log_{10} APC Mean CFU/g (SE)	Log_{10}Coliforms Mean CFU/g (SE)	Log_{10}*Enterococcus* Mean CFU/g (SE)
Arugula	9	12.98 (0.42)	7.61 (1.23)	2.96 (0.68)
Cabbage	12	11.95 (0.48)	2.1 (0.31)	6.31 (0.63)
Cantaloupe	36	14.77 (0.29)	5.45 (0.36)	8.58 (0.39)
Celery	12	12.38 (0.41)	2.08 (0.27)	1.72 (0.12)
Cilantro	34	12.78 (0.41)	2.47 (0.25)	3.95 (0.43)
Collards	6	9.95 (0.65)	1.61 (0.0)	2.66 (0.50)
Dill	6	11.56 (0.20)	5.13 (0.99)	7.77 (0.47)
Greens	31	13.94 (0.36)	4.53 (0.48)	9.24 (0.55)
Parsley	27	11.67 (0.43)	3.81 (0.44)	5.1 (0.54)
Spinach	18	12.92 (0.50)	3.21 (0.49)	4.32 (0.52)
Field				
1	18	11.21 (0.46)	2.25 (0.26)	3.57 (0.68)
2	30	12.05 (0.35)	3.53 (0.43)	5.5 (0.49)
3	24	13.2 (0.50)	3.84 (0.48)	6.19 (0.60)
4	6	14.3 (0.57)	4.95 (1.3)	7.66 (1.30)
5	6	9.81 (0.34)	4.06 (0.67)	2.74 (0.75)
6	3	13.35 (0.52)	3.3 (0.86)	6.29 (0.10)
7	12	14.32 (0.61)	4.85 (0.93)	7.22 (1.21)
8	7	12.65 (0.56)	3.7 (0.61)	5.96 (1.08)
9	12	14.87 (0.32)	5.67 (0.73)	9.51 (0.49)
10	12	11.75 (0.32)	3.51 (0.62)	5.6 (0.51)
11	25	12.28 (0.53)	3.42 (0.49)	6.89 (0.91)
12	3	11.7 (0.71)	1.61 (0.0)	3.27 (0.86)
13	6	17.25 (0.19)	4.66 (1.01)	10.33 (0.63)
14	18	14.06 (0.49)	3.16 (0.44)	5.08 (0.69)
15	9	12.98 (0.42)	7.61 (1.23)	2.96 (0.68)

