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Evaluation of the Microbial Quality of Cantaloupe Fruit Produced on Raised or Flat Beds Following a Flooding Event

Isaack Kikway
Louisiana State University and Agricultural and Mechanical College, kikisaack@gmail.com

EVALUATION OF THE MICROBIAL QUALITY OF CANTALOUPE FRUIT PRODUCED ON RAISED OR FLAT BEDS FOLLOWING A FLOODING EVENT

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ABSTRACT

Floodwater can contain microbial contaminants such as plant and foodborne pathogens and can compromise the quality of fresh fruit and vegetables produced in Louisiana. The goal of this research was to determine the impact of flooding on microbial quality (foodborne and plant pathogens) of cantaloupe fruit produced on raised or flat beds. Cantaloupe fruit produced on 30 cm raised beds or flat ground, were flooded with a mixture of surface and well water spiked with three generic *Escherichia coli* strains (ATCC 23716, 25922, 11775). Mean baseline generic *Escherichia coli* and total coliform populations in flood water (mixture of spiked well and pond water) were 5.1±0.4 and 6.2±0.1 $\log_{10}$MPN/100ml respectively. There were no significant differences (p=0.7509 or p=0.4041 $\log_{10}$MPN/cm^2 and $\log_{10}$MPN/100ml respectively) in generic *Escherichia coli* on fruits surface from raised or flat beds. Independent of bed type, total coliform populations on fruit surface were consistent (p=0.2324 or p=0.1865 $\log_{10}$MPN/cm^2 and $\log_{10}$MPN/100ml respectively) over 72 hours, while generic *Escherichia coli* populations decreased significantly (p=<0.0001 or 0.0001 $\log_{10}$MPN/cm^2 and $\log_{10}$MPN/100ml respectively). There were no significant differences in the number of fruits positive for *Salmonella* spp. over time (RapidChek, p=0.3916; Xylose lysine desoxycholate (XLD), p=0.0634; polymerase chain reaction (PCR), p=0.4100), and between flooded and non-flooded plot (RapidChek, p=0.3916; XLD, p=0.0634; PCR, p=0.4100). Fruits positive for *L. monocytogenes* did not differ significantly over time and between flooded and non-flooded plots based on listeria semi-selective agar medium (LSA, p=0.9196) and polymerase chain reaction (PCR; p=0.9289) and between flooded and non-flooded plots (LSA, p=0.5056 and PCR, p=0.4966). Independent of bed type, mean fruit rot incidence

caused by *Sclerotium rolfsii* or *Phytophthora* spp. increased significantly by 17.6% (p=0.0001) and 20% (p=0.0001) respectively one week after flooding. No significant differences were detected in mean percent fruit rot incidence for Southern Blight (*Sclerotium rolfsii*) (p=0.4231) or Phytophthora fruit rot (*Phytopthora capsici*) (p=0.2657) between fruit produced on raised beds or flat ground. There is evidence that the quality of cantaloupe fruit might drop significantly with or without floods due to foodborne and plat pathogen contamination in production hence presenting a major public health risk to consumers.

1. CHAPTER I

1.1 Literature Review

Fresh fruits and vegetables are important components of healthy and balanced diet. Today their consumption is being encouraged globally by government health agencies to curb and alleviate a wide range of dietary illnesses such as heart disease, blood pressure, cancer, Type 2 diabetes, kidney stones, and obesity (USDA, 2008; WHO, 2003; Park et al., 2007; Zhang and Fu, 2011). Increased awareness of these health benefits has led to an increase in fruit and vegetable consumption (CDC, 2013; Naanwab and Yeboah, 2012). At the same time, the number of reported foodborne illness outbreaks linked to fresh produce has increased (CDC, 2013). A number of factors have been correlated with this increase, including changes in production and processing practices to meet the demand for ready-to-eat products such as bagged salads, increased global distribution of fresh produce in order to meet market demands, improved surveillance by health agencies, and an aging population (Buck et al., 2003; Broglia and Skapel., 2011; Sivapalasingam et al., 2009).

Foodborne illnesses from the consumption of fresh produce are dependent upon many factors. The produce must come into contact with a pathogen and the pathogen must be able to survive (but does not always need to reproduce on the product) at population levels sufficient to cause illness (Harris et al., 2003). For example, Norovirus is unable to multiply outside of a human host but can attach to and survive on lettuce and spinach at sufficient levels to cause illness (Esseili et al., 2012; Wang et al., 2012; Hirneisen and Kniel, 2013). The surface of plants is considered to be a hostile environment for many microorganisms, especially bacteria (Lindow and Brandl, 2003;

Martinez-Vaz et al., 2014). Until recently, it was assumed that human pathogens could not easily survive or reproduce in this hostile environment. However, recent research has demonstrated that enteric human pathogens can not only colonize plant tissue but can induce plant immunity responses (Erickson, 2012; Roy et al., 2013). Although it is still not well understood, the ability of these pathogens to adapt and possibly thrive on or within plants could be one explanation for the increase in foodborne illness outbreaks linked to fresh fruits and vegetables.

A wide range of fresh fruits and vegetables has been implicated in outbreaks of foodborne illnesses. Cantaloupe, tomatoes, strawberries and other berries, and leafy greens, all of which are commonly consumed raw, have served as vehicles for human infections by *Salmonella*, *Listeria monocytogenes*, enterotoxigenic and enterohemorrhagic *Escherichia coli* (pathogenic *E. coli*), Norovirus, *Clostridium botulinum*, *Campylobacter*, and parasites such as *Cyclospora* spp. (Berger et al., 2010, Bowen et al., 2006, Guo et al., 2002; Brassard et al., 2012; Ortega et al., 1997). For example, cantaloupe has been implicated in a number of foodborne related illnesses due to contamination with human pathogens (Bassam et al., 2005; Hanning et al., 2009), with the most devastating outbreak (to date) being linked to the consumption of cantaloupe produced by a farm in Colorado, US (FDA, 2011). In this outbreak, four strains of *L. monocytogenes* were associated with the contaminated fruit. Across 28 states, 146 illnesses, 30 deaths and one miscarriage were confirmed (FDA, 2011). Fruits (from the field and cold storage) and numerous environmental samples collected from the source farm were found to be contaminated with the four strains of *L. monocytogenes*. For this particular outbreak, the FDA (2011) cited several factors as those that likely contributed

to the introduction and spread of *L. monocytogenes* including: low level sporadic *L. monocytogenes* in the production field, close proximity of a cattle operation to the area where trucks used to haul cantaloupes were parked and standing water on the floor of the packing facility. The FDA's findings also highlighted the importance of on-farm good agricultural and management practices.

Water, which is the focus of this dissertation, is arguably the most important route of contamination of fruits and vegetables as the production of these crops is water intensive; utilizing water at nearly every stage of production. Pre-harvest cross contamination of cantaloupe, as well as other fruits and vegetables, with human pathogens can occur when the fruit come into contact with irrigation water or agrochemicals contaminated with animal or human feces, runoff water from livestock areas, and flood water (Hammack et al., 2004; Beuchat, 1997; Suslow, 2003; Steele, 2004). Factors that influence the potential for water to contaminate produce with microbial pathogens include water source, temperature and pH, irrigation methods, microbial quality of water, soil type, and the characteristics of the fruit or vegetable crop that is being irrigated (reviewed by Pandey, 2014). For example, the microbial quality of water depends on the source. Surface water is considered the poorest in terms of microbial quality, followed by rain or ground water and municipal (city) water (Bihn et al., 2013; Suslow, 2010; James, 2003; Pachepsky et al., 2011). Depending on the location of the edible portion of the crop, drip irrigation has the lowest level of risk associated with it in terms of the potential for cross contamination (Suslow et al., 2003). Overhead and flood irrigation methods dispense water directly onto the crop and are considered to be high risk practices for crop contamination with foodborne pathogens, especially when

the crop is irrigated close to the time of harvest (Suslow et al., 2003; Allende and Monaghan, 2015).

In 2011, the Food Safety Modernization Act (FSMA) was signed into US law. This law consists of multiple rules that address all aspects of the food chain of custody. The "Growing, Harvesting, Packing, and Holding of Produce for Human Consumption" rule (also called the Fresh Produce Safety rule), which was finalized in September 2016, focuses on pre- and postharvest standards for the safe growing, harvesting, packing, and holding of fruits and vegetables grown for human consumption. Water is a key requirement in the law and two sets of criteria for water quality, both of which are based on the presence of generic *E. coli*, have been established. The absence or low concentration of generic *E. coli*, which is an indicator microorganism, implies that the produce has not been exposed to conditions that would permit the contamination of the product by a foodborne pathogen. The first rule (criterion) states that no detectable generic *E. coli* are allowed in water used to directly contact produce (including ice) during or after harvest. The second criterion is for agricultural water that is directly applied to a growing crop. This includes irrigation water, water used to apply agrochemicals and water used for frost protection. The post-harvest criterion is based on two values: the geometric mean (GM) and the statistical threshold (STV). The GM of 100 ml water samples must be 126 or less CFU of generic *E. coli* and the STV must be 410 CFU or less CFU of generic *E. coli* per 100 ml water sample (FDA, 2016). If water does not meet these criteria, a die-off or reduction rate may be applied. A die-off rate of 0.5 log per day "to achieve a calculated log reduction of the GM and STV to meet the microbial quality criteria" can be applied for the time interval (days) between the last

application of irrigation water and harvest, or the last day that water comes into contact with the edible portion of the crop and the end of storage (Code of Federal Regulations Title 21, Section 112.45). One study indicates that the 0.5 log per day die-off rate corresponds to a 68.3% reduction in contaminants after one day, 90% after two days and 99% after four days (Bihn et al., 2016). A second study suggests that a 90% reduction in *E. coli* would require 1.5 to 6 days, depending on ambient conditions (Meals et al., 2013). Both of these scenarios provide growers with at least four days to trace-back the potential source of contamination and implement corrective measures or change the water source to prevent a future contamination event.

Good Agricultural Practices (GAPs) and FSMA both aim to ensure that the US food supply is safe for human consumption by reiterating the need for the use of production practices that prevent and minimize food safety hazards rather than utilizing responsive strategies to food contamination in the food chain of custody. However, neither program addresses contamination of fruits and vegetables by floodwater. In 2011, the FDA US Food, Drug, and Cosmetic Act issued guidance for handling fruits and vegetables exposed to floodwater; but these guidelines are conservative and do not address the diversity and complexity of fruit and vegetable production. Within these guidelines flooding is described as the "flowing or overflowing of a field with water" that is not within the grower's direct control, and that will result in crop loss or crop contamination by physical, chemical or microbial contaminants (FDA, 2011). Crops exposed to flood water present a substantial health risk, especially if the flood water has come into contact with sewage, animal waste, agricultural run-off water or other sources of pathogenic microorganisms (Howard et al., 2003). The FDA guidance for edible

portions of a food crop that have come into direct contact with flood water states that it should be "considered adulterated" and "should not enter human food channels." (FDA, 2011). Although unsubstantiated, the FDA warns that "there is no practical method of reconditioning the edible portion of a crop that will provide a reasonable assurance of human food safety" and therefore crops exposed to flood water should be "disposed of in a manner that ensures they are kept separate from crops that have not been flood damaged" to stop cross contamination to unaffected crops. Because this is a guidance, and not a rule, there are no standards established in the guidance for addressing hazards associated with crop following flooding. The guidance does however recommend that growers can test their product to determine if it is suitable for human consumption, but given the high costs associated with testing fresh produce and the prolonged time required to test the product, testing is not a viable option for most producers in the US.

In Louisiana (LA), among other Gulf coast states, hurricanes, tropical storms and torrential rains are common and often result in crop flooding. Flooding events pose a threat to agricultural production and can result in indirect economic losses to the producer (http://www.lsuagcenter.com/). Understandably then, the recommendation to destroy the entire crop due to inherent food safety risks can weigh heavily on producers. In 2008 and 2009, 75% and 50% of the sweet potato crop in LA was lost due to flooding from hurricanes Gustav and Ike and torrential rains, respectively (Da Silva, 2013). In 2014, the Florida (FL) panhandle received about 50 cm of rain in less than 24 hours, inundating fresh produce and causing widespread damage. Frequent floods that occurred in March 2016 in LA, resulted in significant crop losses to strawberry growers and the safety of the product was questioned by both growers and consumers (Lewis Ivey, personal

communication); then in August 2016 historic flooding inflicted LA leading to up to 100% crop loss in affected areas (Gutierrez et al., 2016).

In addition to food safety hazards, flooding can predispose fruits and vegetables to phytopathogens, especially soilborne and waterborne pathogens. Southern blight, caused by the soilborne fungus *Sclerotium rolfsii*, and Phytophthora root and crown rot, caused by the soil and waterborne pathogen *Phytophthora* spp., are two diseases that can result in significant yield losses to specialty crops in the southeastern US (Drenth et al 2004; Punja, 1984). Phytophthora fruit and root rot can be caused by several species of *Phytophthora*. In cucurbits, *P. capsici* is the main cause of fruit rot. Fruit rot can occur from fruit set until harvest. *P. capsici* produces asexual and sexual spores, all of which can cause infection when conditions favor spore germination. Sexual oospores can survive in the soil for years (Fry and Grunwald, 2010). Oospores germinate and produce two types of asexual spores-sporangia and zoospores (Fry and Grunwald, 2010). Biflagellate zoospores are released from sporangia when the soil is saturated and are capable of "swimming" toward the plant in any free water that is present in the soil (Babadoost et al., 2009; Drenth and Guest, 2004). Infections are initiated when the zoospores (or sporangia, mycelium or oospores) come into contact with susceptible plant tissue (i.e. roots, crown, shoot or fruit) (Ristano et al., 1988; Bernhardt et al., 1982; Gevens et al., 2007). Disease symptoms generally start on the side of the fruit that is in contact with the soil. Water-soaked lesions that may or may not be sunken, can be seen on portions of the fruit that are in direct contact with the soil and on the upper surface of the fruit when rain, soil or irrigation water carrying sporangia are splashed onto the fruit (Gevens et al., 2011; Babadoost, 2004). Lesions have a powdery appearance, due to the

growth of mycelia across the surface. The disease is more predominant in low areas of the field that retain water (Hausbeck et al. 2004), therefore practices that encourage soil drying and planting in fields with good drainage are recommended for disease management.

Sclerotium rolfsii is a soilborne pathogen that can persist in the soil or on plant debris for several years in the form of sclerotia (Kator et al., 2015; Mullen, 2001). *Sclerotium rolfsii* causes a soft rot on fruit that are in direct contact with the soil. On cantaloupe, fruit rot is commonly associated with a strong offensive fermenting-like odor. Similar to *Phytophthora* fruit rot, symptoms start as large water soaked lesions. These lesions are generally sunken with a light yellow appearance. Coarse white mycelia grow from the lesion forming a mycelial mat that rapidly spreads to cover the soil surface; later smooth, light tan to dark brown mustard seed-like sclerotia are evident on the mycelial mat (Punja, 1985; Jenkins et al., 1986; Mullen, 2001; Xie et al., 2016). Practices that exclude the pathogen from the production fields are the most effective in preventing fruit rot.

Soil moisture levels have a significant impact on the rate of *S. rolfsii* and *Phytoththora* spp. sclerotia and sporangia or zoospore germination, respectively (Jenkins and Avere, 1986; Macdonald and Duniway, 1978). Crop losses due to rots are heaviest following several days of intense irrigation or heavy rains that result in standing water, especially after extended periods of hot and dry weather (Jenkins and Avere, 1986; Macdonald and Duniway, 1978). In the case of flooding, if plants are not destroyed due to oxygen deprivation or mechanical damage due to the flow of water, floodwater can remain for several days, generating conditions favorable for spore or sclerotia

germination. Flood water may also introduce propagules of *Phytophthora* spp. and *S. rolfsii* into the field (Bowers and Mitchel, 1990; Jackson, 2004).

