ABSTRACT

Title of Dissertation:	*FUSARIUM* SPECIES OF CUCUMIS MELO IN THE MID-ATLANTIC REGION OF THE US AND THEIR IMPACT ON *SALMONELLA ENTERICA* NEWPORT SURVIVAL AND INTERNALIZATION ON VARIOUS MELON CULTIVARS
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Fruit rots caused by *Fusarium* spp. can lead to economic yield losses on melon (*Cucumis melo*). However, which *Fusarium* spp. are the most prevalent in Maryland and Delaware has not been documented. Several *Salmonella enterica* subsp. serovar Newport (*S.* Newport) outbreaks on melon have occurred over the past 25 years. *Fusarium* spp. infestation on melon have potential impact on survival and colonization of Salmonella. Our objectives were to identify *Fusarium* spp. infestations on melons within the Delmarva region, and evaluate their impact on survival and internalization of *S.* Newport on various melon cultivars. Fifty-six isolates were molecularly identified, according to Fusarium-ID online database, as *Fusarium* spp. (*Fusarium fujikuroi*-20, Fusarium proliferatum-18, *Fusarium oxysporum*-15, *Fusarium graminearium*-2, *Fusarium verticilloides*-1). Our findings

revealed that most of the *Fusarium* isolates we collected were not pathogenic to melon fruit. We evaluated the impact of four *Fusarium* spp. (*F. armeniacum*, *F. oxysporum*, *F. fujikuroi*, and *F. proliferatum*) on *S.* Newport survival in five melon cultivars; 'Arava' (*C. melo* var. *reticulatus*, Galia), 'Athena' (var. *reticulatus*, muskmelon), 'Dulce Nectar' (var. *inodorus*, honeydew), 'Jaune de Canaries' (var. *inodorus*, Canary), and 'Sivan' (var. *cantalupensis*, Charentais). Impact of *F. proliferatum* on survival and internalization of *S.* Newport was evaluated on honeydew (smooth) and cantaloupe (netted) melons. Generally, *Fusarium* did not impact the survival of *S.* Newport, however greater survival of *S.* Newport was observed on the netted cultivars compared to the smooth surface melons. *Fusarium fujikuroi* significantly enhanced survival of Salmonella when inoculated on riper 'Jaune de Canaries' melons (above ¾ slip). However, when the experiments were replicated with less ripe (about ¾ slip) melon, *F. fujikuroi* did not significantly influence the growth of *S.* Newport. *Salmonella* Newport internalized in all treatments and the cantaloupe and honeydew melons, but variation in population levels were observed across the treatments. Overall, *Fusarium proliferatum* did not impact internalization of *S.* Newport on either melon type. This may be attributed to that *Fusarium* species used during this study were nonpathogenic. *Salmonella* Newport recovered gradually decreased with time. *Fusarium* species on melon, influence *S.* Newport colonization differently. Also, melon rind type affects the ability of *S.* Newport to survive and colonize differently.

Keywords: Melon, Cultivars, *Fusarium* spp., *Salmonella enterica* Newport, Survival, Internalization.

FUSARIUM SPECIES OF *CUCUMIS MELO* IN THE MID-ATLANTIC REGION OF THE US AND THEIR IMPACT ON *SALMONELLA ENTERICA* NEWPORT SURVIVAL AND INTERNALIZATION ON VARIOUS MELON CULTIVARS.

by

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Dedication

This dissertation is dedicated to my daughter, Meredith Chebet Korir, my parents; John and Esther Segem, and all other family members in Kenya.

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Chapter 1: General Introduction

Introduction

1.1. Rationale for this study.

There is little information available on diversity of *Fusarium* spp. causing *Fusarium* fruit rot of melons in Maryland and Delaware. In addition, there is no information available on survival ability of *Salmonella enterica* subsp. enterica serovar Newport on various melon cultivars. A few studies have identified the origin of *Salmonella* infestation on cucurbits (Angelo et al., 2015), however, there is little information on interactions between plant or fruit pathogenic fungi, or fungal saprophytes, and human pathogenic bacteria on or in melon. A few studies have evaluated and reported internalization and survival of *Salmonella* in produce like tomatoes, but not on melon (Gu et al., 2011 & 2013, and Han and Micallef, 2014 & 2016). Therefore, more research focused on ingress of *Salmonella* into produce, and its survival under harsh environmental conditions which are different from the habitat of mammal guts, is needed. There is little information on how infection by *Fusarium* spp., can influence *S*. Newport survival and colonization of melon fruit or on colonization and survival ability of *S*. Newport on various melon cultivars. Improved understanding of pre- and post-harvest colonization and survival of *S*. Newport on produce could help in reducing the prevalence of food borne outbreaks. This study focused on evaluating and identifying prevalent *Fusarium* species infesting melon (*Cucumis melo*) in Maryland and Delaware. To evaluate and identify these *Fusarium* species, a survey was carried out during the 2016 and 2017 growing seasons. Isolates were

morphologically and phylogenetically identified. Secondly, impact of four *Fusarium* spp. (*F. oxysporum, F. fujikuroi, F. armeniacum, F. proliferatum*) presence on the melon rind on *S.* Newport survival was evaluated. To carry out these evaluations, three *Fusarium* spp. (*F. oxysporum, F. armeniacum, F. fujikuroi*) were inoculated on melon during the experimental repeats 1 through 3, and F. armeniacum was replaced with *F. proliferatum* during the fourth repeat of the experiment. Melon cultivars with varying rind topographies that were inoculated with *Fusarium* spp. were also evaluated for *S.* Newport survival. 'Arava' (*C. melo* var. *reticulatus*, Galia), 'Athena' (var. *reticulatus*, muskmelon), 'Dulce Nectar' (var. *inodorus*, honeydew), 'Jaune de Canaries' (var. *inodorus*, Canary) and 'Sivan' (var. *cantalupensis*, Charentais) were evaluated. The fruit of these five melon cultivars were inoculated with one of three *Fusarium* spp. and subsequently inoculated with *S.* Newport. The *Fusarium* spp. strains that were used were collected and isolated from melon 'Sivan' grown in an organic field at University of Maryland Lower Eastern Shore Research and Education Center (LESREC), and from a farmer's field in Delaware (Table 8). These isolates were obtained during a small survey that was conducted in the 2015 and 2016 growing season, where melon fruit that had symptoms of *Fusarium* fruit rot were collected. *Fusarium* spp. were then isolated and identified using morphological and molecular techniques. Eight isolates were selected and molecularly identified. PCR was conducted using primers that targeted translation elongation alpha factors (TEF1&2) and the second largest sub-unit region of RNA polymerase II (RPB2) and the amplified regions were sequenced. Four *Fusarium* spp. (*F. oxysporum, F. fujikuroi, F. armeniacum, F. proliferatum*) were identified and used during this study.

Internalization of *S.* Newport in the presence or absence of *F. proliferatum* in honeydew and cantaloupe type melons was also evaluated. To carry out this study, surface (rind) of smooth (honeydew) and netted (cantaloupe) melons was inoculated with *F. proliferatum* and incubated for four days, thereafter, followed by *S.* Newport inoculation. The surface, exocarp, mesocarp, and endocarp (seed cavity) were separately evaluated for *S.* Newport at 5- and 10-days post-inoculation.

1.2. Melon consumption in the U.S.

Consumption of melons in the US is high, due to their many nutritional and health benefits (USDA-ERS, 2011). The United States is one of the world's leading consumers

of melons. For example, in 2011, 3.95 kg of cantaloupes and 0.68 kg of honeydew consumption per person was reported (USDA-ERS, 2011). The average American consumes about 12.2 kilograms of melons each year (USDA-ERS, 2011). Melon consumption has increased because of increasing health consciousness of consumers, improved year-round availability, creative marketing, and improved varieties (USDA ERS, 2011).

1.3. Melon Production in the US.

The U.S. cantaloupe acreage increased from 26,851 hectares in 2012, to 30,242 hectares in 2013. However, the production decreased from 2013 to 2015. The U.S. cantaloupe production area decreased from 26,851 hectares in 2012 to 20,882 hectares in 2015. Cantaloupe production was 13.4 million cwt in 2015. During the above period, honeydew acreage under production increased. This was 3.5 million

cwt honeydew produced (Agricultural Marketing Resource Center: https://www.agmrc.org/).