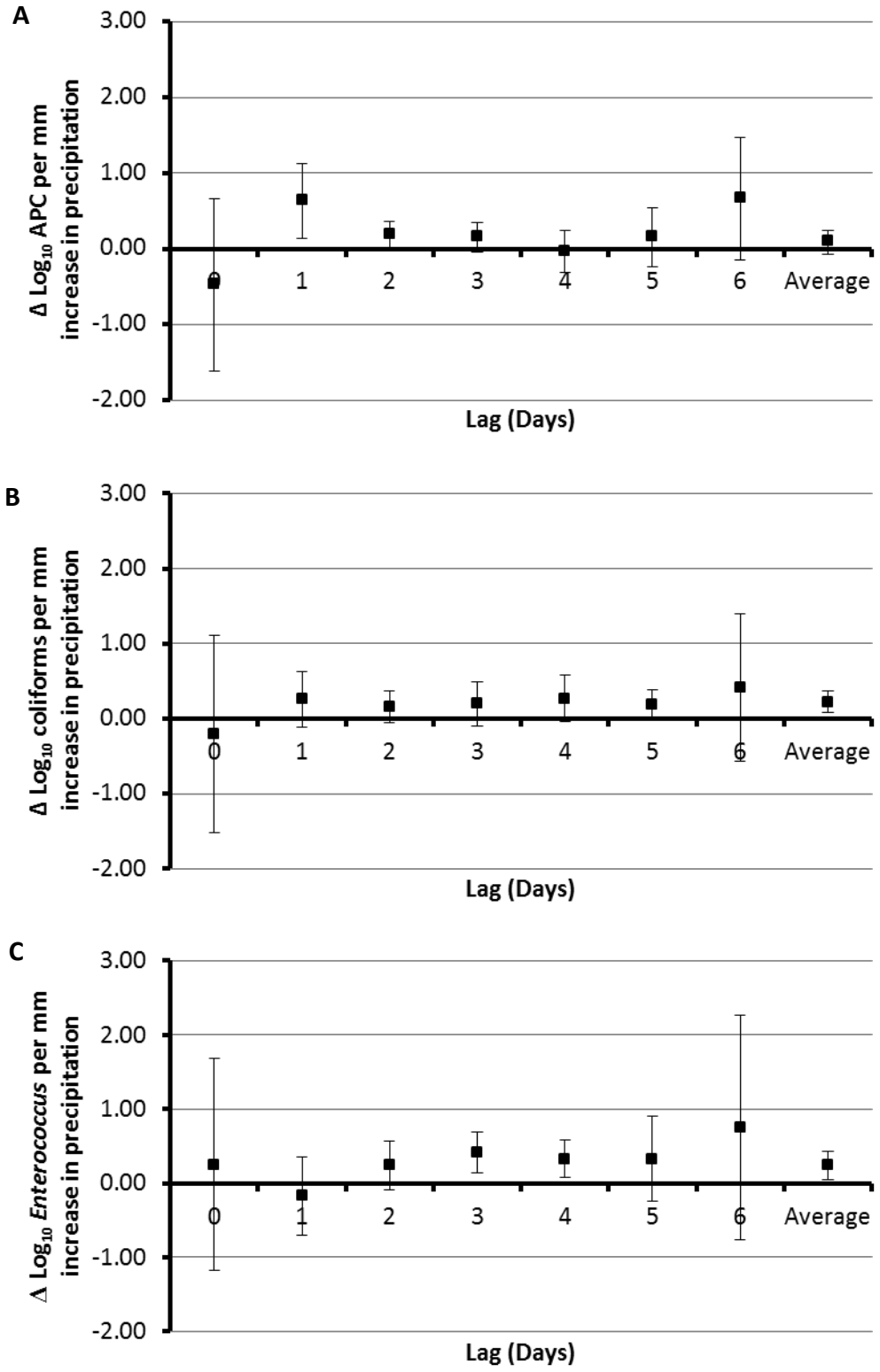

Figure 2. Association between precipitation and the $\log_{10}$ concentration of APC (2A), coliforms (2B), or *Enterococcus* (2C), across all crop types and fields for precipitation on lag days 0 – 6 or the average of lag days 0 - 6.