No single management tactic will provide adequate control of Phytophthora fruit rot or rot from Southern blight. Practices that exclude these pathogens from the soil are the most effective at preventing rots; these include planting disease-free plants, good sanitation practices and crop rotations. Cultural practices, such as mulching and drip irrigation, will minimize soil and water from splashing onto the fruit. Using surface water, which can harbor *P. capsici* propagules (Roberts et al., 2005; Gevens et al., 2007; Lewis Ivey and Miller, 2013), should be avoided as should overhead irrigation. There are no realistic and cost effective practices for preventing flood water from entering the field. The use of berms (Costa, 1978), hedge rows (Dalton, 1996) and other types of barriers can slow the movement of flood water into a field but cannot stop the water completely. Raised beds, with or without mulch, are used to improve soil drainage and reduce standing water in the crop row (Bell et al., 2003). However their effectiveness in protecting fruit from exposure to flood water is not known.

Our knowledge on how to handle fresh produce exposed to flood water in instances where the crop is in contact with the water for a short period of time is minimal. Unless produce shows clear symptoms of rot there is a tendency for growers to try and rescue as much of the crop as possible to try to minimize significant economic loss (Lewis Ivey, personal communication). Fruit and vegetable production fields in LA are more likely to flood because of hurricanes, tropical storms and torrential rains, compared to fields in more northern states. Developing strategies to mitigate food safety and plant disease hazards associated with flooding will require an increase in our understanding of

the persistence of foodborne and plant pathogens on produce while in the natural environment. The goal of this research project is to better understand the impact that a flooding event has on the microbial quality of cantaloupe produced using different cultural practices.

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2. CHAPTER II

Detection and Enumeration of Indicator Microorganisms (Total Coliform Bacteria and Generic *Escherichia coli*) and Human Pathogens (*Salmonella spp.* and *Listeria monocytogenes*) on Cantaloupe Fruit Following a Flooding Event

2.1 Introduction

Since 2004, 643 produce related foodborne outbreaks have been reported in the US, with ~53% of these occurring from the consumption of contaminated leafy greens, tomatoes, melons and berries (Painter et al., 2013). The estimated national cost of these outbreaks is $93.2 billion (Scharff, 2015). Pathogens associated with these outbreaks included Norovirus, *Salmonella* spp., pathogenic *Escherichia coli*, and *Listeria monocytogenes* (Callejon et al., 2015; Lynch et al., 2009). Although these and other pathogens can be transferred into the production environment via the application of inadequately composted manure or sewage (Natviget et al., 2002), feces of wild animals (Rice et al., 1995; Ackers et al., 1998), and insects (Talley et al., 2009); water run-off from contaminated fields and contaminated irrigation water are the most likely sources of fresh produce contamination (Hamilton et al., 2006; Tyrrel et al., 2006). When flood water is exposed to sewage, animal waste, animals, contaminated soil, agricultural runoff or other sources of surface water it can also be a source of human foodborne pathogens and fresh produce contamination (Brackett, 1999; Beuchta and Ryi, 1997; Casteel et al., 2006).

In the Gulf coast states, hurricanes, tropical storms and torrential rains are common and often result in crop flooding, posing a threat to food quality and quantity (Confalonieri et al., 2007). However, limited science-based information on the effects that contaminated flood water might have on the quality and microbial safety of fruits and vegetables hinders our ability to adequately assess or predict food safety risks. In 2005,

hurricanes Katrina and Rita caused severe flooding to many parishes in southern Louisiana (LA) (Schwab et al. 2007; Jonkman et al. 2009). Shortly after hurricane Katrina, the microbial quality of floodwater and water pumped out of the city was evaluated for microbial contamination. Fecal coliform bacteria and total *E. coli* levels recovered from the surface and bottom waters collected along the river banks near Canal Street in New Orleans LA, were as high as 10^8 colony forming units (CFU) per 100 ml and 10^7 CFU per 100 ml of water, respectively (Pardue et al., 2005); indicating a high presence of sewage contamination and associated sewage-borne contaminants. Pardue et al. (2005) indicated that the magnitude of fecal coliform bacteria reported in the floodwater following Katrina, was similar to typical storm water from the area. The large volumes (and hence exposure) of contaminated water distinguished flood water from typical surface runoff.

In late January 2016, Florida (FL) received abnormally high amounts of rainfall, which caused flooding and affected Florida's multibillion dollar agriculture industry via severe destruction of food crops (http://www.growingproduce.com/). In March 2016, flooding from prolonged rains in LA resulted in significant crop losses to strawberry growers, and the safety of the product was questioned by both growers and consumers (Lewis Ivey, personal communication). Five months later, seven trillion gallons of water fell in southern LA over eight days resulting in $110 million in agricultural losses (National Oceanic and Atmospheric Administration; http://www.usatoday.com/pages/interactives/la-floods-august-2016/). The impact that these floods had on soil quality and the microbial quality of salvageable product is still not known.

To study the influence of contamination events, such as flooding, on the quality of fresh produce, indicator microorganism monitoring is used. Indicator microorganisms are detector or marker microbes whose presence in a population, at levels that exceed set standards, are used to indicate possible food adulteration, poor hygiene, presence of pathogenic organisms, pollution or inadequate food processing (FDA, 2001; Feng et al., 2002; Robinson, 2014; Baudisova, 1987; Griffin et al, 1997; Robinson, 2014; Ondonkor et al. 2013). In 1914, the US Public Health Service adopted the use of coliform bacteria as an indicator of fecal contamination in water and water quality standards were established for various types of water (i.e. lakes, rivers, estuaries) in the US (National Research Council, 2004). With the enactment of the Food Safety Modernization Act (2011), standards similar to those for primary use recreational waters were adopted for agricultural water in an effort to reduce the contamination of fresh produce by foodborne pathogens in irrigation water.

The principal indicators for human and foodborne pathogens in water are total coliform, fecal coliform, *Escherichia coli*, and enterococci bacteria. The total coliform group of bacteria are the most widely used indicators of fecal contamination in drinking water, recreational water, shell fish water and agricultural water. However, their suitability as an indicator of fecal contamination of water and fresh produce is often questioned by scientists and regulators. As a result, test methods have evolved to include the fecal coliform test, which only selects for coliforms of fecal origin (Geldreich, 1966) and the *E. coli* test (also called the MUG test) that tests specifically for *E. coli* (Edberg et al., 1988). The enterococci test was developed for use in subtropical and tropical climates to overcome the fact that *E. coli* is often ubiquitous in water in these climates

(Slantez et al., 1955). The feasibility of these tests as indicators of contamination of fresh produce by human and foodborne pathogens is not fully understood, however there are currently no other verifiable tests to evaluate fresh produce quality.

Ideally, the absence or low concentration of an indicator microorganism means that the produce has not been exposed to conditions that would permit the contamination of the product by a foodborne pathogen. However, in addition to population levels of indicators, the type and physiology of the fruit or vegetable, environmental conditions and growing practices need to be considered when deciding if the fruit or vegetable is safe to consume (reviewed by The Food and Drug Administration (FDA), 2001). Heavy rains followed by flooding can reduce the quality of fresh produce, however the extent to which the quality is reduced is not known. In 2011, the FDA published guidelines for handling edible crops exposed to floodwater (FDA, 2011). These guidelines state that "if the edible portion of a crop is exposed to flood waters, it is considered adulterated" and "should not enter human food channels" (FDA, 2011). The FDA recommends that adulterated crops be disposed of in a way that ensures the safety of non-adulterated crops. For edible portions of a crop that were not in direct contact with floodwater, growers must evaluate the safety of the crop on a case-by-case basis. Although these recommendations are important for minimizing the entry of contaminated product into the food chain, there are limited science-based data to support them.

In LA, on-farm flooding is not an uncommon event, especially during hurricane season. Understandably, the decision to destroy an entire crop due to inherent food safety risks can weigh heavily on producers. There is a significant gap in our quantitative knowledge of the impact that a flooding event has on the microbial quality of fresh

produce. Without this knowledge, it is very difficult for growers or regulators to develop food safety management plans or regulations that are science-based, easily adopted and economical. The objectives of this research are to evaluate 1) the incidence and persistence of coliform bacteria and generic *E.coli* indicator microorganisms and 2) the incidence of *Listeria monocytogenes* and *Salmonella* spp. on the surface of cantaloupe grown on raised beds or flat ground, following a flooding event.

2.2 Materials and Methods

2.2.1 Seedling and fruit production Cantaloupe seeds (cv. Ambrosia) were sown into 72-cell flats containing Fafard Fine Seedling Mix (Sun Gro Horticulture, Agawam, MA) and transplants were produced in the Louisiana State University (LSU) research greenhouses in Baton Rouge, LA. Seedlings were grown using general standard conditions for transplant production (Kelley, 2010). Plants were exposed to natural light conditions (~12 hr daylight) and average day and night temperatures were 24.4±1.2 and 18.0±1.2 degrees C respectively. Plants were watered twice a day, once in the morning and once in the late afternoon. Fertilizer was applied once a week beginning when cotyledons were present with (20:20:20, N:P:K, 1.0 g/L) (Everris NA Inc., Dublin, OH). Seedlings were transplanted into the field at the two-true leaf stage (approximately three weeks old). Plants were produced at the LSU AgCenter Burden Center (Baton Rouge, LA) on alluvial alkaline sub soils. Prior to planting, plots were treated with the pre-emergence herbicides Curbit (ethalfluralin; 0. 7 kg/ha) and Command (clomazone; 0.2 kg/ha) and amended with synthetic fertilizer (13:13:13, N:P:K; 360 kg/ha) (Arysta Life Science Inc. Broadway, NY). Transplants were hand planted into raised beds (~30 cm)

without black plastic or into flat ground. Plants were spaced 0.45 m apart and each plot consisted of two raised bed rows and two flatbed rows (Figure 2.1).

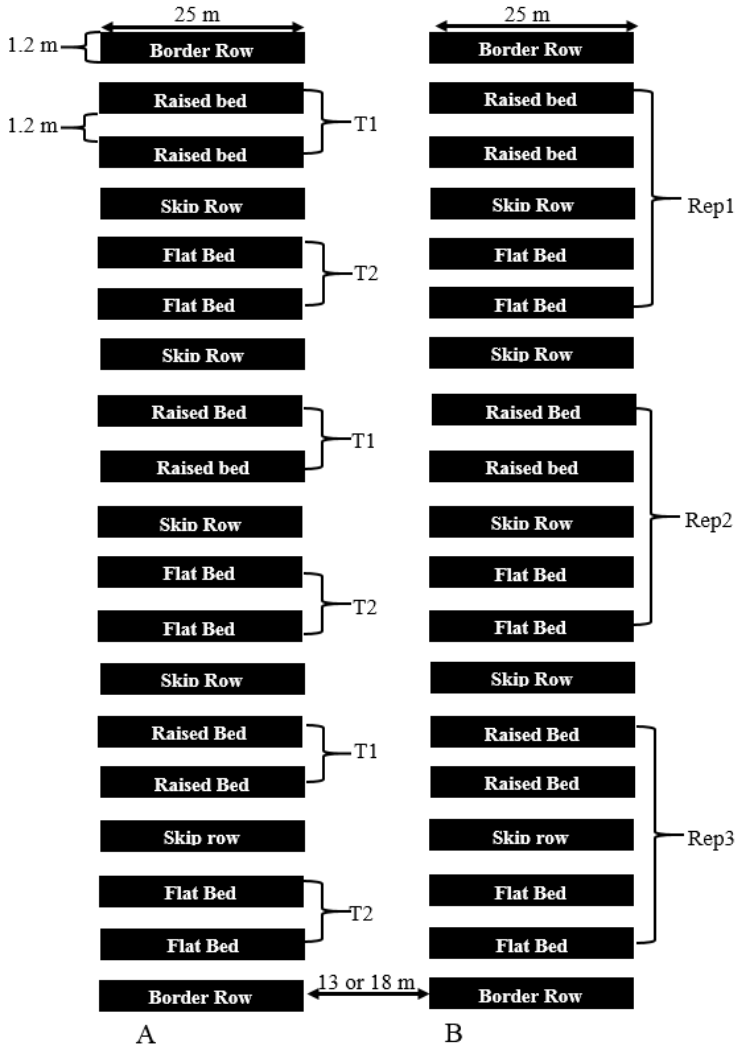


Figure 2.1. Experimental field design. Plots A and B, separated by 13 m (2015) or 18 m (2016), represent flooded and non-flooded plots, respectively. Each plot consisted of two rows-raised beds (T1) and flat beds (T2), each measuring 25 m by 1.2 m. A single raised bed separated the bed types (skip row) and a border row was included at the end of each plot (A and B). Each plot was comprised of three replications (Rep 1 to 3).

Rows were on 1.2 m centers and 24.5 m in length. Skip rows having the same dimensions as treatment rows were placed between the two bed types. Each experiment included treatment (flooding) and no-treatment (non-flooded control) plots (Figure 2.1).

The distance between flooded and non-flooded plots was 13 m (summer 2015) or 18 m (spring 2016). The fields were quarantined off with 1.2 m safety fencing (Uline, Pleasant Prairie, WI) to deter visitors from entering the field. Plots were replicated twice based on bed type, for a total of three replications. Three independent experiments were conducted in order to replicate flooding; one during the summer of 2015 and two during the spring of 2016.

During the growing season, yellow nutsedge and broadleaf weeds were managed using Sandea 75G (halosulfuron-methyl; 12 g/acre), according to the product label. Hand weeding was also conducted to supplement the herbicide treatments. Plants were overhead irrigated with ~2.5 cm well water as needed using a travelling gun overhead sprinkler system (Kifco Inc, Water Reel, Havana, IL). Powdery and downy mildew were controlled by planting cv. Ambrosia, which is tolerant to both diseases. Insecticides were not applied.

2.2.2 *Escherichia coli* inoculum preparation Three strains of generic *Escherichia coli* (ATCC® 23716™, ATCC® 25922™, ATCC® 11775™) were prepared to establish a baseline level of indicator microorganisms in the flood water. Each strain was recovered from -20 degrees C by direct streaking onto nutrient rich Luria-Bertani (LB) agar (Sigma-Aldrich, Co, St. Louis, MO) and incubating at 37 degrees C for 24hr. Initially each strain was prepared separately by transferring one loop full (approximately 100 μl) of the bacterium growing on solid medium to 9 ml of tryptic soy broth (TSB) (Sigma-Aldrich, Co, St. Louis, MO) and then vortexing the cultures and incubating them without shaking for 24hr at 37 degrees C. One ml of the liquid culture was then transferred into 9 ml of

TSB. The final inoculum for each strain was prepared by transferring 4 ml into 396 ml of TSB and incubating the suspension at 37 degrees C for 24hr to achieve a final concentration of ~10^8 CFU/ml. To estimate the concentration (CFU/ml) of the inoculum, the bacterial solution was 10 fold serial diluted in sterile deionized water and 100 µl of the 10^{-5}, 10^{-6}, and 10^{-7} dilutions were spread plated, in duplicate, onto tryptic soy agar (TSA) (Sigma-Aldrich, Co, St. Louis, MO). The plates were incubated at 37 degrees C for 24hr and plates with between 20-200 colonies were counted.