1.4. Melon Production in Maryland.

Melon is an economically important crop in the USA, and Maryland is ranked among the top producers in the nation. Maryland ranked 8th in national muskmelon production in 1997 and accounted for about 1% of total U.S. production (USDA-NASS, 1997). During 1997 and 1998, Maryland farmers planted an average of 607 hectares and harvested an average of 567 hectares of muskmelons for the fresh market. The average cash value of fresh market muskmelons in Maryland for the same period was $2,709,000.00 (Maryland Extension Bulletin, 2003; USDA-NASS, 1997). Although melon is an economic crop both locally and nationwide, it has various production challenges. Those challenges include farm labor shortages, strong competition in export markets, pressure in domestic markets from low-cost imports, competition for land and water from both urban encroachment and alternative crops, rising inputs, and food safety concerns (Ali and Lucier, 2011). According to USDA-ERS (2011), melon production accounted for 72.2% of all the fruit produced in Maryland.

1.5. *Salmonella* outbreaks linked to melon.

Melon consumption has been associated with an increased *Salmonella* outbreaks (CDC website: https://www.cdc.gov/*Salmonella*). Previously, most food borne outbreaks associated with *Salmonella* were associated with food products of animal origin, such as beef and poultry (Natvig et al., 2002). Human pathogens had been thought to live and thrive in the gut of vertebrate hosts, and to be introduced to

produce only through either manure application, contaminated irrigation water, or animal intrusions in the farm as surface contaminants. Contrary to these assumptions, human pathogens have been discovered to enter, adhere to, multiply, and even move to produce leaves and fruits from the growth medium (Islam et al., 2004, Angelo et al., 2015; Wang et al., 2009). Moreover, in recent years, there has been rise in foodborne illnesses and outbreaks that are linked to fresh produce. Most of these produce outbreaks have been due to *Salmonella* and pathogenic *Escherichia coli*. For example, there were several *Salmonella* outbreaks from July 3, 2015 to February 29, 2016 that were associated with consumption of cucumber imported from Baja, Mexico and sold in several states in the US. According to Center for Disease and Prevention and Control (CDC), and Food and Drug Administration (FDA) 907 people infected with the outbreak strains of *Salmonella* Poona have been reported from 40 states. These outbreaks resulted with six deaths from Arizona (1), California (3), Oklahoma (1), and Texas (1) (CDC website: https://www.cdc.gov/*Salmonella*). Three additional multistate outbreaks of *S*. Poona infections associated with eating cantaloupe imported from Mexico occurred in the spring of 2000, 2001, and 2002 (CDC, 2002). *Salmonella* contamination on produce is a national problem in the U.S. and, occasionally linked to international outbreaks. The CDC confirmed and reported cases of *Salmonella* linked to cantaloupes in the following years: 2008 (*Salmonella* Litchfield), 2011(*Salmonella* Panama), 2012 (*Salmonella* Newport and Typhimurium). Additional *Salmonella* outbreaks that were linked to produce like apples and oranges have been reported and documented (Buchanan et al., 1999 and US. FDA, 1999 & Barton et al., 2011). Locally, salmonellosis cases, were linked to

cucumbers produced in Delmarva and sold in twenty-nine other states of the US (Angelo et al., 2015).

1.6. Microbial contamination of produce.

Factors in field production, like the use of a cover crop, organic matter application, field, crop rotation, and type of mulch can also influence the microbial community and survival of bacteria in the soil (Nair and Ngoujio, 2012; Ponge et al., 2013 and Venter et al., 2016). Recent studies indicate that common cover crops grown prior to produce production, can influence survival of *E. coli* and *Listeria* in the soil (Reed-Jones et al., 2016). Reed-Jones et al. (2016), reported that a mixture of cover crops, hairy-vetch plus rye, in the field influenced the *E. coli* and *Listeria innocua* population.

1.7. *Fusarium* spp. and Cucurbits.

Fusarium spp. have been widely studied and reported to infect and cause cucurbit diseases leading to yield loss in most parts of the world (Chehri et al., 2011; Wade and Morris, 1982; Leslie et al., 1992 &1996). The fungal genus *Fusarium* is one of the most studied fungal groups because of its importance as a plant pathogen and mycotoxin producer (*F. solani*, *F. oxysporum*, *F. graminearum*, *F. moniliforme*, *F. sambucinum*, *F. culmorum*, and *F. equiseti*), and a human pathogen (Seremi and Okhovvat, 2006). There are twelve *Fusarium* spp. reported to cause melon fruit rots in the US: *Fusarium acuminatum* Ellis & Everh.; *F. avenaceum* (Fr.: Fr.) Sacc.; *F. culmorum* (Wm. G. Sm.) Sacc.; *F. equiseti* (Corda) Sacc.; *F. graminearum* Schwabe (syn. Gibberella zeae (Schwein.) Petch); *F. graminum* Corda; *F. incarnatum* (Desm.)

Sacc. (syn. *F. semitectum* Berk. & Ravenel); *F. oxysporum* Schltdl.: Fr. *f.* sp. *melonis* W. C. Snyder & H. N. Hansen; *F. petroliphilum* (Q. T. Chen & X. H. Fu) Geiser, O'Donnell, Short & N. Zhang (syns. *F. solani* (Mart.) Sacc. *f.* sp. *cucurbitae* W. C. Snyder & H. N. Hansen race 2; *Nectria* h*aematococca* Berk. & Broome MP V); *F. scirpi* Lambotte & Fautrey; *F. solani* (Mart.) Sacc. *f.* sp. *cucurbitae* W. C. Snyder & H. N. Hansen race 1 (syn. *Nectria haematococca* Berk. & Broome MP I); *F. verticillioides* (Sacc.) Nirenberg (syn. *F. moniliforme* J. Sheld.); *Fusarium* spp. (Elmer, 1996; Keinath et al., 2018; Rivas-Garcia et al., 2018). Melon fruit are prone to cracking due to extreme fluctuations in moisture and temperature, which make them more susceptible to infection by *Fusarium*. Many *Fusarium* species are known to be either saprophytic or weak and opportunistic pathogens and can only cause secondary infections. Conditions such as physical injury or stress on melons due to cracking, drought or insect damage, may make them more susceptible (Palmer and Kommedahl, 1960). *Fusarium* infection on fruit, apart from causing rots, may make them inedible because they produce mycotoxins (Plattner, 2000). *Fusarium* species; *F. fujikuroi* and *F. oxysporum*, which are known to cause melon fruit rots, also may produce toxic fumonisin (Bezuidenhout et al., 1988; Branham and Plattner, 1993a&b; Van Wyk et al., 1987). *Fusarium* is heterogeneous and comprises different morphological characteristics, plant hosts, and production of toxins like fumonisins and tichothecenes that affect humans and animals if ingested through feeds (Fisher et al., 1982). Several taxonomic systems have been proposed for *Fusarium*, which vary in total number of species from 9 to 78 (Nelson, 1991), to over 1,000 species (Taylor et al., 2000). These classical taxonomic systems are based on morphological

characters such as the size and shape of macroconidia, the presence or absence and the shape of microconidia and chlamydospores, and the structure of conidiophores (Guadet et al., 1989).

1.8. Objectives.

Most food safety research has focused on sources and distribution of contamination of fresh produce by human pathogenic bacteria, which include manure, irrigation water, wildlife, and contamination during produce transportation, processing, and handling (Barak et al., 2005, 2007, 2008, & 2011; Bowen et al., 2006; Brandl, 2008; Chisholm, 2006; Mandrell, 2009; Beuchat, 1999 and Korir et al., 2016). However, there are limited studies on human pathogenic bacterial interactions with fungi. The overall goal of this study was to investigate and identify associated *Fusarium* species on *Cucumis melo* in Delmarva region, and their effects on the subsequent survival of the human pathogenic bacterium, *Salmonella enterica* Newport. Four factors were evaluated, 1) the diversity of *Fusarium* spp. of melon fruit in Maryland and Delaware; 2) the impact of the presence of four *Fusarium* spp. (*F. armeniacum*, *F. fujikuroi*, *F. oxysporum*, *F. proliferatum*) on *Salmonella* Newport survival on melon fruits; 3) the impact of five melon cultivars (‘Arava’, ‘Athena’, Dulce nectar, ‘Jaune’, ‘Sivan’) on survival of *S.* Newport in the presence of the four different *Fusarium* spp. (*F. armeniacum*, *F. fujikuroi*, *F. oxysporum*, and *F. proliferatum*) and 4) the impact of *F. proliferatum* on internalization of *S.* Newport in smooth (‘honeydew’) and netted (cantaloupe) surface melon.