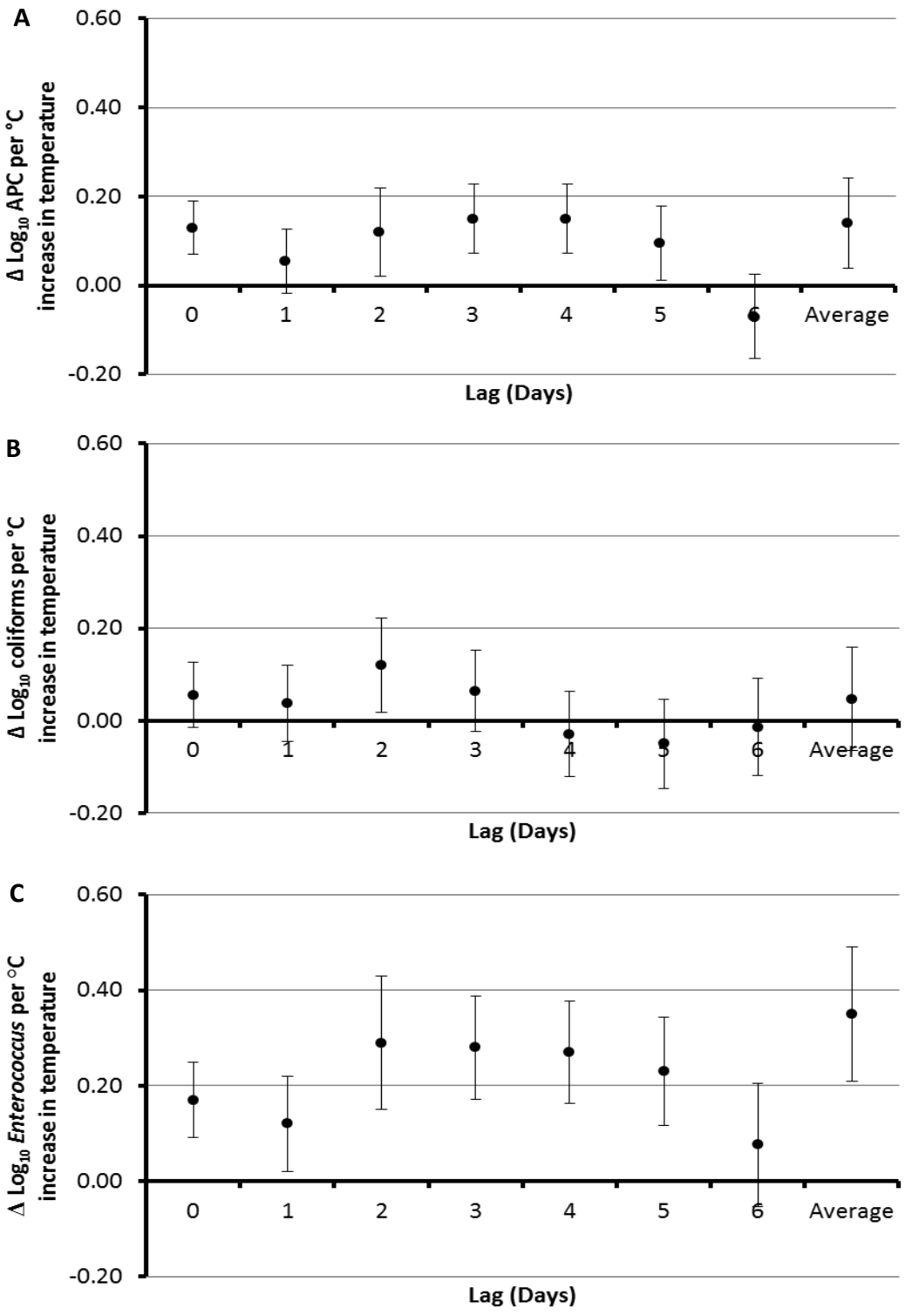

Figure 3. Association between temperature and the $\log_{10}$ concentration of APC (3A), coliforms (3B), or *Enterococcus* (3C), across all crop types and fields for temperature on lag days 0 – 6 or the average of lag days 0 – 6.