2.2.3 Flooding Surface water from an irrigation pond and well water were used to flood the treatment plots. Immediately prior to flooding, 1,000 L of well water was collected in a 1,325 L food grade high density polyethylene tank (Snyder Industries, Inc, Lincoln, NE) and the water was spiked with 400 ml of each strain of *E. coli* prepared as described above. To ensure the inoculum was homogeneous in the water, horse pipe with 10.2 cm diameter was inserted into the bottom of the tank was used to mix the spiked well water while filling the tank. To flood the treatment plots, water was pumped simultaneously from the spiked well water and pond water onto the field using two overhead 360^0 adjustable rain spray guns (Kifco Inc, Havana, IL), one for each water source. Approximately 30.5 cm of water was applied to the flooded plot at a rate of 3.0 cm/hr. No water was applied to the non-flooded control plots.

2.2.4 Water sampling and testing To ensure measurable counts of generic *E. coli* in the flood water and establish a baseline population of generic *E. coli*, well water, spiked well water, and pond water, were collected and total coliform and generic *E. coli* were enumerated as described below. Water was also collected during the application of flood

water by placing five 20 L plastic buckets randomly throughout the field. For the summer 2015 experiment, for each type of water, 500 ml samples were collected, placed on ice, and transferred to the lab for testing. For the spring 2016 experiments, two 100 ml water samples were collected for each water type. The pH and temperature of all water samples were measured (spring 2016 only) using a hand held pH/temperature combination meter (Model HI98121, Hanna Instruments, Inc.). Total coliform bacteria and generic *E. coli* were enumerated using the Quanti-Tray 2000 Most Probable Number (MPN) system (IDEXX Laboratories, Inc., Westbrook, ME) according to the manufacturer's instructions. For each water type, two 100 ml samples were tested. Each sample was diluted 100X (two 10-fold dilutions) and each stock sample (undiluted) and diluted samples were transferred into individual sterile 120 ml clear plastic bottles (IDEXX Laboratories. Inc., Westbrook, ME). One blister pack of Colilert-18 substrate reagent (IDEXX Laboratories. Inc., Westbrook, ME) was added to each sample, mixed well by hand shaking, and transferred to individual 97-well Quanti-Tray 2000 trays. The trays were sealed using the IDEXX Quanti-Tray 2000 sealer (Model 2X; IDEXX Laboratories. Inc., Westbrook, ME) and incubated at 37 degrees C for 22 hr. The number of small and large yellow cells (indicative of the presence of total coliform bacteria) and cells fluorescing under ultraviolet light (λ=365 nm) (indicative of the presence of generic *E. coli*) were counted and the MPN/100 ml sample was determined using MPN tables provided by the manufacturer (IDEXX Laboratories. Inc., Westbrook, ME). Final MPN values were adjusted according to the respective dilution factor (1, 10 or 100).

2.2.5 Fruit sampling Fruits were harvested 24, 48 and 72 hr after the flood water was applied. Four fruits from each bed type were randomly selected and the diameter of each

fruit was measured (spring 2016 only) using a 20 cm stainless steel digital caliper with a fractional and decimal display (Neiko Tools US, Chesterton, IN**)**. The surface area (cm^2) of each fruit was then calculated and recorded. Individual fruits were placed in zip-seal bags (26.7 x 27.8 cm; 3.9 L; S.C Johnson Inc., Racine, WI), sealed, labelled according to flood status, bed type, and replication, and transported in chilled coolers to the laboratory. All samples were processed immediately following sample collection.

2.2.6 Enumeration of total coliform bacteria and *E. coli* on fruit Fruit weight (kg) was measured using a digital balance (Scout Pro SP6000, Ohaus Corp., Pine Brook, NJ). Sterile 1X phosphate buffer saline (PBS, pH 7.4) (Ambion Corp., Naugatuck, CT) was added to each sample bag until each cantaloupe fruit was submerged in the buffer. The final weight (kg) was measured and recorded. To dislodge bacteria on the surface of the cantaloupe fruit each sample was placed on an orbital shaker (Advanced Orbital Shaker Model 5000, VWR Int., Radnor, PA) for 2 min at 250 revolutions per minute (rpm). Two 100 ml samples of rinsate from each sample was transferred into individual sterile 120 ml clear plastic bottles (IDEXX, Laboratories. Inc., Westbrook, ME) and two 10-fold serial dilutions were made using sterile deionized water. Total coliform bacteria and generic *E. coli* were enumerated using the Quanti-Tray 2000 Most Probable Number system (IDEXX Laboratories, Inc., Westbrook, ME, US) as described above. Most probable number values were adjusted based on the dilution factor and the concentration of coliform and *E. coli* bacteria washed from each sample, per fruit weight and per surface area (cm^2) were calculated and recorded.

2.2.7 *Salmonella spp.* enrichment and isolation Enrichment procedures adapted from the Food and Drug Administration Bacteriological Analytical Manual (FDA-BAM) (FDA, 2016; Ozkalp, 2011; Andrews and Hammack, 2007) were used to isolate *Salmonella* spp. from the surface of cantaloupe fruit. Twenty-five ml of the rinsate from each sample was added to 225 ml of Universal Pre-enrichment Broth (UPB) (Neogen Corp., Lansing, MI) and mixed by swirling for 2 min. The samples were then incubated for 24hr at 35 degrees C and 1 ml of the culture was transferred to 10 ml (in duplicate) of tetrathionate broth (TTB) (Himedia Laboratories Ltd. Vadhani, Mumbai, India) and further incubated for 24hr at 42 degrees C without shaking. One hundred µl of each enriched sample was spread plated, in duplicate, onto xylose lysine deoxycholate (XLD) semi-selective medium (Nye et al. 2002; Maddocks et al. 2002) and incubated for 24hr at 35 degrees C. After 24hr, XLD plates with red colonies with black centers, indicative of *Salmonella* spp., were recorded as presumptive positive. Single presumptive colonies (red with black centers) were subcultured from XLD into Luria-Bertani (LB) agar (Sigma-Aldrich, Co, St. Louise, MO), and incubated for 24hr at 35 degrees C. A loopful of the bacterial suspension was transferred to 1 ml cryogenic tube containing nutrient broth (NB) and 15% glycerol (v:v, 1:1). Cultures were stored for future testing at -80 degrees C. In addition to culturing the *Salmonella* spp., RapidChek® (Romer Labs Technology Inc, Newark, DE) assay was used according to the manufacturer instructions to confirm the presence of *Salmonella* spp. on the surface of cantaloupe fruit. One hundred and fifty µL of the enriched sample from each fruit (described above) was transferred to sterile plastic tubes supplied by the manufacturer, and a RapidChek® test strip, also supplied by the manufacturer, was inserted into each sample. Within 10 min the strips were scored as

positive or negative for the detection of *Salmonella* spp. Samples with one lower red line were scored as negative and samples with two red lines were scored as positive.

2.2.8 *Salmonella spp.* confirmation Purified presumptive isolates of *Salmonella* spp. isolated from cantaloupe fruit (see *Salmonella* spp. enrichment and isolation above) were confirmed by polymerase chain reaction (PCR) with *Salmonella*-specific PCR primers (Shanmugasamy et al., 2011). Isolates were recovered from -80 degrees C storage by streaking a loopful of bacteria onto LB agar and incubating the plates at 35 degrees C for 24hr. DNA was extracted from each isolate using cetyltrimethyl ammonium bromide (CTAB) extraction procedures (Wilson, 1987). Bacteria growing on LB were scraped from the plate and suspended in 300 µl Tris EDTA (TE) (pH 7.4, 10 mM Tris, 1 mM EDTA) buffer by vortexing. CTAB buffer [2% CTAB (w/v), 100mM Tris (pH 8.0, 1M), 20mM EDTA (pH 8.0), 1.4 M NaCl, 1% polyvinylpyrrolidone (PVP)] (250µl) was added to each sample, the samples were vortexed for ~30 sec and then incubated at 65 degrees C for 15 min. After cooling to room temperature, 250 µl of 24:1 chloroform-isoamyl alcohol was added to each sample, the samples were vortexed for ~30 sec and then centrifuged at 14,000 x g for 10 min. The aqueous upper phase was transferred to a sterile 1.5 ml microcentrifuge tube and 300 µl of 100% isopropanol was added to precipitate the DNA. After gentle mixing, the sample was incubated at -20 degrees C for 10 min, centrifuged (14,000 x g, 10 min) and the supernatant was discarded. The pelleted DNA was suspended in 30 µl of TE buffer (1X, pH 7.4) and the absorbance at 260 nm was measured using spectrophotometry with a Nano Drop spectrophotometer (NanoDrop Technologies, Inc. Wimington, DE). The concentration (ng/µl) of DNA was calculated and DNA was diluted to 50 ng/µl in sterile dH_2O for use with PCR. Each 25 µl PCR

mixture contained 12.5 µl of 2X Promega GoTaq® Green Master Mix (Promega, Corporation, Madison, WI), 0.5µl of each *Salmonella*-specific primer (5´- GTG AAA TTA TCG CCA CGT TCG GGC AA -3´ and 5´- TCA TCG CAC CGT CAA AGG AAC C -3´), 1.5 µl of DNA template, and 10 µl nuclease-free sterile water. DNA from *Salmonella typhymurium* strain ATCC 19585 was used as positive control template in each PCR assay. DNA from generic *E. coli* strain ATCC 11775 and sterile nuclease-free water were used as negative controls. Amplification was performed in a C1000 Touch™ thermocycler (Bio-Rad Laboratories, Inc., Foster City, CA) with the following cycling conditions; an initial incubation at 94 degrees C for 60 sec, followed by 35 cycles of denaturation at 94 degrees C for 60 sec, annealing at 64 degrees C for 30 sec, elongation at 72 degrees C for 30 sec, and a final extension period for 7 min at 72 degrees C. Amplified products were separated using agarose (1.2%) gel electrophoresis (120 V for 60 min) and DNA was visualized under high wavelength ultraviolet (UV) light with a ChemiDoc It2 imager with a UV trans-illuminator (UV Products LLC, CA). Samples were scored positive for *Salmonella* spp. based on the presence of a 284bp amplicon and negative based on the absence of a 284 bp amplicon.

2.2.9 *Listeria spp*. enrichment and isolation Enrichment procedures adapted from FDA-BAM (FDA, 2016; Andrews and Hammack, 2007, Gasanov et al., 2005) were used to isolate *Listeria* spp. from the surface of the cantaloupe fruit (spring 2016). Twenty-five ml of the rinsate from each sample was added to 225 ml of buffered *Listeria* spp. enrichment broth (BLEB) (Sigma-Aldrich Co., St. Louise, MO, US) and incubated for 48 hr at 30 degree C. A 100 µl aliquot of the enrichment culture was then streaked onto *Listeria* spp. semi-selective Oxford agar medium (Oxoid Ltd, Basingstoke, Hants, UK)

and incubated at 30 degree C for 24hr. Single presumptive colonies (grey with black halos) were sub-cultured onto LB agar and stored at -80 degree C as described above for *Salmonella* spp.

2.2.10 *Listeria monocytogenes* confirmation Purified presumptive isolates of *L. monocytogenes* isolated from cantaloupe fruit (see *Listeria monocytogenes* isolation section above) were confirmed by PCR with *L. monocytogenes*- specific PCR primers (Border, 1990). Whole cell extracts were used as template in the PCR assay. Isolates from -80 degrees C were streaked onto LB agar and incubated for 48 hr at 30 degrees C. Whole cell template was prepared by mixing a loopful of bacteria from the LB plates with 500 ml sterile deionized water and then freeze shocking at -20 degrees C for ~18 hr. Prior to setting up the PCR assays the whole cell extracts were thawed completely. Each 25 µl PCR mixture contained 5 µL of 5X PCR buffer, 1.5 µL of 25 mM $MgCl_2$, 0.2 µL of deoxynucleosidetriphosphate (dNTP) mix (10 mM), 0.3 µL of Taq DNA polymerase, 0.5µl of each *L. monocytogenes*-specific primer (LM1: 5′-CCT AAG ACG CCA ATC GAA-3′ and LM2: 5′-AAG CGC TTG CAA CTG CTC-3′) (1.0nM), 14 µL of sterile nuclease-free water, and 2 µL of thawed whole cell culture. Total genomic DNA from *L. monocytogenes* LCDC 81-861 serotype 4b (Pangloli and Hung, 2013) was extracted as using the CTAB method described above and used as positive control template in each assay. DNA from generic *E. coli* strain ATCC 11775 and sterile nuclease-free water were used as negative controls. Amplification was performed in a Veriti 96 Well Thermal Cycler (Bio-Rad Laboratories, Inc., Foster City, CA) with the following cycling conditions; an initial incubation at 94 degrees C for 5 min, followed by 30 cycles of denaturation at 94 degree C for 30 sec, annealing at 53 degrees C for 1 min, elongation at

72 degree C for 2 min, and a final extension period for 7 min at 72 degrees C. Amplified products were separated using agarose (1.5%) gel electrophoresis (120 V for 60 min) and DNA was visualized under high wavelength ultraviolet (UV) light as described above. Samples were scored positive for *L. monocytogenes* based on the presence of a 702 bp amplicon and negative based on the absence of a 702 bp amplicon.

2.2.11 Data analysis Three independent experiments were conducted in order to replicate flooding; one during the summer of 2015 and two during the spring of 2016. Total coliform bacteria counts and generic *E.coli* counts (from water and fruit samples) from each independent experiment and replication were combined, fruit surface was calculated in MPN/100ml and MPN/cm^2 and log_{10} transformed prior to statistical analyses. Data were analyzed with SAS/STAT® software (SAS Institute Inc., Cary, NC) using General Linear Model (Jupp and Mardia, 1979). Mean differences in indicator microorganism levels based on sampling and between raised and flat beds were separated using Tukey Honest Significant Difference test (Tukey, 1953) at α=0.05.

Salmonella spp. and *L. monocytogenes* incidence data were compared using Pearson's Chi-squared test (Pearson, 1900) and mean differences between flood status, and sampling time were separated using Fishers' exact test of independence (Fisher, 1954) using SAS/STAT® (SAS Institute Inc., Cary, NC).

2.3 Results

2.3.1 Total coliform and generic *Escherichia coli* baseline levels in water Source well water prior to inoculation contained no detectable generic *E. coli* and an average of 3.6±0.2 log_{10}MPN/100ml total coliform bacteria at the time of sampling (Table2.1).

Table 2.1 Baseline population levels of generic *Escherichia coli* and total coliform bacteria and mean water temperature and pH in source water and simulated flood water used in this study.

Source	Generic *E.coli*[3] log_{10}MPN/100ml	Coliform bacteria[3] log_{10}MPN/100ml	Temperature[4] (°C)	pH[4]
Well water	0.0±0.0	3.6±0.2	27.9±0.4	8.3±0.1
Well water spiked with generic *E. coli*[1]	6.4±0.3	6.4±0.2	27.9±0.4	6.6±0.2
Pond water	3.1±0.3	4.7±0.2	36.2±1.7	6.6±0.2
Flood water[2]	5.1±0.3	6.2±0.1	34.1±1.7	7.5±0.7

[1]Well water was spiked with a mixture of generic *E. coli* strains ATCC® 23716, 25922 and 11775 to achieve a concentration of ~10^8 CFU/ml.
[2]Flood water is a 1:1 (vol:vol) mixture of well water spiked with generic *E.coli* strains ATCC® 23716, 25922 and 11775 and pond water.
[3]Values are plus or minus the standard error of three replicated experiments.
[4]Values are the mean temperature or pH plus or minus the standard error of three replicates for two experiments (2016 experiments).