1.9. The goal of this project was to test four main hypotheses:

1: Several *Fusarium* spp. cause *Fusarium* fruit rot of melon or are associated with melon fruit in Maryland and Delaware.

2: Survival of *Salmonella enterica* Newport varies among melon cultivars.

3: *Fusarium* spp. differentially affect the ability of *Salmonella enterica* Newport to establish in melon fruit.

4: *Fusarium* spp. (*F. proliferatum*) influence the survival and internalization of *Salmonella enterica* Newport on melon fruit. The depth of colonization of *S.* Newport into the flesh of the melon is influenced by the presence of *F. proliferatum* and the melon surface type (smooth-honeydew vs netted-cantaloupe).

Chapter 2: Literature Review

2.1. *Salmonella* outbreaks on cucurbits.

Per capita consumption of fresh fruits and vegetables has increased in the US for the past few decades (CDC: https://www.cdc.gov/*Salmonella*/reporting-timeline.html). This increase has coincided with increasing reports of foodborne illnesses associated with fresh produce. During the past 20 years, *Salmonella* has been one of the most common pathogens responsible for foodborne outbreaks that are associated with produce in the US (Mukherjee et al., 2007; Lynch et al., 2009; Sivapalasingam et al., 2004). Some salmonellosis outbreak cases have been linked to produce in Delmarva region. For example, Angelo et al. (2015) reported incidences of salmonellosis that were linked to consumption of cucumber during May through September 30, 2014. During this *S*. Newport outbreak, a total of 275 cases were reported from 29 states and the District of Columbia. A PFGE pattern from infected persons indicated that the origin of outbreak was the Delmarva region (Angelo et al., 2015). The same PFGE pattern report had been linked to consumption of tomatoes harvested from Virginia's Eastern Shore in 2002 (333 persons), 2005 (72 persons), 2006 (115 person), 2007 (65 persons), and 2010 (51 persons). In addition to the outbreaks on cucumber and tomato, many cases of salmonellosis outbreaks associated with melon have been reported (CDC, 1991, 2002; Munnoch et al., 2008; Bowen et al., 2006; Ries et al., 1990, and Hanning et al., 2009). A survey conducted by Castillo et al. (2004), reported that *Salmonella* was found in 0.5% and 0.3% of melon samples evaluated in Texas and Colima, Mexico respectively. Identifying the sources of, and factors that contribute to pathogenic bacteria contamination on produce, and especially melon

fruit is important in developing intervention measures to manage food borne outbreak risks.

2.2. Phytopathogens and Human Pathogens on Plants (HPOPs).

Plant surfaces are naturally infested and colonized by bacteria during plant growth and decay. Many saprophytic and pathogenic microorganisms can grow on healthy plants, and even reach large population sizes (Fokkema et al., 1983; Fokkema, 1991). Human pathogenic bacteria have the capability to survive the harsh environmental conditions during fresh produce processing. For example, the life cycle of *Salmonella* Typhimurium has been reported to comprise an infection and persistence phase within the host, and to survive in the external environment while transitioning to a new host (Klerks et al., 2007).

Emerging evidence suggests that *Salmonella enterica* and pathogenic *Escherichia coli*, which cause most fresh produce outbreaks, can adhere to and form biofilms on produce like cantaloupe (Annous et al., 2005), and parsley (Lapidot et al. 2006), leading to resistance to disinfection treatments (Scher et al., 2005). *Escherichia coli*, *Salmonella*, *Campylobacter*, *Listeria*, and *Shigella* have been found to form distinct biofilms on the surface of produce (Costerton et al., 1999; Lamas et al 2016; Trmcic, et al., 2018). *Salmonella* serotypes that form strong biofilms on produce, tend to attach better to the produce's surface compared with those serotypes that produce weak biofilms (Patel and Sharma, 2010; Saggers et al., 2008; Yaron and Rombling, 2014). Wild type *Salmonella* serotype, *S. enterica* Enteritidis colonizes alfalfa sprouts better than its mutant (Barak et al., 2007). Additionally, *Salmonella enterica* has been reported to easily attach to mangoes (Mathew et al., 2018). Vegetables and fruits

support growth of a wide range of microorganisms due to high levels of nutrients, moisture, and the near neutral pH (Watt and Merrill, 1950). Most fresh vegetables and fruits have water activity (aw) values that are close to the optimum growth level of most microorganisms (0.97 - 0.99, Montville and Mathews, 2001; Mossel et al.,1995).

Bacterial growth on plant surfaces has been reported to occur even when nutrient sources are absent. Plants are reported to be colonized by high numbers of bacteria, which can reach 10^5 to 10^7 cfu per g of leaf under favorable environmental conditions, such as when high relative humidity or free water are present (Hirano and Upper, 1989, 1993). Pathogenic bacteria can be introduced within fresh produce at different points during production in the field environment (Allende, 2008). Contamination may occur through seeds, soil, and irrigation water (Solomon, et al., 2002). Materon et al. (2007), reported the presence of *Salmonella* and *Listeria* on cantaloupe rinds, which was introduced through irrigation water. They also reported that *Salmonella* and *Listeria* could be recovered from cantaloupe rinds after the disinfection process, which could have been due to re-introduction of the bacteria during packing. Unhygienic workers at produce processing plants can be one way of cross contamination. Materon et al. (2007) also detected up to a load of 2.7 $\log_{10}$ cfu cm−2 of the bacteria on hands of harvest workers across 10 fields.

Survival and adaptation of human pathogens, like *Salmonella enterica* on produce outside their primary host may be due to the enabling role of other microbial communities present in the plant, and other mechanisms they employ to adopt (Gourabathini et al., 2008). Fungal activity on produce influences adaptation and

survival of human bacteria on produce. Fungi can cause damage, release nutrients, and change the pH thus creating a conducive environment for human bacteria to thrive (Riordan et al., 2000). Human pathogenic enterobacteria may therefore attach to and proliferate better on damaged tissue because of enzyme or nutrient leakage. Wounds have also been reported to act as sites of coinfection with other microorganisms that can alter microenvironment (Riordan et al., 2000). Plant pathogenic fungi that infect either leaves or fruits contribute to the proliferation of human enterobacteria (Riordan et al., 2000 and Simko et al., 2015). For example, downy mildew lesions caused by *Bremia lactucae* on lettuce, were reported to promote the growth of both *E. coli* O157: H7 and *Salmonella enterica* (Simko et al., 2015). Growth of *E. coli* O157:H7 population was reported in apples inoculated with *Glomerella cingulata*. This could be attributed to the rise in pH from 4.1 to 6.8 (Riordan et al., 2000). In a separate investigation, more *Salmonella* Thompson was detected in cilantro that exhibited fungal lesions compared to the uninfected control (Well and Butterfield, 1999).

Bacteria and fungi can form some symbiotic relationships. For example, *Staphylococcus aureus* can adhere to *Candida albicans* hyphae during mixed biofilm growth (Peters et al., 2012). The presence of plant pathogenic infections has been implicated in the survival and increase of co-inhabiting human pathogens. Presence of soft rot plant pathogen, *Erwinia carotovora* on fruit was associated with *Salmonella* (Deering et al., 2012; Wells and Butterfield, 1999). Wells and Butterfield (1999), also reported that co-inoculation of injured tomatoes, potatoes, and onions with fungal pathogens (*Geotrichum*, *Botrytis*, *Rhizopus*), increased incidences of *Salmonella*.

Human pathogens, including *Salmonella* may benefit from the presence of plant associated bacterial and fungal organisms on produce, which provide carbon and energy sources through the degradation of plant cell wall polymers. Fungal lesions in plants may create a microenvironment that is more favorable to the survival and replication of human pathogens, such as *Salmonella* spp. and *E. coli* O157:H7 (US Food and Drug Administration).