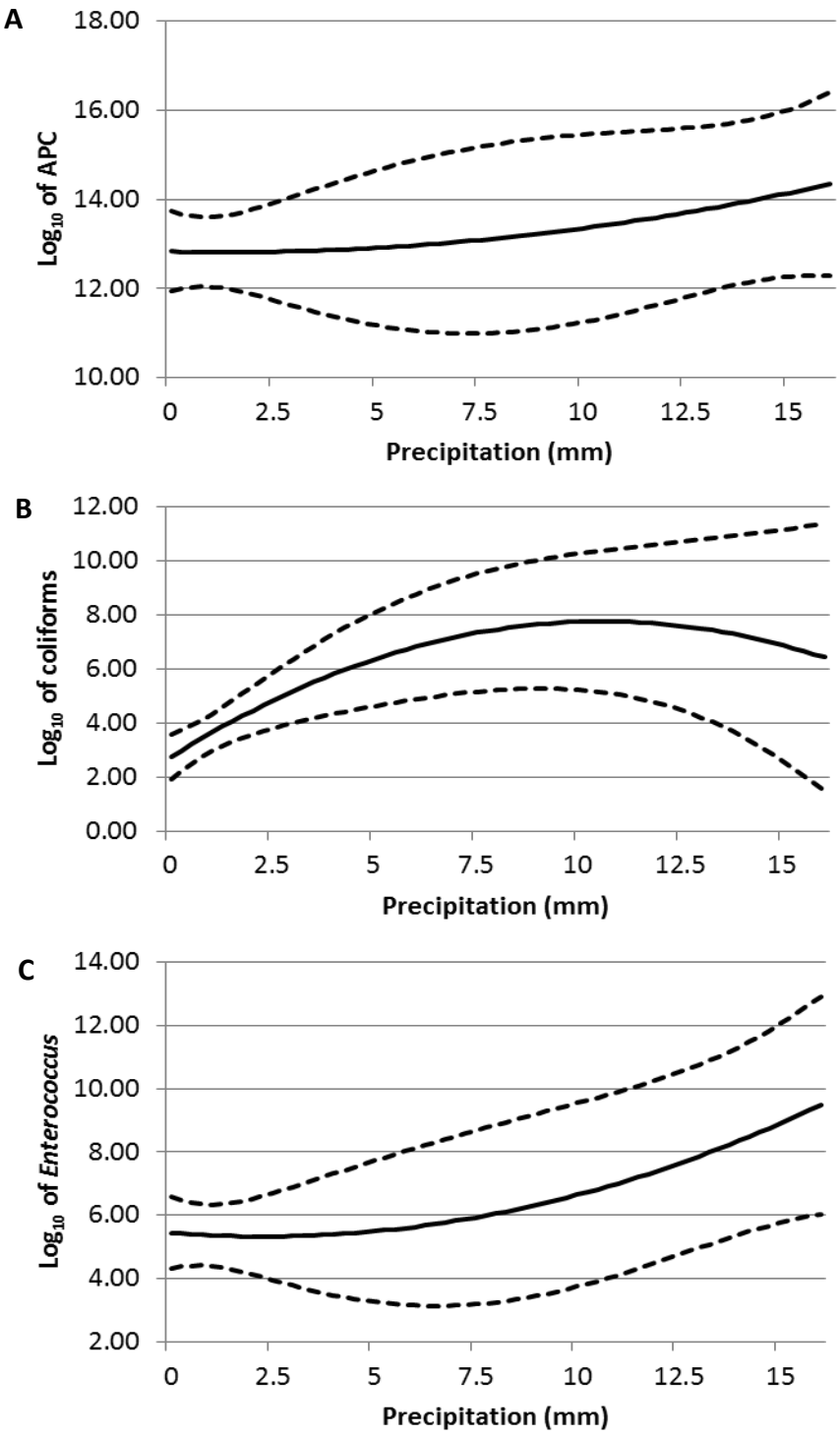

Figure 4. Quadratic model fit between precipitation and $\log_{10}$ of indicators: APC (4A), coliforms (4B), or *Enterococcus* (4C) across all crop types and fields for precipitation over the average of lag days 0 – 6. 95% confidence interval indicated by dotted lines.