The mean baseline levels of generic *E. coli* and total coliform bacteria in well water spiked with the three ATTC strains of generic *E. coli* were 6.4±0.3 and 6.4±0.2 log_{10}MPN/100ml, respectively (Table 2.1). Pond water contained an average of 3.1±0.4 log_{10}MPN/100ml generic *E. coli* and 4.7±0.2 log_{10}MPN/100ml coliform bacteria (Table 2.1). Mean baseline generic *E. coli* and coliform bacteria populations in the flood water (mixture of spiked well water and pond water) were 5.1±0.4 and

6.2±0.1 log_{10}MPN/100ml respectively (Table 2.1). Mean water temperatures (degree C) at the time of sampling (2016 only) were 27.9 (well), 27.9 (spiked well water), 36.2 (pond), and 34.1 (flood) (Table 2.1). Mean pH values (2016 only) of well (not spiked), well (spiked), pond, and flood waters were 8.3, 6.6, 6.6, and 7.5 respectively (Table 2.1).

2.3.2 Generic *Escherichia coli* levels on the surface of cantaloupe fruit from flooded and non-flooded plots Generic *E. coli* populations based on surface area (log_{10}MPN/cm^2, Table 2.2) and rinsate volume (log_{10}MPN/100ml, Table 2.3) on cantaloupe fruit exposed to the flooding treatment decreased significantly over a 72 hr period on fruit produced on raised beds ($p=0.0025$ and $p<0.0001$) and flat ground ($p=0.0025$ and $p<0.0001$) (Table 2.2 and 2.3 respectively).

Table 2.2 Mean generic *Escherichia coli* and total coliform population levels based on surface area (log_{10}MPN/cm^2) present on the surface of cantaloupe fruit that were produced on raised beds or flat ground and exposed to the flood water treatment.

	Bed Type					
	Raised Bed		Flat ground		p-value	
Time (hr)	Generic *E.coli*[1] log_{10}MPN/cm^2	Coliform[1] log_{10}MPN/cm^2	Generic *E.coli*[1] log_{10}MPN/cm^2	Coliform[1] log_{10}MPN/cm^2	Generic *E.coli*[3]	Coliform[3]
24	3.8±0.29 a[2]	5.9±0.19 a[2]	3.8±0.29 a[2]	5.9±0.19 a[2]	0.8325	1.0000
48	3.3±0.29 ab	5.8±0.19 a	3.1±0.29 ab	5.9±0.19 a	0.4559	0.9992
72	2.7±0.29 b	5.9±0.19 a	2.1±0.29 b	5.9±0.19 a	0.1091	0.8623
p-value	0.0025	0.3035	0.0025	0.3035		

[1]Values are mean population levels plus or minus the standard error of 144 fruit samples (n=144) for two experiments (2016 experiments only).
[2]Values within a column followed by the same letter are not significantly different at $p<0.05$.
[3]P-values correspond to comparisons between bed type for generic *E. coli* and coliform population levels at each time interval (within a row). No significant differences were detected at $p<0.05$.

Table 2.3. Mean generic *Escherichia coli* and total coliform population levels present in rinsate (log_{10}MPN/100ml) from the surface of cantaloupe fruit that were produced on raised beds or flat ground and exposed to the flood water treatment.

	Bed Type					
	Raised Bed		Flat ground		p-value	
Time (hr)	Generic *E.coli*[1] log_{10}MPN/100ml	Coliform[1] log_{10}MPN/100ml	Generic *E.coli*[1] log_{10}MPN/100ml	Coliform[1] log_{10}MPN/100ml	Generic *E.coli*[3]	Coliform[3]
24	4.7±0.33 a[2]	7.2±0.20 a[2]	4.9±0.33 a[2]	7.1±0.20 a[2]	0.4829	0.6357
48	4.3±0.33 ab	7.1±0.20 a	3.8±0.33 b	7.1±0.20 a	0.1419	0.9057
72	3.4±0.33 b	7.1±0.20 a	2.4±0.33 c	7.1±0.20 a	0.2073	0.9202
p-value	<0.0001	0.8340	<0.0001	0.8340		

[1]Values are mean population levels plus or minus the standard error of 216 fruit samples (n=216) for three experiments.
[2]Values within a column followed by the same letter are not significantly different at $p<0.05$.
[3]P-values correspond to comparisons between bed type for generic *E. coli* and coliform population levels at each time interval (within a row). No significant differences were detected at $p<0.05$.

No significant interaction was observed between the three main effects (bed type*sampling time*flooding) for generic *E. coli* based on surface area or rinsate volume (Table 2.4).

Table 2.4. Fixed effects of flooding by bed type by sampling time for generic *Escherichia coli* and total coliform population levels based on the enumeration method.

	Enumeration Method	
Indicator microorganisms	Surface Area (log_{10}MPN/cm^2)	Rinsate Volume (log_{10}MPN/100ml)
Generic *E.coli*	0.9460[1]	0.4670
Coliform bacteria	0.0695	0.9002

[1] P-value $p>0.5$ represent no significant interaction of the main experimental fixed effects.

Independent of bed type (i.e. data from raised beds and flat ground were combined), generic *E. coli* based on surface area (log_{10}MPN/cm^2) and rinsate volume (log_{10}MPN/100ml) on cantaloupe fruit exposed to the flooding treatment decreased significantly ($p<.0001$ and $p=0.0001$) over a 72 hr period compared to populations on fruit that were not exposed to flood water (Figure 2.2).

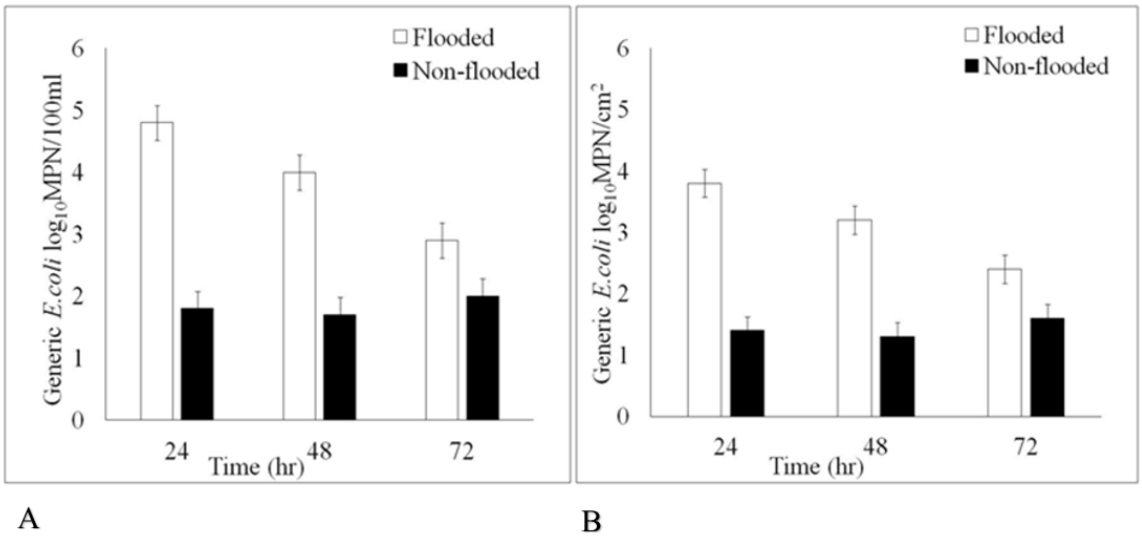


Figure 2.2. Mean level of generic *Escherichia coli* (log_{10}MPN/100ml) (A) and generic *E.coli* log_{10}MPN/cm^2 (B) on cantaloupe fruit from flooded (white bars) and non-flooded control plots (black bars) 24, 48 and 72 hr post flooding. Error bars indicate the standard error of the mean values.

Generic *E. coli* populations on cantaloupe fruit that were produced on raised beds and exposed to flood water that had a baseline *E. coli* level of 5.1 log_{10}MPN/100 ml (Table 2.1), declined to 3.8, 3.3, and 2.7 log_{10}MPN/cm^2 or 4.7, 4.3, and 3.4 log_{10}MPN/100ml after 24, 48 and 72 hr respectively (Figure 2.3, solid lines). On fruit exposed to flood water and produced on flat ground they reduced to 3.8, 3.1, and 2.1 log_{10}MPN/cm^2 and 4.9, 3.8, and 2.4 log_{10}MPN/100ml after 24, 48 and 72 hr respectively (Figure 2.3, dotted lines).

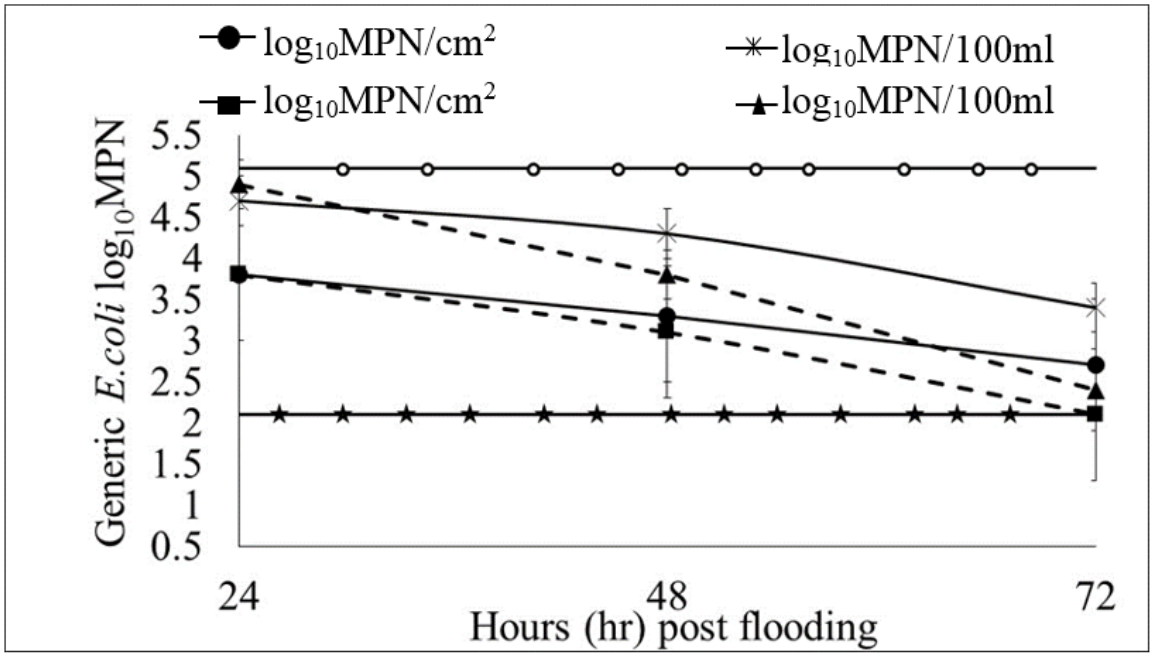
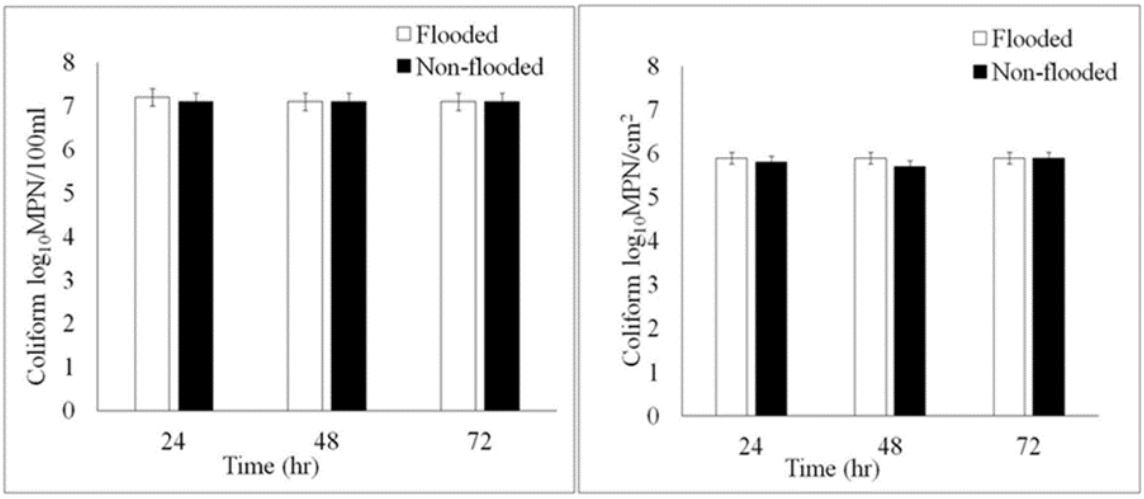


Figure 2.3 Mean generic *Escherichia coli* levels detected on the surface of cantaloupe produced on raised beds (solid lines) or flat ground (dotted lines) from flooded plots 24, 48 and 72 hr post flooding. The mean level of generic *E. coli* in the flood water was 5.1 $\log_{10}$MPN/100ml (—○—). The Food Safety Modernization Act-Fresh Produce Safety Rule threshold (—★—) for generic *E.coli* (2.1 $\log_{10}$MPN/100ml) permitted in irrigation water based on geometric mean of five samples (Bihn et al., 2016). The error bars indicate the standard error of the mean values.

2.3.3 Total coliform levels on the surface of cantaloupe fruit from flooded and non-flooded plots In flooded plots, coliform population levels on fruit produced on raised beds or flat ground remained constant over the same time period (Table 2.2 and Figure 2.4) and no significant interaction was observed between the three main effects (bed type*sampling time*flooding) based on surface area or rinsate volume (Table 2.4).



A B

Figure 2.4. Mean levels total coliform bacteria log_{10}MPN/100ml (A) and coliform bacteria log_{10}MPN/cm^2 (B) on cantaloupe fruit from flooded (white bars) and non-flooded control plots (black bars) 24, 48 and 72 hr post flooding. Error bars indicate the standard error of the mean values.

On fruits that were not exposed to flooding (non-flooded control plots) coliform and generic *E.coli* population levels on fruit produced on raised beds (p=0.6042 and 1.000 respectively) or flat ground (p=0.1372 and p=1.0000 respectively) did not significantly change over a 72 hr period when populations were based on surface area (log_{10}MPN/cm^2)

(Table 2.5). Additionally, fruit that were not exposed to flooding generic *E.coli* and total coliforms populations did not differ significantly between raised and flat beds at 24hr (p=0.1356 and 1.000 respectively), 48 hr (p=0.5094 and p=0.9783 respectively) and at 72 hr period (p=0.6756 and p=0.7643 respectively) (Table 2.5).

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Table 2.5 Mean generic *Escherichia coli* and total coliform population levels based on surface area ($log_{10}MPN/cm^2$) present on the surface of cantaloupe fruit that were produced on raised beds or flat ground and not exposed to the flood water treatment

	Bed Type					
	Raised Bed		Flat ground		p-value	
Time (hr)	Generic *E.coli*[1] $log_{10}MPN/cm^2$	Coliform[1] $log_{10}MPN/cm^2$	Generic *E.coli*[1] $log_{10}MPN/cm^2$	Coliform[1] $log_{10}MPN/cm^2$	Generic *E.coli*[3]	Coliform[3]
24	1.2±0.29	5.8±0.19	1.7±0.29	5.8±0.19	0.1356	1.0000
48	1.4±0.29	5.7±0.19	1.4±0.29	5.8±0.19	0.5094	0.9783
72	1.6±0.29	5.9±0.19	1.5±0.29	5.9±0.19	0.6756	0.7643
p-value[2]	0.6042	1.0000	0.1372	1.0000		

[1] Values are mean population levels plus or minus the standard error of 144 fruit samples (n=144) for two experiments (2016 experiments only)

[2] P-values corresponds to comparisons of generic *E.coli* of coliforms after 72 hr period (down the column)

[3] P-values correspond to comparisons between bed type (raised and flatbed) for generic *E. coli* and coliform population levels at each time interval (within a row). No significant differences were detected at $p>0.05$.