Salmonella enterica was also detected on cantaloupe fruits with water-soaked lesions of *Erwinia tracheiphila*, but not on uninfected fruit (Gautam et al., 2014). *Salmonella* Thompson was reported to have a lower epiphytic fitness on cilantro plants compared to the common plant bacteria, *Pantoea agglomerans* and *Pseudomonas chlororaphis* (Brandl and Mandrell, 2002). Likewise, *Salmonella enterica* persistence on tomato leaves was influenced by the presence of *Xanthomonas perforans* (Potnis et al., 2014) and survival of *Salmonella* strains on and in healthy, non-infected tomatoes was lower. Some *Salmonella* spp. are known to survive and persist in harsh environmental conditions outside their primary host, and may exist as contamination on plants, and then be ingested by animals. Furthermore, *Salmonella* Typhimurium can be recognized by plants, and even reported to activate the plant immune system (Meng et al., 2013). *Salmonella* Weltevreden can survive for long period of time on spinach leaves, it has been recovered for up to 21 days post inoculation (Arthurson et al., 2011).

Cantaloupe fruit is very susceptible to contamination by human pathogens (Richards and Beuchat, 2005a&b; Bowen et al., 2006; Gautam et al., 2012). However, how contamination occurs in cantaloupe is not well documented. Fortunately, few studies

have demonstrated strategies and behavior of some human bacteria on other produce plants. Survival of *Escherichia coli* O157:H7 during a storage period (at 4^{o}C) up to 15 days, is attributed to their ability to establish in lettuce's trichomes and stomata, which have been identified as a source of nutrition for the bacteria (Beuchat, 1999). Additionally, bacteria like *E. coli*, due to its surface charge and hydrophobicity, can strongly attach to the cantaloupe rind, making it difficult to remove during the wash process (Ukuku and Fett, 2002).

2.3. Internalization of human bacteria in plants/produce.

Produce's physiological conditions play a role on survival of human pathogenic bacteria. For example, some surveys on commercial produce found that there was an increased likelihood of finding *Salmonella* in association with tissue that is damaged by soft rot pathogens when compared with healthy tissue (Gourabathini et al., 2008). Human pathogens and plant pathogens on fresh vegetables and fruits can establish a close relationship (Gagliardi and Karns, 2002; Natvig et al., 2002; Ingham et al., 2005). The presence of *Cladosporium cladosporiodes* and *Penicillium expansum* in cantaloupe rind, increased the pH, and consequently, influenced internalization of *Salmonella* into inner tissues (Richards and Beuchat, 2005a, and Erickson, 2010). The pathogen *S.* Poona was also recovered on the wounded and inoculated rind surfaces of cantaloupe fruits stored at 20^{o}C. Richards and Beuchat (2005b), additionally reported that the presence of *C. cladosporiodes* and *P. expansum* facilitated the migration of *S.* Poona 3-4 cm below the cantaloupe rind wound surface into underlying mesocarp tissues. Both *Cladosporium cladosporoides*, and *Penicillium expansum* were recovered from the inoculated rind and underlying tissues throughout storage at 20^{o}C

for 10 days. Likewise, Potnis et al. (2015), found that the population of *Salmonella enterica* on tomato leaves pre-inoculated with plant pathogenic fungi, *Xanthomonas* species (*X. Euvesicatoria, X. gardneri, X. perforans*) increased after 8 days of inoculation.

2.4. *Fusarium* species Infection.

The genus *Fusarium* is one of the most studied fungal genera because of its importance as a plant pathogen, a mycotoxin producer (*F. solani, F. oxysporum, F. graminearum, F. moniliforme, F. sambutinum, F. culmorum,* and *F. equiseti*), and as a human pathogen (Seremi and Okhovvat, 2006). *Fusarium* spp. are widely reported to infect most economically important crop plants from around the world (Mahovic et al., 2004). They can exist as either saprophytes or pathogens on plants, animals, and humans.

Most *Fusarium* species are reported to be opportunistic pathogens on both plants and animals (Espinel-Ingroff et al., 2016; Tortorano et al., 2014). *Fusarium oxysporum, Fusarium solani,* and *Fusarium* giberrella *fujikuroi* complex, are the three most reported common species of *Fusarium* causing infections on plants (Tortorano et al., 2014). They are associated with wide range of plants in their natural habitats (Mahovic et al., 2004; Burgess, 1981; Burgess et al., 1994, 1989, & 1992; Nelson et al., 1990). Several *Fusarium* spp. cause postharvest rotting on a wide range of vegetables crops. *Fusarium* fruit rot on melon causes both pre-harvest and post-harvest losses. It has been reported as one of the most common causes of post-harvest fruit rots on vegetables; cucurbits, and tomatoes are frequently affected (Nuru et al., 2009; Mohammad and Zitter, 2009; McGovern, 1994; Fletcher, 1994). Fruit infection

occurs in the field before or during harvest, symptoms may also develop in storage. The infection of softer tissues such as melon fruit develops quickly and is characterized by pink soft tissues (Ignjatov et al., 2015), lesions that extend into the center of the fruit, which become water-soaked, and have white, yellow or pinkish mycelium (Champaco et al., 1993).

Fusarium species, which can be saprophytic or opportunistic pathogens, have also been isolated from soils in various climatic conditions (tropical, Mediterranean, arid; Backhouse and Burgess, 1995 & 2002).

Melon fruit are susceptible to *Fusarium* infections from the time of fruit set through storage (Carter, 1979). Common *Fusarium* spp., for example: *F. proliferatum*, *F. semitectum*, *F. verticillioides*, and *F. subglutinans* have been associated with fruit rots of cucurbits (Izzati et al., 2011, and Kenny, 2010). Outbreaks of pumpkin fruit rot have been reported in the USA (Elmer, 1996; Elmer et al., 2007; Castroagudin et al., 2009). *Fusarium* spp. infection on fruits also produce mycotoxins and make fruit inedible (Mule et al., 1997). Mycotoxins called trichothecenes are produced by several *Fusarium* species (*F. equiseti*, *F. graminearum*, *F. moniliforme*, and *F. sporotrichioides*) (Mule et al., 1997& 2004). *Fusarium* species infection of cereal crops like wheat are prone to production of these mycotoxins (Adler et al., 1995). *Fusarium fujikuroi* and *F. oxysporum* have been reported to produce fumonisins and bikaverin, whereas, *F. graminearum* and *F. pseudograminearum* are known to produce fusavielin and zearalenone as their secondary metabolites (O'Donnell et al., 2013). Additionally, *Fusaria* as opportunistic human pathogen, have been reported to cause *Fusarium* keratitis outbreaks in immunocompromised persons (Chang et al.,

2006 & 2013). Fusaric acids are produced by most of the *Fusarium* spp. in infected food products (Brown et al., 2012).

Melon fruit are susceptible to *Fusarium* infections because they are prone to cracking due to fluctuations in moisture and temperature in the field. Pathogenic *Fusarium* can infest these small cracks or infect at the soil-melon interface. Under the above conditions, infected melon fruit develops small lesions, surrounded with a green margin. Also, as the lesions mature, larger cracks develop along the fruit, allowing the *Fusarium* to enter. Under high humidity, white to pink fungal growth may also be observed near the crack or when the infected fruit is cut open (University of Kentucky Extension, 2010). The ability of these saprophytes and opportunistic pathogens to infest is made easier by the present of natural cracks and netting common in most melon cultivars (Fernandez-Trujillo, 2007).

Fusarium spp. have been widely reported to cause post-harvest rots of cantaloupes and to be highly pathogenic (Wade and Morris, 1982). However, some *Fusarium* spp., like *F. solani*, which may be saprophytic on muskmelon's roots and stems, can also be extremely pathogenic on fruits (Champaco et al., 1993). *Fusarium solani* infection on melon fruit may be facilitated by natural cracks or netting, physical injury from insects and wind-blown sand (Champaco et al., 1993). *Fusarium proliferatum* has been isolated from melon production fields in Northern-Western Mexico (Rivas-Garcia et al., 2018). Rivas-Garcia et al. (2018), molecularly identified all the *Fusarium* isolates collected from infected melons as *F. proliferatum*. Rivas-Garcia et al. (2018), were the first to report *Fusarium proliferatum* infection on melon fruit in that region of Mexico.