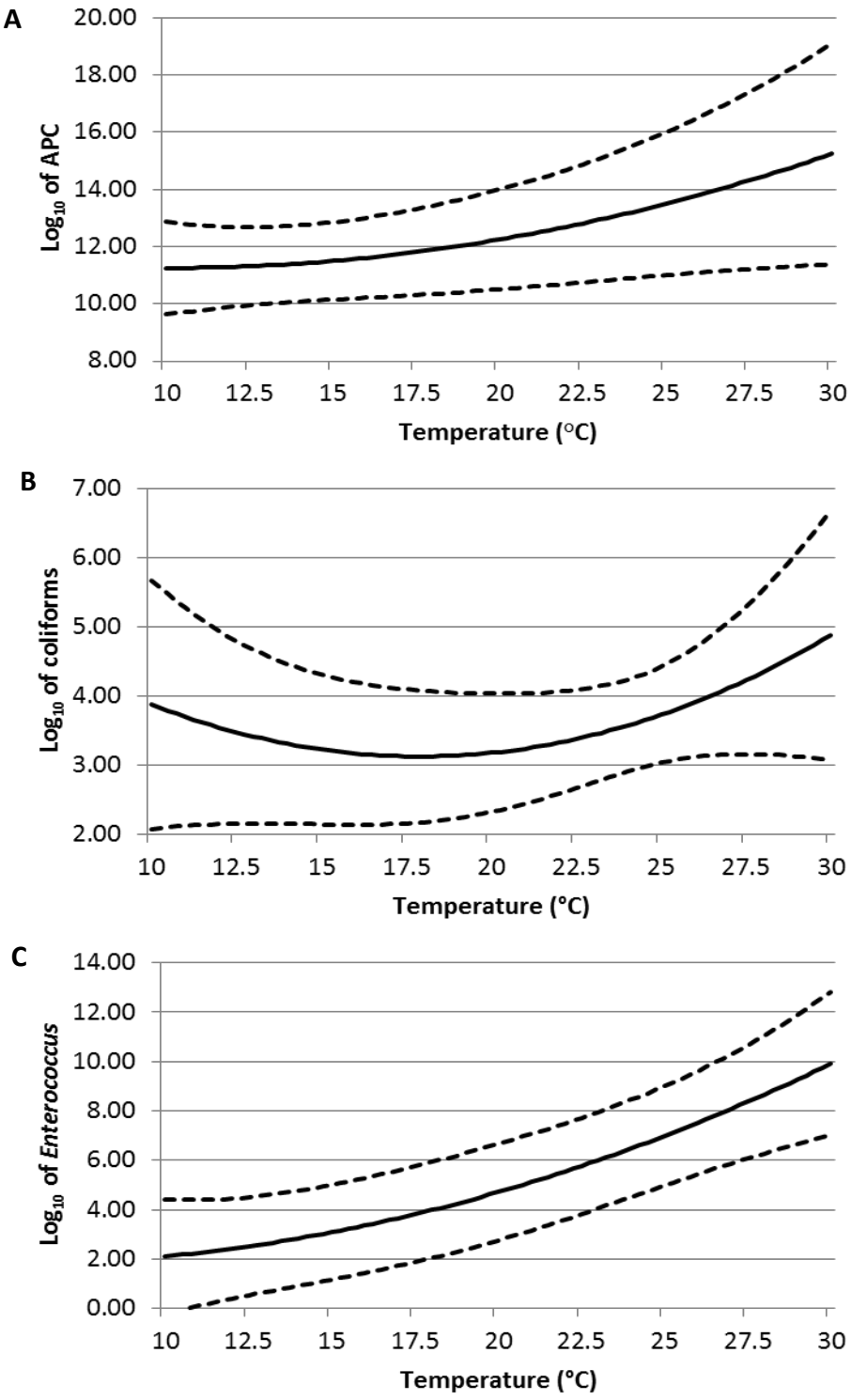

Figure 5. Quadratic model fit between temperature and $\log_{10}$ of indicators: APC (5A), coliforms (5B), or *Enterococcus* (5C) across all crop types and fields for precipitation over the average of lag days 0 – 6. 95% confidence interval indicated by dotted lines.

DISCUSSION

To evaluate the relationship between weather and fecal contamination on produce in the field, we examined the association between each of the fecal indicators (aerobic plate count (APC), coliforms, and *Enterococcus*) and weather variables using a hierarchical modeling approach. The association between indicators and average daily temperature or daily total precipitation was estimated for each day, up to a week, prior to sample collection. The average daily temperature was significantly associated with indicator concentrations on multiple lag days, from lag day 0 through lag day 5. However, the lag days were not significantly different from one another, as evidenced by the overlapping confidence intervals. The daily total precipitation was generally significantly associated with indicator concentrations on one or two lag days prior to harvest.