Based on fruit rinsate volume ($log_{10}MPN/100ml$) generic *E.coli* and total coliforms populations did not significantly change after 72 hr period for fruit produced on raised beds (p=0.1372 and p=1.0000 respectively) or flat ground (p=0.5231 and p=0.9399 respectively) (Table 2.6). Generic *E.coli* and total coliforms populations ($log_{10}MPN/100ml$) did not differ significantly between raised and flat beds at 24hr (p=0.6525 and p=0.8091 respectively), 48 hr (p=0.9985 and p=0.6571 respectively) or at 72 hr period (p=0.1.0000 and p=0.7768 respectively) (Table 2.6).

Table 2.6. Mean generic *Escherichia coli* and coliform population levels present in rinsate from the surface of cantaloupe fruit that were produced on raised beds or flat ground and not exposed to the flood water treatment.

	Bed Type					
	Raised Bed		Flat ground		p-value	
Time (hr)	Generic *E.coli*[1] log_{10}MPN/100ml	Coliform[1] log_{10}MPN/100ml	Generic *E.coli*[1] log_{10}MPN/100ml	Coliform[1] log_{10}MPN/100ml	Generic *E.coli*[3]	Coliform[3]
24	1.8±0.33	7.0±0.20	2.2±0.30	7.1±0.20	0.6525	0.8091
48	1.9±0.33	7.0±0.20	1.8±0.30	7.1±0.20	0.9985	0.6571
72	2.0±0.33	7.0±0.20	2.0±0.30	7.1±0.20	1.0000	0.7768
p-value[2]	0.1372	1.0000	0.5231	0.9399		

[1]Values are mean population levels plus or minus the standard error of 144 fruit samples (n=144) for two experiments (2016 experiments only).

[2] P-values corresponds to comparisons of generic *E.coli* or coliform bacteria after 72 hr period (down the column). No significant differences were detected at $p>0.05$.

[3]P-values correspond to comparisons between bed type for generic *E. coli* and total coliform population levels at each time interval (within a row). No significant differences were detected at $p>0.05$.

2.3.4 Actual and predicted die-off values of generic *Escherichia coli* on raised or flat flooded plots over time Based on the half log die-off rate assumption (Bihn et al., 2016), predicted *E. coli* population levels in floodwater and on cantaloupe fruit harvest from raised beds (Table 2.7) and flat beds (Table 2.8) were calculated and compared to the actual population levels.

Table 2.7. Predicted and actual (MPN/cm^2 and MPN/100ml) reduction rate of generic *Escherichia coli* on cantaloupe fruits surface sampled from raised beds and flood water over time after flood event.

Die-off rate (Percent reduction[1])	Days Post flooding	Floodwater[1] MPN/100ml	Predicted[1] MPN/cm^2	Actual[3] MPN/cm^2	Predicted[1] MPN/100ml	Actual[3] MPN/100ml
0	0	125892	6309[2]	6309	50119	50119
68.4	1	39807	1995	1995	15848	19953
90.0	2	12589	631	501	5012	2511
96.8	3	3978	199	-	1584	
99.0	4	1259	63	-	501	

[1]Predicited values calculated based on the assumption that the half log die-off rate equates to 68.38% die off of *E. coli* over one day, 90% over two days, 96.84% over three days, or 99% over four days (Bihn et al., 2016).
[2]Mean generic *E. coli* population at 24hr post flooding, which corresponds to the initial mean levels of *E. coli* on the cantaloupe or day 0 post flooding.
[3]Actual generic *E. coli* levels enumerated on the surface of cantaloupe fruit post flooding.

Table 2.8. Predicted and actual (MPN/cm^2 and MPN/100ml) reduction rate of generic *Escherichia coli* on cantaloupe fruits surface sampled from flat grounds over time after flood event.

Die-off rate (Percent reduction)	Days Post flooding	Floodwater[1] MPN/100ml	Predicted[1] MPN/cm^2	Actual[3] MPN/cm^2	Predicted MPN/100ml	Actual[3] MPN/100ml
0	0	125892	6309[2]	6309	79433	79433
68.4	1	39807	1995	1259	25116	6309
90.0	2	12589	631	126	7944	251
96.8	3	3978	199	-	2510	-
99.0	4	1259	63	-	795	-

[1]Predicited values calculated based on the assumption that the half log die-off rate equates to 68.38% die off of *E. coli* over one day, 90% over two days, 96.84% over three days, or 99% over four days (Bihn et al., 2016).
[2]Mean generic *E. coli* population at 24hr post flooding, which corresponds to the initial mean levels of *E. coli* on the cantaloupe or day 0 post flooding.
[3]Actual generic *E. coli* levels enumerated on the surface of cantaloupe fruit post flooding.

Reduction levels were also determined for combined data from raised and flat beds. After 4 consecutive days, *E. coli* populations in flood water were predicted to decline from 125,892 MPN/100 ml (equivalent to 5.1 $\log_{10}$MPN/100 ml, which is the actual mean population of *E. coli* in the flood water at the time of flooding (see Table 2.1) to 1259 MPN/100 ml. On fruit from raised beds (Table 2.7), generic *E.coli* mean levels were predicted to decline from the initial mean level of 50119 MPN *E.coli* /100ml or 6309 MPN *E.coli*/cm^2 to 501 MPN *E.coli*/100ml or 63 MPN *E.coli*/cm^2 respectively, after four consecutive days (Table 2.7). The actual mean *E. coli* populations on fruit from raised beds were 6309 MPN/cm^2 and 50119 MPN/100ml and after three consecutive days the mean generic *E. coli* were 501 and 2511 MPN/cm^2 and MPN/100ml respectively (Table 2.7). On fruit harvested from flat beds (Table 2.8), generic *E.coli* levels were predicted to decline from the initial mean level of 79433 MPN *E.coli* /100ml or 6309 MPN *E.coli*/cm^2 to 795 MPN *E.coli* /100ml or 63 MPN *E.coli* /cm^2 respectively, after four consecutive days. The actual mean *E. coli* populations on fruit from flat beds were 6309 MPN/cm^2 and 79433 MPN/100ml and after three consecutive days generic mean *E. coli* populations had declined to 126 and 251 MPN/cm^2 and MPN/100ml respectively (Table 2.7). When data from the flat beds and raised beds were combined actual mean *E. coli* populations were 251 or 794 MPN/cm^2 or MPN/100ml respectively (Table 2.9).

Table 2.9. Combined (raised beds and flat ground) predicted and actual (MPN/cm^2 and MPN/100ml) reduction rate of generic *Escherichia coli* on cantaloupe fruit surface and flood water over time after flood event.

Die-off rate (Percent reduction)	Days Post flooding	Floodwater[1] (MPN/100ml)	Predicted[1] (MPN/cm^2)	Actual[3] (MPN/cm^2)	Predicted MPN/100ml	Actual MPN/100ml
0	0	125892	6310[2]	6310	63096	63096
68.4	1	39807	1995	1585	19951	12589
90.0	2	12589	631	251	6310	794
96.8	3	3978	199	-	1994	-
99.0	4	1259	63	-	631	-

[1]Predicited values calculated based on the assumption that the half log die-off rate equates to 68.38% die off of *E. coli* over one day, 90% over two days, 96.84% over three days, or 99% over four days (Bihn et al., 2016).
[2]Mean generic *E. coli* population at 24hr post flooding, which corresponds to the initial mean levels of *E. coli* on the cantaloupe or day 0 post flooding.
[3]Actual generic *E.coli* levels enumerated on the surface of cantaloupe fruit post flooding.

2.3.5 Incidence of *Salmonella spp.* on cantaloupe fruit from flooded and non-flooded plots (independent of bed type)

Presumptive *Salmonella* colonies on XLD medium were red with black centers. When culturing was conducted using XLD medium there was no significant difference in *Salmonella* incidence on fruit over the sampling period for the flooded (p=0.7610) or non-flooded (p=0.0634) plots (Table 2.10).

Table 2.10. Percentage of cantaloupe fruit (n=432) from flooded and non-flooded control plots that were positive for the presence of *Salmonella* spp. based on the RapidChek immunoassay, culturing on xylose desoxycholate (XLD) medium, or *Salmonella*-specific PCR.

	Flooded Plot			Non-flooded Control Plot		
Time (hr)	RapidChek[2]	XLD[3]	PCR[4]	RapidChek	XLD	PCR
24	18.1%	52.8%	17.2%	9.7%	30.6%	8.1%
48	13.9%	47.2%	20.3%	12.5%	50.0%	17.4%
72	15.2%	52.8%	16.6%	5.6%	41.7%	10.4%
p-value[1]	0.844	0.761	0.365	0.392	0.063	0.410
Chi-square value	0.489	0.593	2.288	2.094	5.676	1.945

[1]P-values corresponds to fruits samples positive for *Salmonella* spp. over time for each test (down the column).
[2]Values are percentage of fruits positive for *Salmonella* spp based RapidChek test
[3]Values are percentage of the fruits positive for *Salmonella* spp. based on xylose desoxycholate (XLD) semi-selective and differential medium.
[4] Values are percentage of the fruits positive for *Salmonella* spp. based on *Salmonella* spp. invA gene-primer specific PCR (Shanmugasamy et al., 2011).

Twenty-four hr post flooding an average of 52.8% of the sampled fruit had colonies presumed to be *Salmonella* on their surface. After 48 hr the average incidence dropped to 47.2% and after 72 hr the incidence increased to 52.8% (Table 2.10). Of the fruit samples with presumptive *Salmonella*, 17.2%, 20.3%, and 16.6% were confirmed to be *Salmonella* spp. using *Salmonella*-specific PCR after 24, 24 and 72 hr respectively but no significant differences were observed between sampling times (p=0.3650) (Table 2.10). *Salmonella* was detected less frequently using the *Salmonella* RapidChek assay compared to culturing. Using the *Salmonella* spp. RapidChek assay, *Salmonella* spp. was detected on 18.1%, 13.9% and 15.2% of the cantaloupe fruit collected from flooded plots 24, 48, and 72 hr post flooding respectively. No differences in incidence between sampling times were detected (p=0.8439).

For the control plots (non-flooded) fruit were sampled at the same time as those in the flood plots. Presumptive *Salmonella* was recovered from 30.6%, 50.0% and 41.7% of the sampled fruit by culturing 24, 48 and 72 hr after the treatment crop was flooded (Table 2.11). Of the fruit samples with presumptive *Salmonella*, 8.1%, 17.4%, and 10.4% were confirmed to be *Salmonella* spp. using *Salmonella*-specific PCR. An average of 9.7%, 12.5% and 5.6% of fruits sampled from non-flooded plots were positive for *Salmonella* 24, 48 and 72 hr using RapidChek assay. For all the test methods utilized *Salmonella* spp. incidence on fruit did not significantly change over time (RapidChek: p=0.3916; XLD: p=0.0634; PCR: p=0.41) (Table 2.10). Independent of sampling time (Table 211.),

Table 2.11. Chi-square comparison for *Salmonella* spp. (n=432 fruits) *and Listeria monocytogenes* (n=288 fruits) incidence between flooded and non-flooded plots based on RapidCheck, Xylose lysine desoxycholate, Listeria Selective Agar and polymerase chain reaction.

Detection methods	n	Chi-square Value (χ^2)	df	P-value[3]
Salmonella spp.[1]				
RapidCheck	432	1.7840	1	0.1817
XLD	432	0.3472	1	0.5557
PCR	432	0.8417	1	0.3587
L. monocytogenes[2]				
LSA	288	0.4432	1	0.5056
PCR	288	0.4623	1	0.4966

[1]RapidCheck, Xylose lysine desoxycholate (XLD) culture medium and polymerase chain reaction (PCR) detection methods for *Salmonella* spp.
[2] Listeria Selective Agar (LSA) and polymerase chain reaction *L.monocytogenes* detection methods
[3]P-values corresponds to comparisons of fruits positive for *Salmonella* spp. and *L. monocytogenes* between flooded and non-flooded plots based on individual detection methods. No significant differences were detected at p>0.05.

Salmonella spp. incidence on fruit did not significantly differ between flooded and non-flooded plots for the RapidChek test (n=432, χ^2 =1.7840, df=1 P=0.1817) and culturing on XLD (n=432, χ^2 =0.3472, df=1, P=0.5557) or by PCR (n=432, χ^2 =0.8417, df=1, P=0.3587).

2.3.6 Incidence of *Listeria spp. and L. monocytogenes* on cantaloupe fruit (independent of bed type) Presumptive *Listeria* colonies on Oxford LSA medium were grey with black zones surrounding the colonies. When culturing was conducted using Oxford LSA medium, 27.1% of the sampled fruit from flooded plots had colonies presumed to be *Listeria* spp. on their surface 24 and 48 hr post flooding (Table 2.12).

Table 2.12. Percentage of cantaloupe fruit (n=288) from flooded and non-flooded plots positive for *Listeria* spp. and *Listeria monocytogenes* based on Listeria selective agar (LSA) medium, or *Listeria monocytogenes* primer specific polymerase chain reaction.

	Flooded Plot		Non-flooded Control Plot	
Time (hr)	LSA[2]	PCR[3]	LSA	PCR
24	27.1%	2.2%	29.2%	2.4%
48	27.1%	3.4%	22.9%	1.9%
72	31.3%	3.3%	22.9%	0.5%
P-value[1]	0.9196	0.9289	0.8082	0.3933
Chi-square value	0.2728	0.5455	0.6667	2.1333

[1]P-values corresponds to comparison of fruits samples positive for *Listeria* spp. *and Listeria monocytogenes* over time. No significant differences were detected at p>0.05.
[2] Percentage of the fruits positive for *Listeria spp.* based on Listeria Selective Agar medium (LSA).
[3] Percentage of the fruits positive for *Listeria monocytogenes* based on hlyA gene-primer specific polymerase chain reaction (PCR) (Border, 1990).

Seventy-two hr post flooding the number of fruit with detectable *Listeria* spp. increased to 31.3%. Of the fruit samples with presumptive *Listeria* spp., 2.2% (24hr), 3.4% (48 hr), and 3.3% (72 hr) were confirmed to be *L. monocytogenes* using *L. monocytogenes*-

specific PCR *Listeria* spp. (p=0.9196) and *L. monocytogenes* (p=0.9289) incidence on fruit did not significantly change over time (Table 2.12). For the control plots (non-flooded) fruit were sampled at the same time as those in the flood plots. Incidence of *Listeria* spp. on fruit sampled from these plots was 29.2%, 22.9% and 22.9% 24, 48 and 72hr post flooding respectively (Table 2.12). Of the fruit samples collected from the control plots that were positive for *Listeria* spp., *L. monocytogenes* was confirmed on 2.4% (24h) , 1.9% (48 hr) and 0.5% (72 hr) of the fruit using *L. monocytogenes* specific PCR (Table 2.12). Over time *Listeria* spp. (p=0.8082) and *L. monocytogenes* incidence did not differ significantly (Table 2.12).

Independent of sampling time, *Listeria* spp. (n=288, χ^2 =1.7840, df=1, P=0.5056) and *Listeria monocytogenes* (n=288, χ^2 =0.3472, df=1, P=0.4966) incidence on fruit did not significantly differ between flooded and non-flooded plots (Table 2.11).

2.3. Discussion

Flood water can introduce foodborne bacterial pathogens into crop production systems and increase the food safety risk of any edible portions that come into direct contact with the water (Castro-Ibáñez et al., 2015). This study was initiated in order to assess the microbial safety of cantaloupe fruit after exposure to floodwater. Vegetable and fruit production fields in LA are prone to flooding, and cantaloupe, which can be produced on raised beds or flat ground, are considered high risk crops for contamination by foodborne pathogens (Confalonieri et al., 2007). In this study, cantaloupe fields with mature, harvestable fruit were flooded and the populations of generic *E. coli* and total coliform bacteria on the surface of fruit, and the incidence of fruit contaminated with *Salmonella* spp., *Listeria* spp., and *L. monocytogenes* were determined. Generic *E. coli* and total coliform bacteria were selected as indicators of fecal contamination and *Salmonella* and *Listeria* were chosen because of the potential health hazard of these pathogens posed to humans who may consume contaminated cantaloupe.