Both *Fusarium* and *Salmonella* are endemic in the cucurbits' production fields of the Eastern Shore Maryland, USA (Egel and Martyn, 2007, Angelo et al., 2015). Currently, there is limited information on interactions between *Fusarium* spp. that cause fruit rot in melon fruits and human pathogenic bacteria such as *Salmonella* in/on melon fruits.

Chapter 3: *Fusarium* spp. of *Cucumis melo* in the Mid-Atlantic Region of the U.S.

Abstract

Melons (*Cucumis melo*) are hosts to many fruit rots including those caused by *Fusarium* spp. However, which *Fusarium* spp. cause cucurbit fruit rots in the mid-Atlantic region of the U.S, especially in the states of Maryland and Delaware, has not been documented. Our objective was to identify *Fusarium* spp. causing fruit rot on melon within the region. A survey was conducted in five fields in Maryland and two in Delaware during the months of July and August, in 2016 and 2017. One hundred and forty-three (71in 2016, and 72 in 2017), putative *Fusarium* isolates were obtained from the two hundred and fifty fruits collected. To carry out pathogenicity tests, isolates were inoculated on healthy honeydew melon. Honeydew melon fruit were inoculated in five sites (3-center, stem scar, blossom end). The lesions sizes (width and depth, mm) formed by the isolates were measured and compared to an isolate *Fusarium oxysporum* f.sp. *niveum* (control which infects the roots and stems of watermelon but not cantaloupe plants; $p \leq 0.05$). Following the pathogenicity screen, selected isolates (67), exhibiting significant larger lesion sizes at least one location on the fruit were selected for phylogenetic identification. To carry out the phylogeny identification, PCR was conducted using primers that targeted the translation elongation alpha factors (TEF1&2) and the second largest sub-unit region of RNA polymerase II (RPB2). According to *Fusarium*-ID online database, 56 isolates could be molecularly identified as *Fusarium* spp. (*F. fujikuroi*-20, *F. proliferatum*-18, *F. oxysporum*-15, *F. graminearium*-2, *F. verticilloides*-1). Our pathogenicity tests indicated that most of the *Fusarium* isolates we collected were not pathogenic to

melon fruit. Therefore, we can conclude that saprophytic and opportunistic pathogenic *Fusarium* species are present in rotten melons in the field.

Keywords: Melon, Cultivars, *Fusarium* fruit rots, Pathogenicity, *Fusarium* spp., Delmarva.

Introduction

Fusarium species are one of the most common pathogens that cause post-harvest fruit rots on vegetable fruit (Saseetharan et al., 2014; Nuru et al., 2009; Fletcher, 1994). In fruits, *Fusarium* rot is characterized by its superficial white mycelium and sporodochia masses resulting in lesions on the surface (Fletcher, 1994 & Burgess et al., 1994) that often extended into the center of the fruit. Infected fruit surfaces may become water soaked and covered by white, yellow, or pinkish mycelium (Champaco et al., 1993). *Fusarium* rot of cucurbits was first identified in squash and described in detail in 1932 from South Africa. It is common in many countries around the world, and most cucurbits are susceptible, including melons (Doidge and Kresfelder, 1932). Melon fruit become infected by *Fusarium* especially if they are bruised or wounded, or in contact with the soil. Fruit rot symptoms can vary considerably depending on moisture levels, but most fruit rots begin small, and progress to large sunken lesions that may extend into the flesh (Champaco et al., 1993).

Fusarium infections on pumpkins can lead to up to 60% yield loss (Elmer, 1996). Elmer (1996), identified *F. Gibberella acuminatum, F. equiseti* (Corda) Saccador, and *F. graminearum* species as the main cause of type 1 dry, and hard rot of pumpkins.

Through other available literature, *F. graminearum, F. equiseti, F. avenaceum,* and *F.*

solani has been identified as common *Fusarium* spp. causing post-harvest rots of cucurbits. Even though *F. solani* is identified as a weak pathogen to nonpathogenic species on melon seedlings, it can cause extensive fruit rots (Champaco et al., 1993). Additionally, *F. culmorum*, *F. fujikuroi*, *F. oxysporum*, *F. scirpi*, and *F. semitectum*, are the five other common *Fusarium* spp. reported to cause fruit rot of melon in the US (Babadoost and Zitter, 2009 & Elmer, 1996). The pathogen *F. solani* f. sp. *cucurbitae* race 1 has been determined to cause a root, seedling, and fruit rot, whereas race 2 causes only fruit rot in cucurbit crops (Booth and Waterston, 1964).

Few evaluations have been carried out on *Fusarium* spp. causing fruit rot on melon, and other cucurbit fruits in the US (Elmer, 1996; Babadoost; 2009; Carter, 1979, 1981, and Waraitch and Nandpuri, 1975). Even though *Fusarium* fruit rot is one of the most important disease of muskmelon in Maryland (Maryland Extension Bulletin, 2003), *Fusarium* spp. evaluation on cucurbits in the Delmarva region of the US is minimal.

Previous researchers evaluated the *Fusarium* spp. causing wilt on watermelon and muskmelon in region (Zhou and Everts, 2003; Dutky et al., 1986), but not fruit rot. Therefore, to identify the *Fusarium* spp. present that cause fruit rot on melon, and to provide information to the producers in Maryland and Delaware, a survey was carried out in both states during summer season of 2016 and 2017.

Materials and Methods

3.1. Sample Collection.

A survey of *Fusarium* fruit rot of melon was conducted during the months of July and

August 2016 and repeated in the same months during 2017, at three locations in Maryland and two in Delaware. The locations in Maryland were: The University of Maryland's Lower Eastern Shore Research and Education Center, Salisbury (LESREC)- two fields, A and B, where field A was an organic production field and field B was farmed with conventional practices; the University of Maryland's WYE Research and Education Center in Queenstown (WYEREC), and a grower's field in Baltimore County. In addition, two fields were surveyed in Laurel, Delaware, in 2016. During 2017, the locations were: LESREC-A, and B, WYEREC, University of Maryland Central Maryland Research and Education Center, Upper Marlboro facility (CMREC), and the two fields in Laurel, Delaware. Fruits were collected during the second half of crop harvest. Melon cultivars sampled included: LESREC-A- 'Sivan' (Charentais type); LESREC- B: 'Arava' (Galia type), 'Athena' (Cantaloupe type), 'Dulce nectar' (honeydew type), 'Jaune de Canaris' (Canary type), 'Sivan' (Charentais type); WYEREC: 'Edens Gem' and 'Athena' (Cantaloupe type, 'Snow Mass' (honeydew type), 'Escorial' and 'Sivan'(Charantais type), 'Spanish Sun' (Canary type) ; Laurel, Delaware: 'Ariel' (Cantaloupe type) and 'Athena' (Cantaloupe type). Diseased fruits had one or more of the following symptoms: sunken lesion, mycelial growth, or pink or white area on the surface excluding obvious sunscald (Appendix 7i). Symptomatic whole melon fruit were transported in coolers (4^{o}C) to the lab for processing.

3.2. Isolation of *Fusarium* spp.

Infected fruits were stored in a walk-in cooler (4^{o}C) until processing. To prepare samples for isolation, fruit were lightly brushed and washed with sterile deionized

water to remove dirt and any other foreign particles on the fruit rind. The washed melon fruits were then blotted dry with paper towels and left to air dry for 5 mins. Twenty 0.5 cm sections of fruit, which included the edge of the lesion area, were excised and surface disinfested in a 1.12% sodium hypochlorite (NaOCl) solution for 3 mins. After 3 minutes, the sections were transferred into petri dishes containing sterile deionized water for an additional 3 mins, repeated once, to wash off traces of NaOCl. Five sections of the diseased fruit were placed on a quarter strength Potato Dextrose Agar (QPDA) and incubated at room temperature under constant fluorescence light. The plates were examined every 2 days for mycelium growth and sub-cultured when growth was visible (Appendix 7.ii). Sub-cultures were incubated at room temperature for 7 days.