The results of this study indicated that temperature generally had a significant, positive association with fecal indicators APC and *Enterococcus* over nearly a week-long period prior to sampling. We speculate that concentrations of indicator organisms on the crops increased as the average air temperature neared the optimal temperatures for bacterial growth. In the days leading up to and during the study period in which samples were collected, the average daily temperature did not rise above 30°C, and 90% of the daily temperatures were above 10.5°C. APC constitutes a wide range of aerobic bacteria, and the optimal range for growth of APC organisms is considered to be 30°C – 37°C [56]. Based on our quadratic models, APC had a positive association with temperature once the weekly average temperature exceeded approximately 15°C.

Likewise, *Enterococcus* appeared to thrive in warm temperatures. Several *Enterococcus* spp. strains grow optimally at 45°C, but they have been observed to grow at a wide range of temperatures, from 0°C – 50°C [57] (reviewed in [15]). The association detected in the present work between *Enterococcus* and weekly average temperature was strong over a wide range, with a positive association starting around 10°C. For both APC and *Enterococcus,* the association with temperature was sustained for nearly a week prior to sample collection. As expected, the results indicated that sustained warm temperatures were conducive to fecal indicator growth.

In contrast to APC and *Enterococcus,* coliforms were only significantly associated with temperature on one lag day. Coliforms grow optimally between 35°C – 45°C (reviewed in [17]), which is greater than the temperatures observed in this study. The lack of a sustained relationship between temperature and coliforms may be due to other conditions not included in this study, such as solar radiation [58].

Daily total precipitation was positively associated with APC and *Enterococcus* concentrations on one or two days, respectively, in the week prior to sample collection. Precipitation, as well as its duration, may affect indicator proliferation in multiple ways. For example, precipitation may provide moisture necessary for growth [59]. In modeling the relationship between daily average precipitation and APC, precipitation on lag day 1 was the only significant lag. As APC is a general measure of aerobic bacteria, the results indicated that moisture may encourage bacterial growth in the short term (i.e. one day prior to harvest), while precipitation earlier in the week may have limited influence on the current concentration. The significant association found in the present work between precipitation on lag days 3 and 4 with *Enterococcus* may also be attributed to the moist

and humid conditions that typically follow rainfall, which are ideal for growth of *Enterococcus* [59].

However, precipitation may also introduce bacteria to the field. For instance, heavy rainfall may cause contaminated water to wash onto a field, or it may spread contamination already present throughout the field (reviewed in [4]). The indicators we measured are present in environmental reservoirs (reviewed in [15-17]), and precipitation may play a role in spreading bacteria from these sources. If precipitation introduced bacteria into fields or onto crops, we would expect a small delay in between rainfall and bacteria proliferation on the crop. Previous studies indicated that gastrointestinal illness was associated with rainfall 4 days prior to illness, which was likely attributed to the lag between rainfall and runoff contamination reaching drinking water sources [45].The significant association we identified between *Enterococcus* and precipitation on lag days 3 and 4 may be indicative of rainfall introducing contamination to the crops; on the other hand, the rainfall may be creating ideal conditions for growth of *Enterococcus* as we discussed previously.

In addition to stimulating growth and introducing contamination to the field, precipitation may also remove bacteria from crops via a rinsing effect during rainfall (reviewed in [39]). Based on our models, sustained precipitation was not associated with indicator concentrations, and this may be due to sustained precipitation washing bacteria from the plant surfaces. In contrast, short periods of precipitation may be insufficient to remove bacteria, while at the same time providing ideal conditions for growth. Our quadratic precipitation models lend support to this hypothesis in that APC and *Enterococcus* concentrations increased once weekly average precipitation reached

approximately 3 mm, which may be sufficient to create moist conditions conducive to bacterial growth. Below these thresholds, indicator concentrations decrease, which may be partially due to bacteria being washed off of the plants at a rate faster than they are introduced. As with temperature, coliforms were not significantly associated with precipitation in our models.