Currently the FSMA-Fresh Produce Safety Rule recommends using *E. coli* as an indicator of agricultural water microbial quality (FDA, 2016). In this study generic *E. coli* was not detected in the well water used for flooding (Table 2.1). However, *E. coli* levels in the pond water, which was mixed with the well water, were 10 fold higher than the 126 CFU/100ml rolling geometric mean (GM) standard for agricultural water required by the FSMA-Fresh Produce Safety Rule (Table 2.1). This was not a surprising finding as surface water (i.e. ponds, streams, rivers and lakes) is considered to be the poorest in microbial quality compared to well water or municipal water (Suslow, 2010; James, 2003; Pachepsky et al., 2011). The mean water temperature at the time of

sampling was 36.2 degree C and the mean pH was 6.6, conditions that favor optimal growth of *E.coli* (Van et al., 2011: Don, 2008). In addition, goats graze on the surrounding land and wildlife such as deer and coyotes use the pond as a water source, which may have contributed to the high *E. coli* counts in the pond water. The floodwater used in this study contained an average of 5.1 $\log_{10}$MPN/100 ml of generic *E. coli* (Table 2.1), 1000 times the standard threshold level permitted by the FSMA-Fresh Produce Rule for agricultural water. Similar to the pond water, the mean temperature (34 degree C) and pH (7.5) of the water were ideal for pathogen growth. Coliform bacteria levels in the well water, pond water and flood water exceeded US Environmental Protection Agency (EPA) standards (1000 MPN/100 ml) for recreational water (EPA, 2000).

Total coliform populations on mature cantaloupe fruit were present at high levels (>$\log_{10}$5.7 MPN/cm^2 or $\log_{10}$7.1 MPN/100 ml) on fruit from both flooded and non-flooded plots (Figure 2.3) and populations did not differ based on whether or not they were produced on raised beds or flat ground (Table 2.3). Total coliform levels on cantaloupe were also consistent over a 72 hr time period (Figure 2.3). These results support our current understanding that total coliform bacteria are not suitable indicators of fecal contamination of fresh produce, including cantaloupe. As early as 1980, the use of total coliform bacteria as indicators has been challenged. Splittstoesser et al. (1980) showed that total coliform bacteria were present in over 90% of frozen vegetable packages that they sampled, but that only 0-28% of those same samples contained *E. coli*. A longitudinal microbiological survey that included 63 farms and 2029 pre-harvest produce samples, consisting of 13 types of produce, demonstrated that mean fecal coliform populations on the samples did not differ significantly over a two year period

and that all counts were within a 0.2 $\log_{10}$ MPN/g standard error (Mukherjee et al., 2006). None of the fruit and vegetable samples in their study tested positive for *Salmonella* or *E. coli* 0157:H7 (Mukherjee et al., 2006); indicating that total coliform bacteria are not suitable indicators of foodborne pathogens on many different types of fruits and vegetables. After a natural flooding event in Spain, lettuce heads exposed to floodwater were sampled for seven weeks to determine the levels of coliform bacteria on the lettuce (Castro-Ibanez et al., 2015). While populations of fecal coliform bacteria declined significantly on the lettuce samples, a significant decline was not observed until 3 weeks after the flood (Castro-Ibanez et al., 2015). An ideal indicator should be present and detectable at any time the target pathogen may be present and it should be at concentrations similar to those of the target pathogen (Buchanan, 2000). In our studies, total coliform bacteria did not meet either of these criteria.

In contrast, generic *E. coli* populations were significantly higher on cantaloupe fruit harvested from flooded plots compared to non-flooded plots (Figure 2.2) and populations decreased significantly over 72 hr on cantaloupe sampled from the flooded plots (Table 2.2). However, a significant decrease in populations was only observed on the third day following exposure to the floodwater. After 72 hr, generic *E. coli* levels were 794 MPN/100 ml ($\log_{10}$2.9 MPN/100 ml) or 251 MPN/cm^2 (2.4 log MPN/cm^2). Despite this decline, generic *E. coli* populations still exceeded the FSMA-Fresh Produce Safety Rule rolling GM standard of 126 CFU/100 ml. Although this standard is specific to irrigation water there are currently no standards for fruit or vegetables and thus comparisons to water standards can only be made at this time. After flooding in Spain in 2012, Castro-Ibanez et al. (2015) were unable to detect *E. coli* on lettuce heads three and

five weeks after flooding with a detection limit of 10 CFU/100 ml. Using a regression analysis, Castro-Ibanez et al., (2012) determined that *E. coli* counts decreased exponentially with day length and exposure to solar radiation, providing support that solar radiation plays a role in the natural reduction of bacteria in the field. In a recent study that tracked the movement of *E. coli* from floodwater across a horizontal plane of soil in a field planted with spinach, Callahan et al. (2016) detected *E. coli* within one day of flooding on spinach leaves and up to 14 days, dependent on the location of the plants relative to the edge of the flood zone. However, *E. coli* populations were not enumerated on the spinach leaves making it extremely difficult to make direct comparisons between *E. coli* presence on spinach compared to lettuce (in the case of the Castro-Ibanez et al. (2012) study or cantaloupe (this study). Overall however, *E. coli* appears to be a much better indicator of a potential contamination event by human pathogens compared to total coliform as demonstrated by this study and the flooding study by Castro-Ibanez et al. (2012).

Raised beds have long been recommended to improve soil drainage and manage soilborne plant diseases throughout the world (Thurston, 1990), however to the best of our knowledge, no published studies have been conducted to evaluate the potential of raised beds in protecting fruit from becoming contaminated with human pathogens carried in flood water. In this study, *E. coli* levels on the surface of cantaloupe were not different between those produced on raised beds and those produced on flat ground. The fact that the quality of the fruit was similar between beds types was somewhat surprising given that there is an abundance of literature that indicates that raised beds can reduce plant and fruit disease severity significantly (review by Sanogo and Ji 2013 and Kousik,

2011). In our studies the height of the beds was ~30 cm, the same height at which the floodwater reached. It would seem obvious then that the recommended bed height should be higher in order to protect the cantaloupe from direct exposure to floodwater. However this is probably not practical for several reasons: 1) small-scale bed makers and shapers that would be used by most growers in LA cannot be easily adjusted to accommodate bed heights over 30 cm, 2) beds that are too high do not hold moisture well and thus can cause drought-like symptoms and reduce yield (Hwang and Kim, 1995), 3) for growers who want to use black plastic, plastic laying equipment does not accommodate high ridge beds, and 4) there is no guarantee that floodwater will not exceed the height of high ridge beds. Rather than recommending an increase in bed height more rigorous vine and fruit training may be the better strategy. Because of the cantaloupe plant vines morphology, fruit set and development was not confined to occurring on top of the raised bed and thus the raised beds did not protect most of the fruit from exposure to the flood water. The FSMA-Fresh Produce Safety Rule (FDA, 2011) has implemented a microbial die-off rate that can be used to predict an appropriate harvest day interval in the case that irrigation water microbial quality exceeds thresh-hold criteria ($\leq$126 CFU/100 ml) outlined in the rule. If a water source, in this case flood water, does not meet the quality standards set in the FSMA-Fresh Produce Safety Rule, the assumption that microbes die-off at a rate of 0.5 $\log_{10}$ per day, for up to four days, can be invoked. Given the volume of contaminated water that can enter a field during a flood, we wanted to determine if generic *E. coli* populations on cantaloupe contaminated by floodwater would decrease by at least 0.5 $\log_{10}$ per day for upto 4 days, thus confirming the die-off rate assumption. The concentration of generic *E. coli* in the floodwater in this study was $\log_{10}$5.1 MPN/100 ml

(1.2 X 10^5 CFU/100ml). Assuming the half log die-off rate compares to 68.4% die off of *E. coli* over one day, 90% over two days, 96.8% over three days, or 99% over four days (Bihn et al., 2016), predicted *E. coli* populations in the floodwater would remain above the standard of ≤126 CFU/100 ml after four consecutive days (Tables 2.7-2.9). In this case the cantaloupe exposed to the floodwater would not be considered safe for consumption. However, predicted *E.coli* levels on the fruit by day four would be 63 MPN/cm^2 or 631 MPN/100ml (Table 2.9). Therefore, whether or not the cantaloupe are deemed safe for consumption would depend on which predicted unit of measure is interpreted (Table 2.9). These same observations were observed when these data were separated based on bed type (Tables 2.7 and 2.8). Interestingly, the actual die-off of *E. coli* was larger than the predicted die-off based on both the surface area (per cm^2) and volume (per 100 ml) measurements for fruit harvested from flat ground but not fruit from raised beds. Independent of bed type, the actual die-off of *E. coli* was greater than the predicted die-off for two consecutive days.

Clearly there are still many uncertainties and contributing factors as to whether or not the 0.5 $\log_{10}$ *E. coli* per day die-off with respect to floodwater is an adequate measure of product safety. However, in our study we provided evidence that the microbial quality of cantaloupe following direct exposure to floodwater slowly improves over four days. Provided other quality issues such as fruit rots or chemical residues don't reduce the quality of the fruit, the 0.5 $\log_{10}$ *E. coli* per day die-off rule may be more appropriate to assess product safety than the strict FDA US Food, Drug, and Cosmetic Act issued guidance for handling fruits and vegetables exposed to floodwater which requires that

any flood exposed produce is deemed “adulterated” and no way of reconditioning and should not be allowed into food chain.

Ideally, the detection, identification and enumeration of human bacterial pathogens such as *Salmonella, E. coli* 0157:H7 and *L. monocytogenes* on or in fresh produce would be the best indicator of product safety. However, current testing methods lack the specificity or sensitivity to detect pathogens that may be present in low numbers. Consequently, pathogenic bacteria are rarely detected on fresh produce (International Commission for the Microbiological Specifications of Foods, 2002) unless enrichment techniques are used. Because enrichment is required to detect human pathogens in environmental samples, quantification cannot be done. In our study we hypothesized that fruit exposed to floodwater would have a higher incidence of *Salmonella* sp. and *L. monocytogenes* than cantaloupe that were not exposed to floodwater.

Salmonella spp. was detected on cantaloupe fruit from flooded and non-flooded plots using three different detection methods. The accuracy of *Salmonella* detection methods, specifically PCR and culturing, can vary depending on the sample type (Koyuncu et al., 2010), however overall PCR-based assays have been shown to be more sensitive than the culture method, and the culture and PCR-based assays more specific than immunoassays (Koyuncu et al., 2010; Fratamico, 2003; Eriksson and Aspan, 2007; Maciorowski et al., 2006). For all three tests a pre-enrichment step was included, which increases testing time but ensures the detection of *Salmonella*, which is generally present in low numbers on fresh produce (Jeddi et al., 2014; Dennis et al., 2016). In addition, enrichment reduces the concern that PCR detects both live and dead cells and thus can overestimate to presence of viable cells (reviewed by Cangelosi and Meschke, 2014).

This is important because viability, defined as the ability to replicate and produce progeny, can directly impact the food safety risk of the product being tested. In agreement with the assessments described above, in our study *Salmonella* spp. incidence was underestimated using the RapidChek immunoassay strip tests and overestimated by culturing on semi-selective medium assuming *Salmonella*-specific PCR is the most sensitive and specific detection method; although *Salmonella* incidence was not significantly different between the three test methods. It was not surprising that the RapidChek immunoassay underestimated the presence of *Salmonella* on the fruit because this test (and other immunoassay tests) has a detection limit of only 10^4–10^5 ml^{-1}. However, in the absence of culturing, immonoassays are still important because they are capable of detecting viable and non-culturable *Salmonella* cells (Lee et al 2015; Maciorowski et al., 2006), unlike PCR. In addition, the sensitivity of immunoassays can be reduced depending on the sample background micro-flora, sample quality, and inhibitory substances (Alakomi and Saarela, 2009; Lee et al. 2015) and in food where the initial background microflora is high, competing microorganisms may outgrow *Salmonella* spp. during the enrichment process reducing the overall sensitivity of the test (Mozala, 2006; Naraveni and Jamil, 2005). Given that cantaloupe are in direct contact with the soil and the surface of the rind of the cantaloupe variety used in this study (cv. Ambrosia) is netted, it is plausible that there were high populations of microflora on the surface and that they may have reduced the overall sensitivity of the RapidChek test.

It was also not surprising that the culturing medium used in this study may have inflated *Salmonella* incidence. Although pre-enrichment steps encourage the growth of *Salmonella*, XLD medium is semi-selective and thus other enteric bacteria with similar

growth requirements to *Salmonella* can also grow on the medium. For example, *Proteus* and *Citrobacter* species can grow on XLD and they have similar morphological characteristics as *Salmonella* on this medium (Park et al., 2012; Eigner et al., 2001; Cook et al., 1999; Rambach, 1990; Tate et al., 1990). For this reason, colony confirmation using *Salmonella*-specific PCR is often done, especially when populations are low, so that false positives are not reported. This is extremely important because a positive test for *Salmonella* on produce could result in an entire lot of product being destroyed unnecessarily. In our flooded plots (at 72 hr post flooding) approximately 36.2% of the cantaloupe samples were false positives when compared to PCR results. Similarly, in the non-flooded plots approximately 31.4% of the cantaloupe samples were false positives. In a study conducted by Kumar et al. (2015) the prevalence of *Salmonella* serovars on cantaloupe with different rind netting characteristics was evaluated. They found that 16% of the samples were false positives using culturing on XLD medium compared to using biochemical serovar testing. The sensitivity of the biochemical test (API 20E) compared to PCR is not mentioned however, serovar-specific PCR is not routinely used for initial detection of the pathogen in food. Uyttendaele et al. (2014) used XLD culturing to determine *Salmonella* spp. incidence on lettuce, strawberry and from soil but, did not confirm *Salmonella* spp. using PCR. However, the incidence of lettuce (42%), strawberry (28%) and soil (42%) samples with *Salmonella* spp. was similar to the incidence of *Salmonella* spp. on cantaloupe (18%) from non-flooded fields that we found in our study.

In addition to *Salmonella* spp., *Listeria* spp. and *L. monocytogenes* were detected on cantaloupe fruit from both the flooded and non-flooded plots but no significant

differences in incidence between the plots were identified. Among the fruit from the flooded plots that tested positive for *Listeria* spp., about 3.1% also tested positive for *L. monocytogenes* whereas only 1.8% of the fruit with *Listeria* spp. from the non-flooded plots also tested positive for *L. monocytogenes*. Even though there was not a significant difference in fruit from flooded or non-flooded plots with *L. monocytogenes*, there were more fruits from the flooded plots with *L. monocytogenes*. The genus *Listeria* consists of six species of which two are pathogenic, however only *L. monocytogenes* is considered a foodborne pathogen (reviewed by Schlech, 1996). The detection of *Listeria* spp. on the rind of cantaloupe was not alarming since all *Listeria* species are ubiquitously distributed in nature and can often be found in soil, decaying plants, sewage, and water (Weis and Seeliger, 1975; Beuchat and Ryu, 1997; Jamali et al., 2013; Linke et al., 2014). What was alarming however, was the high percentage of fruit with *L. monocytogenes*. This was alarming because *L. monocytogenes* has a low infectious dose and high fatality rate, particularly in immuno-compromised populations, it is well adapted to a wide variety of environments as it can grow at temperatures as low as 4 degrees C and as high as 43 degrees C, and it can colonize most surfaces easily. Fruits contaminated with *L. monocytogenes* are a source of post-harvest contamination including in processing environments, packing sheds and the home kitchen. While there have been several outbreaks of *L. monocytogenes* on cantaloupe and other fruit and vegetables (Walsh et al., 2014) determining the incidence on product in the field that caused the outbreak is unlikely to occur; although testing surfaces and soil after the fact frequently occurs. Additionally, an a *priori* risk assessment based on fruit quality alone is not logistically or economically feasible as testing requires several days and is expensive. Furthermore,

determining how many fruit to sample to get a representative sample is challenging (ICMF, 2002). The fact that upto 3% of the cantaloupe in our study were contaminated with *L. monocytogenes* and that product sampling is not feasible emphasizes the importance of pre-harvest prevention measures.