Monoconidial cultures were obtained by rinsing spores off from the mycelium on the QPDA plates into a tube containing 0.1% water agar. Micro and macro spores were counted using a Hemocytometer (Fisher scientific) and serially diluted to 10 spores per ml. The spore dilution was plated unto water agar (10% Bacto Agar) for 24 hr. Plates were observed under a light microscope and a single germinated spore was identified and transferred onto slant tubes of QPDA and incubated at room temperature for 7 days. Thereafter, 4 mL of 15% of Glycerol solution was dispensed into slant tubes, slightly shaken for mycelia to release spores, and 2 ml pipetted into sterile cryogenic tubes and stored in the -80^{o}C until use.

3.3. Pathogenicity test of *Fusarium* isolates; inoculum preparation.

To prepare *Fusarium* inoculum, isolates were removed from storage and placed in 100 mL Potato Dextrose Broth (PDB) medium in a 250-mL Erlenmeyer flask (Fisher

Scientific) and incubated with constant shaking (150 RPM) at 25^{o}C for seven days. After the seven days, mycelium was strained through a 4-layered sterile cheese cloth. The conidial number was determined using Hemocytometer under a light microscope. The culture was then serially diluted to obtain a spore density of 1x 106 mL^{-1}.

A total of one hundred and forty-three isolates, a control (*Fusarium oxysporum* f.sp. *niveum*: F063-2), and the water control were in pathogenicity tests. The location label and number of isolates used during the pathogenicity test were as follows: from Baltimore County (B-18), University of Maryland WYE Research and Education Center (WYEREC-9), University of Maryland Lower Eastern Shore Research and Education Center organic field (LESREC-A-14), University of Maryland Lower Eastern Shore Research and Education Center conventional field (LESREC-B-15), and farmer's field in Laurel, Delaware (V-14), in 2016.

In 2017, isolates tested were from the University of Maryland Central Research and Education Center, Upper Marlboro (CMREC-15), WYEREC (22), LESREC-A (13), UM-LESREC-B (13), and Farmer's field in Laurel, Delaware (V-8). Ripe honeydew-type melon (cultivar unknown) purchased from a local retail store in Salisbury, MD, were surfaced sterilized using 1.12% NaOCl. Five wounds, 7mm in diameter and depth were made on surface of each fruit with ethanol-flamed cork borer. Wounds were located at the stem scar end (one), three locations around the circumference/center, and one at blossom end. At each wound location, the plug of fruit was removed and 20 µl (1 x 10^{6} spores mL^{-1}) of fungal inoculum was placed in the exposed flesh. Plugs were replaced to cover the holes after inoculation, and a clear tape used to hold them in place. A single fruit was inoculated with one isolate and

constituted one replicate. There were two one-fruit replicates for each isolate for the first test and three replicates for the second test (Appendix 7iii). Inoculated fruit were kept in an incubator with a 12-h light/dark cycle, and the temperature maintained at 22^{o}C for 10 days, at which time, the means depth and maximum width were recorded. Isolation was made from lesions on fruit, grown on PDA plates and examined to confirm that they were caused by *Fusarium* spp.

Analysis was carried on lesion circumference width, depth, stem scar width, depth, and blossom width, depth, (Champaco, et al., 1993). Lesion width and depth data were subjected to analysis of variance (ANOVA) and means separated using mix model (JMP; Elmer, 1996). Isolates that caused lesions greater than F-063-2 (α=0.05) at one or more locations on the fruit were selected for molecular identification. In addition, some isolates that caused larger lesions than F-063-2, but not significantly, so (α=0.2) were chosen to include more geographical diversity of isolates.

3.4. Identification of *Fusarium* Isolates.

Morphological Identification. During 2016 survey, to identify the isolates morphologically, monoconidial isolates were grown on carnation leaf agar (CLA) at 25^{o}C for 10 days for phenotypic identification according to *Fusarium* manual Leslie and Summerell (2006) and Siddique et al. (2010). Phenotypic characteristics were determined under a light microscope, the cultures were examined for shapes and sizes of the following features: macroconidia and microconidia, number of septa and shapes of the apical and basal cells of the macroconidia, conidiogenous cells, growth rate, and presence of chlamydospore, microconidia chains and sporodochia, and presence of microspores.

3.5. Molecular Identification of *Fusarium* Isolates.

Monoconidial isolates were identified to the species level using molecular techniques. Briefly, mycelia of individual cultures were grown and harvested from the PDA plates after 7 days of incubation. Genomic DNA was extracted using the DNA extraction buffer (EB) (1mM KCl, 100mM Tris-HCl, and 10mM EDTA, adjusted pH to 8.0; 0.5 mL per sample) and Isopropanol (100%) (Chi et al., 2009). Mycelia (20-40 mm2) and conidia were carefully scrapped off the media surface using a sterile inoculating loop and placed into a sterile 2 ml tube with EB and ground using micro pestle (Fisher scientific, Raleigh, NC) until it appeared fine. The fine mycelia sample was then centrifuged at 5000 rpm for 10 min, and the supernatant containing DNA was decanted into a 1.5-mL tube containing 0.3 mL Isopropanol, and the tubes were inverted 5-times followed by centrifugation at 12000 rpm for 10 min. The supernatant was then discarded and pellet containing DNA preserved. Tubes with pellets were kept opened in a sterile hood with constant airflow for one hour. The DNA was re-suspended in 100 µl rehydration solution (Promega Corporation, Durham, NC; Chi et al., 2009). The purified DNA was stored at -20^{o}C until
further analysis.

3.6. PCR Amplifications.

Fusarium species were identified based on their translation elongation factors 1α; Tef15′ ATGGGTAAGGAGGGACAAGAC-3′ and Tef2 5′GGAAGTACCAGTGATCATGTT-3′ (O'Donnell et al., 1998), and RNA polymerase II second largest subunit (RPB2) (5′-GGGGWGAYCAGAAGAAGGC-3′(O'Donnell et al., 2013) and on RNA polymerase II second largest subunit (RPB2-

RP1; 5′GGNGTCATGCARATCATNGC-3′; LeBlanc et al., 2015), PCR products were sequenced.

The PCR amplification was carried out in 25µl reaction volumes using 9.5µl deionized dH_20; 12.5µl of premix Master mix PCR buffer (Go Tag, ThermoFisher scientific.), 1.0µl of Taq DNA polymerase, 1.0µl each of 2.5pmoles/µl primers translation elongating factor alpha and RPB2 (Concentration: 1/10), and 1.0 µl of the target DNA (Concentration: about 20 ng/µl). The PCR mixture was then amplified on a Eppendorf Master Cycler equipped with heated lid and subjected to an initial denaturing at 94^{o}C for 1 min (1 cycle) followed by 35 cycles of 30 sec at 94^{o}C, an annealing temperature at 55^{o}C for 30 sec, extension at 72^{o}C for 1 min, and a final extension at 72^{o}C (1 cycle), and cooling at 4^{o}C until end.

The amplicons were separated by gel electrophoresis using 1% agarose minigels with a 100 bp molecular marker (Fisher Scientific). Electrophoresis was performed in a 1 x TBE (0.045 M Tris-borate and 1 mM ETA, pH 8.2) containing Nucleic Acid Gel Stain (5 µl/100 mL) at 70 V for 1 h. After one hour, the gels were visualized under UV-light and photographed using PhotoDoc-It ® Imaging System, Bench top UV Trans illuminator (Appendix 7).

3.7. DNA Sequencing, Alignment, and Phylogenetic Analysis.

Template DNA (50 µl) for sequencing was prepared and purified, following manufacturer's instructions (QIAquick ®, QIAGEN, USA). The purified products (30 µl) were sent to Macrogen Service Center, Rockville, Maryland for sequencing. The sequences of TEF or RPB2 and 2 regions of the tested isolates were obtained and edited to generate a consensus sequence from the forward and reverse sequence runs

in the amplicon. Out of sixty-seven strains sequenced, only fifty-six could be identified as *Fusarium* spp. (Tables 1& 2). Sequences of PCR fragments were aligned using BLASTn algorithm to the sequences of reference strains belonging to individual *Fusarium* species, deposited in the GenBank Database in both *Fusarium* ID online and NCBI online data bases. They were assigned to the reference species, of which both sequence coverages and nucleotide identities matched the query (Table 1); (www.ncbi.nlm.nih.gov/) and *Fusarium* ID (http://isolate.*fusarium*db.org/blast.php).

3.8. Data Analysis.

Isolate's lesion sizes were evaluated using analysis of variance (ANOVA), and the means comparison using Fishers LSD and the LSD at $p \leq 0.05$. All data analysis was performed using JMP mixed model.