Understanding the association between weather and contamination on crops is important for improving food safety, but one limitation of this study was that we measured fecal indicators rather than foodborne pathogens. The presence of very low concentrations of foodborne pathogens in the environment presents a tradeoff: in order to study the relationship between weather and foodborne pathogens, one must either inoculate the crops with a pathogen at concentrations sufficient for detection [43], which may not accurately reflect the association in a natural setting, or one must assess fecal indicators, which provide indirect evidence of contamination with fecal material and potentially pathogens [12]. Our results were strengthened by our use of three different indicators of fecal contamination. Another major limitation of our study was the short time span of sample collection (November 2000 – May 2002) and the absence of data from the summer months, during which temperatures may have been in the optimal range for growth of several classes of bacteria. Studies examining associations between gastrointestinal disease and weather frequently span six years or more; longer studies may permit the analysis of the effects of extreme precipitation or temperature on bacteria proliferation or disease spread [31, 45, 60]. While equivalent, long-term studies of pathogens on crops are rare, owing to their great expense, our study was strengthened through the use of a hierarchical analysis, which allowed us to explore crop- and field-

specific effects within the short period studied, making our results more widely applicable.

Our results identified multiple associations between weather and fecal contamination, providing vital first estimates that can support future research on the relationship between weather and pathogen proliferation in the field. Such findings can help shape policies to improve food safety, such as by identifying precipitation thresholds at which drainage ditches could be constructed around the fields to reduce runoff water from contaminating the field, particularly if waste sites are located nearby. Furthermore, farmers could take extra precautions to improve food safety, such as an additional produce wash step, when the weather conditions are ideal for pathogen proliferation. In addition, the implications of our results can be viewed in the context of a changing climate. As temperatures are projected to increase and precipitation may become more intense in some areas (reviewed in [26]), conditions may become even more conducive to pathogen proliferation and diffusion, in which case steps may need to be taken to improve the safety of the global food supply.

While previous studies found associations between weather events and plant-pathogens [41, 42], laboratory inoculated human pathogens on plants [43], and foodborne and waterborne diseases [44, 45], this is among the first studies to identify an association between weather and fecal bacteria on crops in a natural environment. With a changing climate and ever increasing demands on the food supply, our results provide a basis for better understanding the association between weather and foodborne pathogens.

PUBLIC HEALTH IMPLICATIONS AND RECOMMENDATIONS

- We identified a new association between weather and fecal contamination on produce on the farm.
    - Sustained warm temperatures may be particularly conducive to bacterial growth. Therefore, extra precautions may need to be taken during warm weather to maintain the quality of the food supply.
    - Precipitation within four days of harvest may encourage bacterial growth. More research is necessary to determine if farmers should wait a certain length of time after rainfall before harvesting crops in order to minimize contamination.
    - These results indicate that weather and climate should be considered when creating food safety policies on the farm.
- Our results could contribute to debates on policies to improve food safety.
    - Weather is associated with proliferation of bacteria that is indicative of fecal contamination. If weather conditions also encourage pathogen growth, then policies should focus on preventing initial contamination from reaching the crops.
    - Additional produce wash steps may be necessary during warm weather.
    - Drainage ditches could be dug around fields to prevent contaminated runoff water from contacting the crops.
- Based upon our results, we suggest the following for future studies:
    - Expand this study over a longer time period (at least 5 years), and include data collection during all seasons

  - Incorporate ‘extreme events’ into the analysis to determine how the association may change during extreme rainfall or heat.
  - Include data from a variety of climates, with a focus on agricultural regions
  - Study the association between weather and enteric pathogens, instead of just indicators, to determine if our results are applicable to pathogens.

- Our results highlight the importance of reducing contamination at the farm level in the context of a changing climate.
  - Due to climate change, temperatures may rise and precipitation may become more sporadic in the future. Our results indicate that fecal indicators are associated with warm temperature, and sporadic rainfall may be sufficient for indicator proliferation. Therefore, as the climate changes, the association between weather and contamination may become more pronounced.

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