Contrary to our hypothesis that flood water would increase the incidence of *Salmonella* spp. and *L. monocytogenes* on cantaloupe we found that there were no significant differences between fruit from flooded and non-flooded plots. Although both pathogens are widely distributed in the environment, this result was surprising given the fact that water plays an important role in the movement of these pathogens through agricultural systems and the temperature of the flood water (34 degrees C) was optimal for the growth of both pathogens. It was also surprising because the pond water used to simulate a flood is a water source for wild animals and birds, both of which are carriers of *L. monocytogenes* and *Salmonella* spp. (Harris et al., 2003: Hellstrom et al., 2008). To our knowledge, only two studies have examined the potential for produce contamination in a field setting after a flooding event and both of these studies evaluated leafy greens. Castro-Ibanez et al. (2015) sampled lettuce for seven weeks after a natural flooding event in southeast Spain and detected *Salmonella* spp. one week after flooding using multiplex-PCR but could not confirm its presence by colony isolation. They also detected *L. monocytogenes* but only on two samples 3 weeks after flooding. The second study (Callahan et al., 2016) evaluated spinach quality following a simulated flood but they only looked a generic *E. coli* prevalence. However, the purpose of this study was to determine the suitability of the Leafy Green Marketing Agreement (LGMA) metrics for harvesting flooded leafy green crops, which states that “leafy green crops within 9 m of

the edge of a flooded field not be harvested due to potential contamination" and that "flooded soils should not be replanted for 60 days". (California Leafy Green Products Handler Marketing Board, 2012). They determined that the 9-m sampling distance may not be sufficient as *E. coli* was detected on spinach leaves at this distance and concluded that "there is the potential for bacteria mobilized by floodwater to contaminate leafy green crops throughout the 9-m buffer zone of crop destruction suggested by the LGMA" (Callahan et al., 2016). They also concluded that the 60 day no replant metric was suitable for crops planted in the spring but that fall plantings should occur 90 days post-flooding. This study, as well as our study, supports the need for more research to validate current FDA and marketing agreement guidelines for preventing contamination during a flood, handling product after a flood and protecting human health, while protecting farms from unnecessary crop destruction and the associated profit losses.

2.4. References

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3. CHAPTER III

Assessment of Phytophthora Fruit Rot and Southern Blight Fruit Rot on Cantaloupe Fruit Following a Flooding Event

3.1. Introduction

The United States (US) produces 30,300 ha of cantaloupe yearly, with a farm value of $325 million (NASS, 2015). In Louisiana (LA) cantaloupe production contributes just under $1 million to the state economy (LSU AgSummary, 2014). Production practices vary across the state and country and depend on field size, soil type, water availability and pest pressure. In the southeastern US, cantaloupes are generally planted in the early spring on bare ground and irrigated as needed using overhead sprinkler systems. In the southern desert valley, mid-bed trenches and slant-bed culture are commonly used (Hartz et al., 1996). Plastic culture production with drip irrigation is expanding across the southeastern US but is still not as cost-effective as planting on bare ground. Growers in the deep southern US states are also hesitant to use black plastic since high temperatures can scorch young cantaloupe plants (Fontenot, K., personal communication). Plastic culture is most popular in states where water is a major limiting factor such as California, Arizona and Oklahoma.

Cantaloupe and other types of melons are susceptible to several diseases that can infect the roots, foliage, and fruit, often resulting in serious crop losses. In LA, two soilborne diseases, Southern blight and Phytophthora crown and fruit rot, are major limiting factors to cantaloupe production. Southern blight, caused by the soil fungal pathogen *Sclerotium rolfsii*, is an economically important disease throughout the subtropics (Aycock, and Aycock1966; Jenkins and Averre, 1986), including LA. *Sclerotium rolfsii* can infect any part of a susceptible plant that comes into contact with

infested soil. In cantaloupe, fruit rot is often the first symptom observed, although plants may also wilt prior to fruit set. Coarse mycelium of the fungus grows over the infected tissue and surrounding soil forming a thick white fungal mat. After approximately seven days, sclerotia form on the surface of mycelial mats (Mullen, 2006). Sclerotia can survive in the soil for many years, serving as primary inoculum in the next growing seasons (Kator et al., 2015; Mullen 2001). The pathogen can also survive as mycelium on dead organic material when living susceptible plant tissue is not present.

Phytophthora crown and fruit rot is caused by the oomycete *Phytophthora capsci* or other species of *Phytophthora*. Similar to Southern blight fruit rot, symptoms initiate on portions of the fruit that are in contact with the soil. However, contaminated water that is splashed onto the fruit can also initiate infections on the upper surface of fruit (Babadoost, 2004). Symptoms begin as water-soaked sunken lesions. The pathogen forms a thin, white, powder-like mycelial layer, containing sporangia, over the sunken lesion (Gevens et al., 2007). *P. capsici* can survive in soil between crops for more than two years, and longer if oospores are produced (Babadoost et al., 2013).

Because *S. rolfsii* and *P. capsici* can survive in the soil for prolonged periods of time, control is difficult once they are introduced into the field. As such, control is rarely achieved through the application of a single method. Management requires the implementation of an integrated management program that utilizes cultural practices including crop rotation, fungicides and biocontrol agents (Mullen, 2001, Xie, 2016; Ristaino and Johnston, 1999; Hausbeck and Lamour, 2004). Effective fumigants and commercial varieties with genetic resistance are not available. Cultural practices aimed at reducing soil moisture and improving soil drainage are recommended for Southern blight

and Phytophthora crown and fruit rot management. Plastic culture with drip irrigation, organic mulch, raised beds and planting on level ground are examples of water management practices that can be used to mitigate Southern blight (García, 1933; Kousik, 2011; Philley and Kaufman, 1982) and Phytophthora crown and fruit rot (reviewed by Sanogo and Ji, 2013) diseases. Raised beds can minimize the impact of soilborne pathogens by improving water drainage, which limits the conditions favorable for disease development. This is important because the survival of *P. capsci* and *S. rolfsii* infectious propagules in the soil is dependent on soil moisture (and temperature) (Sanogo and Ji, 2013; Mullen, 2001).

When production fields are inundated with flood water, the physical, chemical and microbiological characteristics of the soil change (Striker, 2012). For example, oxygen is rapidly depleted, carbon dioxide levels increase and soil nitrogen levels decrease (Striker, 2012). As a result, changes in soilborne pathogen profiles and spatial patterns occur, often increasing the number of disease outbreaks (Munkvold and Yang, 1995; Niem and Inglis, 2012; Strandberg, 1987). Following flooding, soilborne disease outbreaks have been documented from potato fields in Washington state (Niem et al., 2008), sweetpotato fields in LA (Dasilva, 2013), and soybean fields in the north central US (Munkvold and Yang, 1995). The goal of this study was to document the difference in Southern blight and Phytophthora fruit rot incidence on cantaloupe before and after a flooding event. Specific objectives were to: 1) determine the impact of flooding on Southern blight and Phytophthora fruit rot incidence after a flood and 2) determine the effect of bed type on Southern blight and Phytophthora fruit rot incidence after a flooding event**.**

3.2. Materials and Methods

3.2.1 Seedling and fruit production and flooding Seedling and fruit production, plot design (Figure 2.1), and flooding were described in Chapter II (Section 2.2.1).

3.2.2 Plant Disease assessment Fruit rot (Phytophthora fruit rot and Southern blight) was assessed on cantaloupe in flooded and non-flooded plots beginning one week prior to flooding and weekly thereafter (2015 only). During 2016 (spring and summer experiments), fruit rot was assessed one week prior to flooding and one week following flooding. To ensure accurate fruit counts during each fruit assessment, individual fruits were marked with a 45 cm high field flag. The total number of healthy fruit, fruit with Southern blight symptoms and fruit with Phytophthora fruit rot symptoms, from flat beds and raised beds, and in flooded and non-flooded plots, were counted and the proportion of diseased fruit (incidence) was calculated. Following the weekly assessment, diseased fruit were removed from the field and transported to the lab to confirm the presence of the causative pathogen.

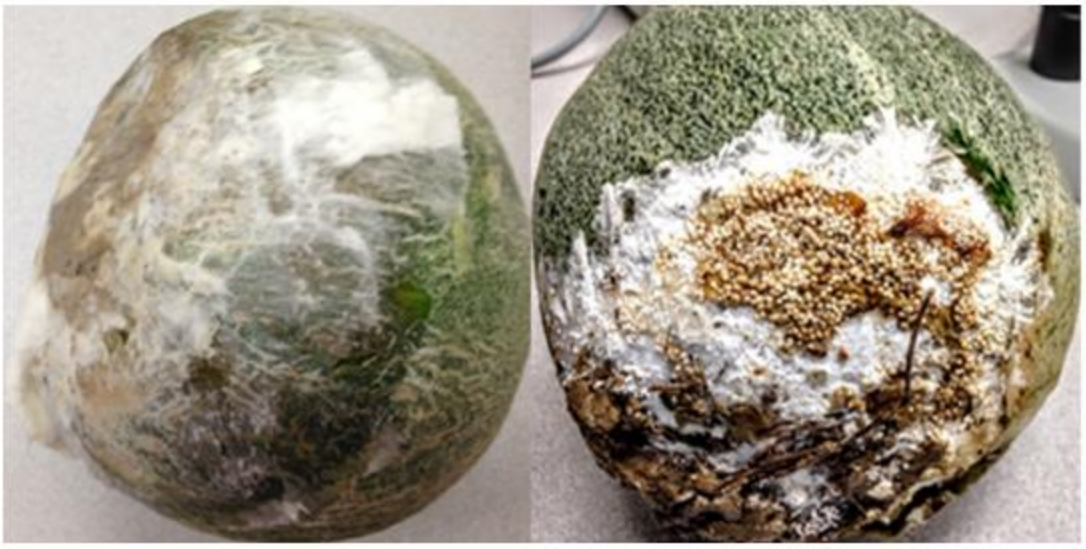
3.2.3 Phytophthora fruit rot and Southern blight fruit rot confirmation Cantaloupe with Phytophthora fruit rot or Southern blight fruit rot symptoms were further evaluated using light microscopy to confirm the symptoms were caused by *Phytophthora* sp. or *S. rolfsii*. Fruit with water soaked lesions or white growth were collected into a sterile zip-seal bag (26.7 x 27.8 cm, 3.9 L, S.C Johnson Inc., Racine, WI) and transported to the lab for disease confirmation. Small pieces (~1 mm) of tissue obtained from the margins of lesions were used to prepare wet mounts for microscopy. For *S. rolfsii* confirmation, the tissue was surface sterilized for 30 sec in 70% ethanol and plated onto acidified potato dextrose agar (aPDA) (Difco, Laboratories, Inc, Sparks, MD). Plates were incubated at

ambient room temperature for 3-15 days. *P. capsici* was confirmed based on the presence of coenocyte mycelia and lemon shaped caducous sporangia with attached pedicels when viewed at 400X magnification with a compound light microscope (Carl Zeiss Inc. Scarsdale N.Y). *Sclerotium rolfsii* was confirmed based on the formation of tan colored sclerotia on aPDA after 14 days and the presence of white septate mycelia with clamp connections by microscopy (1000X magnification).

3.2.4 Data analysis Weekly data for the first trial (2015) and combined Phytophthora fruit rot or Southern blight fruit rot incidence based on flood status, bed type and time were analyzed using the General Linear Model (GLM) procedure with SAS statistical software (SAS Institute Inc., Cary, NC). Means were separated by sampling time, flood status or bed design using Fisher's Least Significant Difference (LSD) test (Fisher, 1954) at α=0.05.

3.3. Results

3.3.1 Phytophthora fruit rot (*P. capsici*) incidence Symptoms on cantaloupe that were indicative of Phytophthora fruit rot included water soaked lesions and lesions with white powder-like growth (Figure 3.1A).



A B

Figure 3.1. Signs and symptoms of Phytophthora fruit rot (*Phytophthora capsici*) (A) and Southern blight (*Sclerotium rolfsii*) fruit rot (B) on a mature cantaloupe fruit one week after flooding**.**

All fruits with Phytophthora fruit rot symptoms were positively confirmed by microscopy. Mycelia were non-septate, and sporangia were caduceus and lemon shaped with a defined papilate. Fruit rot incidence did not differ significantly between raised and flat beds in flooded ($p=0.0644$) or non-flooded plots ($p=0.4879$) (Table 3.1).

Table 3.1. Mean percent incidence of cantaloupe fruit, produced on raised beds or flat ground, with Southern blight (*Sclerotium rolfsii*) or Phytophthora fruit (*Phytopthora capsici*) one week prior to flooding and one week after flooding and in flooded or non-flooded plots.

	Percent Fruit Rot[1] Non-flooded Control Plots				Percent Fruit Rot[1] Flooded Plots				p-value	
	One Week Prior to Flooding		One Week Post Flooding		One Week Prior to Flooding of Flood Plot		One Week Post Flooding of Flood Plot			
Disease	Raised beds	Flat beds	Raised beds	Flat beds	Raised beds	Flat beds	Raised beds	Flat beds	Bed type[3]	Time[4]
Phytophthora fruit rot	1.9±2.2a[2]	4.0±2.2a	7.5±2.2a	6.6±2.2a	2.9±2.2a	3.4±2.2a	13.6±2.2a	15.9±2.2a	0.2657	0.0001
Southern blight fruit rot	10.5±4.3b	11.7±4.3b	13.1±4.3b	11.2±4.3b	8.1±4.3b	6.9±4.3b	22.6±4.3b	26.6±4.3b	0.4231	0.0001

[1] Values are the mean percent incidence of Phytophthora or Southern blight plus or minus the standard error.
[2] Within columns, means followed by the same letter are not significantly at $p<0.05$.
[3] P-value corresponds to comparisons between Phytophthora or Southern blight incidence on raised beds and flat ground.
[4] P-value corresponds to comparisons between Phytophthora or Southern blight incidence before and after flooding.

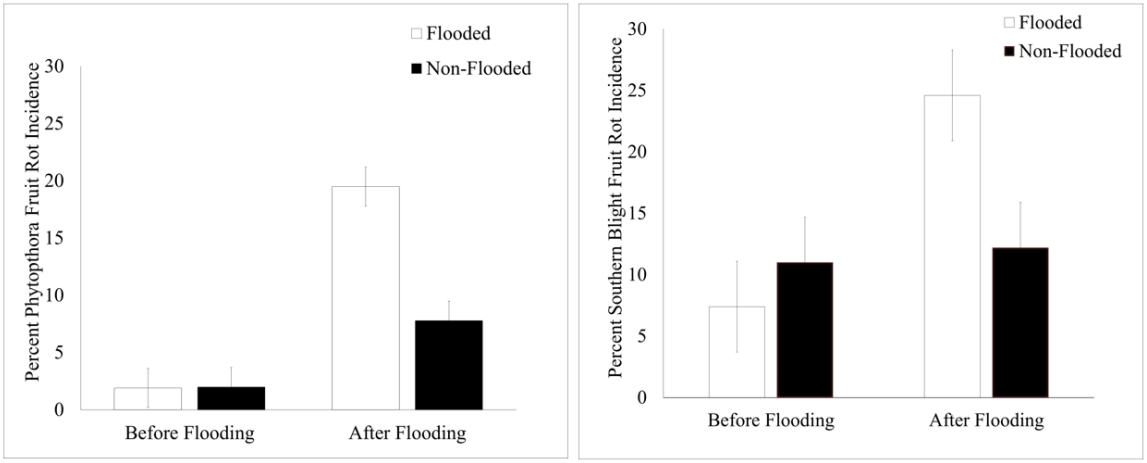
Within the flooded plots, one week after flooding, Phytophthora fruit rot incidence significantly increased from 2.9% to 13.6% in raised beds (P=<.0001) and from 3.4% to 15.9% in flat beds (P=<.0001) (Table 3.1). No significant interaction between flood status, bed type, and sampling time was detected (P=0.2453 and P=0.1599) for 2016 and 2015 trials respectively (Table 3.2).