Results and Discussion.

4.1. Morphological analysis.

From about two-hundred and fifty melon fruit that were suspected to be infested with *Fusarium* and collected in Maryland and Delaware during 2016 and 2017, only one hundred and forty-three gave reddish, purple, or pink pigmentation on PDA with strong indications as being *Fusarium* isolates in preliminary observations. Those isolates were selected for further identification.

Preliminary morphological determination was that most isolates were *F. solani* (29.58%) (Appendix 1-6). However, when the isolates were molecularly identified, the sequencing results revealed the isolates collected in 2016 and 2017, belonged to

only five *Fusarium* spp., and none was *F. solani* as originally thought. Because morphological determinations did not coincide with the molecular identification, only molecular identification was conducted in 2017.

4.2. Macroscopic Characteristics.

The presumptive identification for each isolate from characteristics observed on CLA is given in appendix 1-6. During this identification procedure, generally, the culture grew within two days on CLA; the mycelia were dense, white, and turned either purple, pink, or even some yellow pigmentation. Some isolates showed several shades of red to brown when observed from the bottom of the plate (Appendix 6). Morphological species identification is regarded to be difficult and needs a lot of expertise to differentiate the closely related *Fusarium* species (Herron et al., 2015). Morphological characteristics among different but closely related species of *Fusarium* cannot be easily recognized and they may look more similar than different (Geiser, 2004; Aoki and O'Donnell, 1999; Nireriberg and O'Donnell, 1998). Therefore, our findings are based only on molecular identification of the *Fusarium* spp.

4.3. Isolation and Pathogenicity Test.

Of the total two hundred and fifty melon samples collected, only one hundred and forty-three pure cultures were isolated and identified as presumptive *Fusarium*.

4.4. Molecular Identification.

Based on *Fusarium*-ID online database, only 56 isolates could be molecularly identified as *Fusarium* spp. (*F. fujikuroi*-20, *F. proliferatum*-18, *F. oxysporum*-15, *F. verticillioides*-1, *F. graminearum*-2).

During 2016, from Baltimore County, *F. fujikuroi* (7) and *F. proliferatum* (4) were molecularly identified (Table 1). All the isolates collected and identified from the UMWYREC, during 2016 were *F. fujikuroi* (4), whereas during 2017, the isolates were identified as *F. fujikuroi* (1) and *F. proliferatum* (4; Table 2). In 2016, isolates collected from LESREC-B were: *F. proliferatum* (1), *F. fujikuroi* (4), and *F. oxysporum* (2; Table 3). In 2017, from the same location, isolates identified were: *F. fujikuroi* (1), *F. proliferatum* (1), *F. verticillioides* (1), *and F. oxysporum* (3; Table 4). Isolates collected from LESREC-A in 2016 were identified as *F. oxysporum* (1) and *F. proliferatum* (1; Table 3). In Delaware, isolates collected in 2016, were: *F. proliferatum* (5), *F. oxysporum* (2), and *F. graminearum* (1). In 2017 isolates from this location, were: *F. fujikuroi* (2), *F. oxysporum* (1), *F. proliferatum* (1), *F. graminearum* (1; Table 5). The isolates collected from UPCMREC in 2017, were: *F. oxysporum* (3), *F. fujikuroi* (1), and *F. proliferatum* (1; Table 6). Overall, *F. fujikuroi* (20) and *F. proliferatum* (18) were the two most prevalent species identified and most *F. fujikuroi* (7) were collected from Baltimore in 2016 (Table 1). To the best of our knowledge, *F. proliferatum* has been rarely reported on melon fruit (Rivas-Garcia et al., 2018). However, it has been previously isolated and associated with produce like asparagus (Bargen et al., 2009).

In our study, *Fusarium proliferatum* was one of the most prevalent species found. It has been reported in most parts of the world as a moderately aggressive pathogen of multiple plant species, especially maize, and one the major mycotoxin (fumonisins) producer (Musser and Plattner, 1997; Stepien et al., 2011; Ross et al., 1990). The pathogen can also survive as an endophyte-like organism, without visible disease symptoms in the host (Logrieco, 1995).

In this study, *Fusarium fujikuroi* was identified as a predominant species. In some literature, *Fusarium fujikuroi* complex (FFSC), is reported as one of the larger groups within the genus *Fusarium* and are found in various geographical locations of the world (Jurjevic et al., 2005). With emerging technology and rise in more advanced molecular analysis techniques used in identifying *Fusarium* species, there is likelihood of recognizing more species within the *F. fujikuroi* complex (O'Donnell et al., 2015).

We also identified the species *Fusarium oxysporum* during this study, which has been reported and well-studied in cucurbits (Vakalounakis, D. J., 1996) and as a non pathogenic soil-borne fungus (Lievens et al., 2007).

Fusarium graminearum has been widely reported to cause scab in most cereal crops, where it is extremely destructive, and a producer of the harmful mycotoxin deoxynivalenol (Jansen et al., 2005). *Fusarium graminearum* has been categorized as an epidemic disease in North America (Ward et al., 2008). *Fusarium* verticillioides is commonly associated with Fumonisins productions in cereal crops especially maize and maize-based products however, it has been rarely reported in cucurbits (Proctor et al., 2006).

4.5. Repeat Pathogenicity Test.

After the phylogenetic identification, representative isolates (24), were selected for a confirmation pathogenicity test to determine the isolates' pathogenicity and separate those that were pathogenic from saprophytes. The isolates were inoculated on healthy honeydew melon, as previously described in this study (Elmer, 1996). Inoculation of *Fusarium* species on honeydew melon was effective in reproducing lesions of *F. fujikuroi*, *F. proliferatum*, *F. oxysporum*, *F. graminearum*, and *F. verticillioides* (Table 7). However, most of these selected isolates did not produce significantly different lesions compared to the *F. oxysporum* f. sp. *niveum* (control; Table 7). Therefore, we can conclude that, the five *Fusarium* spp. (*F. proliferatum. F. oxysporum, F. fujikuroi, F. graminearum, F. verticillioides*), obtained from melons, and identified from Maryland and Delaware region may have been saprophytes or opportunistic pathogens on melon (Tables 1-7). Fernandez-Trujillo et al. (2007), found that, the melon fruit with decay and damage in the storage, had lower percentage of *Fusarium* spp. infections. When they were found, they were located on fruit skin and were frequently associated with Alternaria rot.

Conclusion.

Most of *Fusarium* species isolates obtained and identified in our study were saprophytes, or opportunistic pathogens. *Fusarium fujikuroi* and *F. proliferatum* were the two most common species isolated from both the states of Maryland and Delaware. However, previously these species, have rarely been reported to infect melon fruit. They have been widely isolated and associated with cereal crops and asparagus (Stepien et al., 2015).

Fusarium fujikuroi (35.71%) and *F. proliferatum* (32.14%) were the two most common species isolated from melons in our study. *Fusarium* spp. collected from the LESREC-B and Laurel; Delaware had the most diverse population compared to other locations. Isolates collected from LESREC-B in 2017, comprised four species: *F. proliferatum*, *F. fujikuroi*, *F. oxysporum*, and *F. verticillioides*. Those collected from Laurel, Delaware comprised of three species (*F. proliferatum*, *F. fujikuroi*, *F. graminearum*). The knowledge about the occurrence of *Fusarium* pathogens on various cucurbit fruit especially melon fruit in Delmarva region of the US is still limited.

Chapter 4: Interactions between *Salmonella enterica* Newport, *Fusarium* spp. and Melon Cultivars.