Table 3.2. Phytophthora fruit rot (*Phytophthora capsici*) or southern blight fruit rot (*Sclerotium rolfsii*) incidence tests for significant interaction between flood status, bed type and fruit sampling time for experiments conducted in 2015 and 2016.

Effect	P-value of test of fixed effects[1]			
	Phytophthora fruit rot		Southern blight fruit rot	
	2016	2015	2016	2015
Flood status	0.0104	0.1666	0.0029	0.8095
Bed type	0.4231	0.4835	0.2657	0.1794
Flood*Bed Type	0.5064	0.2261	0.4108	0.0674
Sampling time	<.0001	<.0001	<.0001	<.0001
Flood*Sampling time	<.0001	0.3922	0.0004	0.0114
Bed type*sampling time	0.0575	0.7995	0.7905	0.0292
Flood status*bed type*sampling time	0.2453	0.1599	0.4362	0.9448

[1] Test for mean significant interaction between flood status, bed type and sampling time.

Independent of bed type (data combined for raised and flat beds), Phytophthora fruit rot incidence increased significantly from 1.9% to 19.5% one week post flooding in plots that were flooded (p=0.0001). In plots that were not flooded fruit rot increased from 2.0 % to7.8% (p=0.0066) (Figure 3.2a).



A B

Figure 3.2. Mean percent incidence of Phytophthora fruit rot (*Phytophthora capsici*) (A) and Southern blight (*Sclerotium rolfsii*) fruit rot (B) on cantaloupe fruit from flooded (white bars) and non-flooded control plots (black bars) one week prior to flooding and one week post flood. Error bars indicate the standard error of the mean values.

In the 2015 flooded plots, percent fruit rot increased over time and peaked at week seven, which corresponded to one week post flooding. Independent of bed type fruit rot incidence were significantly different (P=0.0022) one week after flooding. In the non-flooded plots fruit rot peaked at week 5 in the flat beds (5.7%) and week 6 in the raised beds (6.4%) (Figure 3.3).

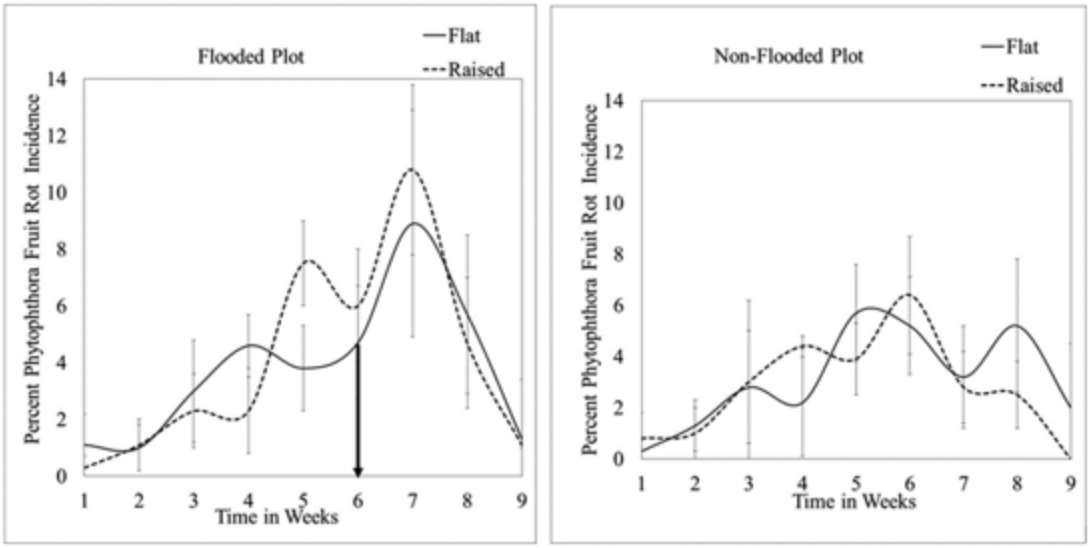


Figure 3.3. Weekly mean percent incidence of Phytophthora (*Phytophthora capsici*) fruit rot on fruit produced on raised beds (dotted line) and flat ground (continuous line) in flooded (A) and non-flooded (B) plots over 9 weeks in 2015. Error bars indicate the mean standard error where n=1660.

3.3.2 Southern blight (*S. rolfsii*) fruit rot incidence Symptoms indicative of Southern blight fruit rot included water soaked lesions and lesions with white cotton-like growth with dark-brown sclerotia (Figure 3.1B). All fruit with Southern blight fruit rot symptoms were positively confirmed by microscopy. Mycelia were septate with clamp connections. Sclerotia formed on aPDA 15 days after plating. Southern blight fruit rot incidence in did not differ significantly between raised and flat beds in flooded (p=0.9553) or non-flooded plots (p=1.000) (Table 3.1). Within the flooded plots, one week after flooding, Southern blight fruit rot incidence significantly increased from 8.1% to 22.6% in raised beds (p=<.0001) and from 6.9% to 26.9% in flat beds (p=<.0001) (Table 3.1). No significant interaction between flood status, bed type, and sampling time was detected (p=0.4362 and P=0.9448) for 2016 and 2015 trials respectively (Table 3.2). Independent of bed type (data combined for raised and flat beds), Southern blight fruit rot

incidence increased significantly from 7.0% to 32.8% one week post flooding in plots that were flooded (p=<.0001). In plots that were not flooded fruit rot increased from 9.0 % to13.8% (p=0.0188) (Figure 3.2b). In the 2015 flooded plots, percent fruit rot increased over time and peaked at week seven, which corresponded to one week post flooding. Independent of bed type fruit rot incidence did not differ significantly (P=0.6422) one week after flooding. In the non-flooded plots fruit rot peaked at week 5 in both raises and flat beds at 22.9% and 9.0% respectively (Figure 3.4).

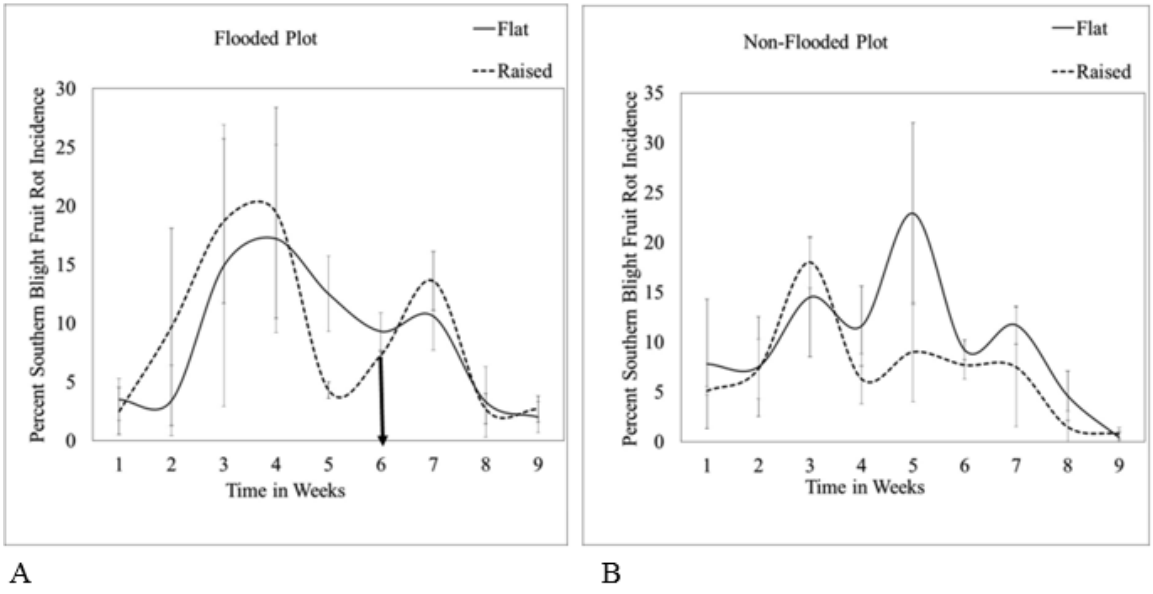


Figure 3.4. Weekly mean percent incidence of Southern blight (*Sclerotium rolfsii*) fruit rot on fruit produced on raised beds (dotted line) and flat ground (continuous line) in flooded (A) and non-flooded (B) over 9 weeks in 2015. Error bars indicate mean standard error where n=16

3.4. Discussion

Southern blight and Phytophthora crown and fruit rot, are major limiting factors to cantaloupe production in LA. When production fields are inundated with flood water, the incidence of disease caused by soilborne pathogens can increase (Niem et al., 2008; Dasilva, 2013; Munkvold and Yang, 1995). In this study, both Southern blight and Phytophthora fruit rot incidence increased significantly one week after simulating a flood but no differences were detected between raised and flat beds. It is well documented that the mobility of *Phytophthora* spp. zoospores increases in saturated soils and that saturated soils predispose plant roots to *Phytophthora* spp. infections (Reviewed by Sanogo et al., 2013). In our studies Phytophthora fruit rot incidence approached nearly 20% just one week after flooding, which in a commercial field would result in major economic losses, especially since the average field size for cantaloupe is 0.7 ha in LA (LSU AgSummary, 2014). Although *P. capsci* has a very wide host range (Erwin and Ribeiro, 1996), this study appears to be the first report to document an increase in Phytophthora fruit rot incidence on cantaloupe following a flood. Other studies on different crops have also demonstrated that flooding contributes to an increase in disease incidence and severity. For example, Bowers et al., (1990) demonstrated that both the frequency of flooding and the amount of time that the production field is exposed to flood water increased the mortality of pepper plants due to infection by *Phytophthora* spp. Browne et al., (1980) and Wilcox and Mircetic (1985) both found a positive correlation of flood duration to the development of Phytophthora crown and root rot of apple and cherry respectively.

Southern blight causes economic losses to a wide range of hosts, particularly in the Southern US (Mullen, 2001; Jenkins, 1986). *Sclerotium rolfsii* is dispersed within a field and between fields by infested seedlings, water, wind, and cultural practices culpable of moving infested soil or plant debris (Xie, 2016; Jenkins, 1986). Epidemics of cantaloupe fruit rot caused by *S. rolfsii* was first reported in the summer months of 1928 in Arkansas and Virginia (Rosen et al., 1929). According to Rosen et al. (1929), *S. rolfsii* infections on cantaloupe fruits and subsequent economic losses may have been attributed to high levels of natural inoculum present in the soil, emergence of new aggressive isolates, warm summer weather with excess rainfall, and destructive floods experienced in the summers of 1927 and 1928. Coupled with favorable summer temperatures and the presence of high levels of natural inoculum in our trial fields, Southern blight fruit rot incidence increased nearly 30% within a week after the treatment plots were flooded. Such a dramatic increase in Southern blight fruit rot incidence following a natural flood could result in substantial economic losses to growers in LA and other flood prone regions.

Cultural management practices that may reduce soil saturation or splash dispersal in the field such as raised beds, mulching, irrigation methods, and avoidance of excess water are recommended to reduce disease incidence and severity (Reviewed by Sanogo and Ji, 2013). Raised beds have been effective in reducing Phytophthora blight of chili and bell pepper (Ristaino and Johnston, 1999; Babadoost, 2005; Hwang et al., 1995), Phytophthora root rot of raspberries (Maloney, 1993) and Phytophthora fruit rot of summer squash (Meyer and Hausbeck, 2012), but none of these studies evaluated bed type with flooding events. In contrast, other studies have also provided evidence that

raised beds (in the absence of flooding) have little to no effect on Phytophthora blight or fruit rot (Kousik et al., 2011). In our study we found that before flooding, Phytophthora fruit rot incidence was about 50% lower on raised beds compared to flat beds (Table 3.1) but that following flooding, incidence did not differ significantly. However, in plots that were not flooded (control plots), Phytophthora fruit rot incidence did not differ based on production on flat beds or raised beds.

No studies have evaluated the effect of using raised beds to manage Southern blight fruit rot. However, in a review Southern blight, Southern stem blight, and white mold diseases by Mullen (2001), good soil drainage is mentioned as an important disease management tactic. Knowing that raised beds can improve soil drainage and reduce moisture retention levels in vegetable and fruit production fields (Reviewed by Sanogo and Ji, 2013) we hypothesized that raised beds may protect cantaloupe fruit from direct exposure to floodwater and also protect the fruit from coming into direct contact with *S. rolfsii* (and *P. capsici*) present in the soil. However, similar to Phytophthora fruit rot, raised beds provided no protection against Southern blight fruit rot infections in flooded or non-flooded plots. In this study we did not utilize black plastic mulch, primarily because the plastic layer available to use could not accommodate 30 cm high beds but also because cantaloupe producers in the deep South do not generally use it for cantaloupe production, especially large producers in states surrounding LA. Because black plastic was not used, the fruit were in direct contact with infested soil in the flat ground and raised bed plots. For this reason the lack of differences between fruit rot in fruit from raised or flat ground was not unforeseen. For the flooded plots we anticipated that the raised beds would provide some protection from fruit rot infections based on the

fact that water is a carrier of both pathogens and thus the inoculum load in the flooded plots would be higher than in the non-flooded plots. However, we did not train the vines so that the vines would be in a position to set fruit on top of the beds and, as a result, fruit were on the beds as well as in between the beds putting them in direct contact with the flood water. Training of cantaloupe plants is time consuming and labor intensive, and without the use of plastic the fruits will ultimately remain in contact with infested soils. Additional studies using plastic mulch or other types of mulch in combination with raised beds may elucidate differences between fruit rot incidence in raised beds compared to flat ground following a flood. Although growers may be hesitant in adopting mulched raised beds they may find that this might be the only sustainable option in lieu of the increasing number of extreme weather events that we are experiencing in the US, particularly those that generate flash floods or extended days of rain.

Consistent with our results and previous studies, there is no single method that can fully manage Southern blight or Phytophthora fruit rot. Integrating cultural practices such as raised beds, plastic mulch, application of organic matter, all of which can improve soil drainage and potentially provide a barrier between the fruit and flood water or soil, along with other strategies such as flood prediction models, may be of great value to cantaloupe producers in LA and other deep south states.

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VITA

Isaack Kikway, received his B.S. in Horticultural Science and Management from the University of Eldoret, Kenya in December, 2013. He was involved in floriculture industry research at the Rosetto Flower Company (Kenya) in 2013. There, he worked in several departments, including those associated with biocontrol, fertigation, and post-harvest and packing house operations. He became more interested in horticulture after working on a flower farm in Kenya since 2013 and consequently decided to pursue a M.S. in Plant Pathology at Louisiana State University, Baton Rouge, LA. Currently Isaack is pursuing his Ph.D. with Dr. Jong Ham at Louisiana State University, Baton Rouge, LA.

Isaack Kikway