Abstract

Melons are perishable fruit, affected by several post-harvest plant pathogens as well as human pathogens, posing a risk to food safety. This study evaluated the relationship between four *Fusarium* spp. of melon and the foodborne pathogen *Salmonella enterica* Newport. In four repeated trials, melon rind discs from 'Arava', 'Athena', 'Dulce Nectar', 'Jaune de Canaries' and 'Sivan' fruit grown in the field (field 1 and 2) and in high tunnels (high tunnel 1 and 2) were inoculated separately with locally-isolated *Fusarium* isolates. These were either *F. oxysporum, F. fujikuroi,* and *F. armeniacum* or *F. proliferatum*, with no *Fusarium* infection serving as a control. *Salmonella Newport* was inoculated onto melon discs four days post-*Fusarium* infection and recovered 24 hours later. Melon cultivar impacted the retrieval of *S.* Newport. In all four replicated experiments, one or more of the netted varieties 'Arava', 'Athena' and 'Sivan' yielded higher *S.* Newport counts that one or both smooth-rind melons 'Jaune de Canaries' and 'Dulce Nectar' ($p<0.05$). *Fusarium* infection did not have a significant impact on *Salmonella* retrieval. The average *S.* Newport count recovered was 5.0 log CFU mL-1 for both infected and uninfected melons. However, in field 2 melons, *S.* Newport counts recovered from *F. fujikuroi*-infected melons were higher than all other treatments (8.6 log CFU mL^{-1}; $p<0.001$), due to high levels of *Salmonella* recovered from 'Jaune de Canaries' compared to other experiments. The minimal *Fusarium* influence observed could in part be attributed to the lack of pathogenicity of the *Fusarium* strains used. The food safety

risk of melon did not appear to be enhanced by post-harvest colonization with local *Fusarium* spp. However, melons with netted rinds appeared to favor *Salmonella* colonization compared to smooth melons. Choice of melon cultivar may be an important consideration in reducing *Salmonella* colonization risk in areas where *Salmonella* may be endemic in the environment.

Key words: Melon food safety, Melon Cultivars, *Fusarium* Fruit Rot, *Fusarium* spp. *Salmonella enterica* Newport, Human Pathogen-Plant Pathogen Interaction, Human Pathogen-Plant Interactions.

Introduction.

5.1. Melon Consumption and *Salmonella* Contamination.

The growing consumption of fresh fruit and vegetables in the U.S. over the past few decades have coincided with increasing reports of foodborne illnesses associated with fresh produce (Callejon et al., 2015). During the past 20 years in the U.S., *Salmonella* has been one of the most frequent pathogens responsible for foodborne illness outbreaks (CDC, 2015). Some of these outbreaks have been associated with cucurbit produce, and many involved melons (Angelo et al., 2015; CDC, 2015; CDC, 2012, CDC, 2011; CDC, 2008; CDC, 2002; Lynch et al., 2009).

5.2. *Salmonella enterica* on/in Plants.

Salmonella enterica can adhere to, persist and even multiply on plant leaves and fruit (Barak et al., 2005, 2007, 2008 & 2011; Barak and Liang, 2008; Golden et al., 1993; Hirano et al., 1982). *Salmonella* internalize in cucurbits (Golden et al., 1993). Vegetables and fruit support growth of a wide range of microorganisms due to

available nutrients and moisture, and water activity (aw; 0.97 - 0.99) values that are close to the optimum level of most microorganisms (Montville and Matthews, 2001). Also, near neutral pH in produce enhance microbe's survival (Webster and Craig, 1976). Additionally, sites of damaged plant tissue may exhibit enhanced bacterial activity due to available nutrients on produce surface (Wells and Butterfield, 1999). Human bacteria can form biofilms on produce to adapt to survive adverse environments (Annous et al., 2005). *Salmonella* and *Listeria monocytogenes* can survive and multiply on cucumbers (Blostein, 1993; Angelo et al., 2015), and may be favored by netting on melon (Blostein, 1993). Melon development characterized by netting formation may support attachment of human bacteria (Keren Keiserman et al., 2004).

Plant pathogens, which cause degradation of plant cell wall polymers, release nutrients which support survival and growth of human pathogens in plants. *Salmonella* are known to be less competitive and have lower survival ability on plant surfaces than resident microbiota and may benefit from presence of plant pathogens (Richards and Beuchat, 2005a & b, Riordan et al., 2000). Surveys carried out to evaluate commercial produce quality found out that there was an increased likelihood of finding *Salmonella* in association with tissue damaged by soft-rot pathogens, when compared with healthy tissue (Meng et al., 2013). Injury and wounds present on produce can also act as sites of co-infection between plant and human pathogens, and can further alter the microenvironment, such that plant pathogens can contribute to the proliferation of human enterobacteria (Riordan et al., 2000, Simko et al., 2015). Presence of downy mildew and *Bremia lactucae* in combination with high humidity

on lettuce, improved growth and survival of *Escherichia coli* O157: H7 and *Salmonella enterica* (Simko et al., 2015).

Apples infected by *Glomeralla cingulata* had higher pH, which supported significantly more growth of *E. coli* 0157:H7 compared to uninfected control (Riordan et al., 2000). A study conducted on supermarket produce to evaluate the impact of *Erwinia carotovora*, which causes soft rot on plants, on *Salmonella* persistence and colonization, revealed that *Salmonella* incidence was more common when *E. carotovora* was present (Mez-lopez, 2013). Richards and Beuchat (2005), evaluated the impact of plant pathogens on wounded and intact cantaloupe rinds and reported that three of the pathogens, *Cladosporium cladosporioides*, *Geotrichum candidum*, and *Penicillium expansum* increased the pH and supported growth of *S.* Poona. However, *Epicoccum nigrum* and *Alternaria alternata* neither impacted the pH nor growth of *S.* Poona.

5.3. *Fusarium* spp. and Melon

Melon fruit is highly perishable in part due to melon susceptibility to post-harvest diseases such as *Fusarium*. *Fusarium* infection on cucurbit fruit results in mycelial growth that penetrates plant tissue, migrating into the center of the fruit and leading to water-soaked areas (Bachi et al., 2004). Melon infection may occur in the field before or during harvest, symptoms may develop in storage and result in both pre-harvest and post-harvest losses (Fernandez-Trujillo et al., 2007). Since infection often proceeds and manifests after purchase by consumers, melons with less severe infection are frequently handled and may even be consumed. Both *Fusarium* and *Salmonella* are indigenous in the agricultural environment of the Maryland Eastern

Shore, a region known for cucurbit production (Angelo et al., 2015; Everts et al., 2012). Our current study evaluated the impact of four *Fusarium* spp. (*F. oxysporum, F. fujikuroi, F. armeniacum,* and *F. proliferatum*), infestation of various melon cultivars (Arava, Athena, Dulce nectar, 'Jaune de Canaries' and Sivan), on *Salmonella enterica* Newport colonization and survival.

Materials and Methods

6.1. Melon Samples Production.

All experiments were carried out on five different cultivars of melon grown at the University of Maryland Lower Eastern Shore Research and Education Center (LESREC), Salisbury, MD facility, either in the field in summer 2016 or in a high tunnel in spring summer 2017; or in a high tunnel at the University of Maryland Wye Research and Education Center, Queenstown, MD (WYEREC) in fall 2016. Melon seeds were purchased from Park Seed Co. (Cokesbury, SC) and Eden Brothers (Arden, NC), in 2016 and 2017.

Five melon cultivars represented different melon types: 'Arava' (*C. melo* var. *reticulatus*, Galia), 'Athena' (var. *reticulatus*, muskmelon), 'Dulce Nectar' (var. *inodorus*, honeydew), '*Jaune de Canaries*' (var. *inodorus*, Canary) and 'Sivan' (var. *cantalupensis*, Charentais). Seeds were first grown in the greenhouse for approximate 22 days at UM-LESREC, 'hardened off' outside the greenhouse for four days, then transplanted to the field or the high tunnel. While the seedlings were being 'hardened off', they were treated with Admire insecticide (ADMIRE® PRO Systemic Protectant, Bayer Crop Science LP, NC; 0.58mL/Liter of water at 29.6mL/A), to

prevent insect pest damage. Melon seedlings were transplanted in the field using a water wheel trans-planter, and in the high tunnel by hand. Melon seedlings were spaced 61 cm apart within and rows spaced 183 cm apart. To lengthen the harvest period, melon seedlings were planted 3 weeks apart for 2 experiments in 2016. Melon cultivars were grown in a completely randomized block design (CRB), arranged in four replicates for a total of twenty plots (3.0 m x 1.8 m), both in the field and the high tunnel. Melons were grown on raised bed/rows covered with 1.25-mil black plastic over a single line of 8-in. emitter spaced drip tape in a one-pass operation in the field whereas in the high tunnel, raised beds were covered with a landscape fabric mat and irrigated with drip lines along each bed.

Fertigation was done using 1.1 kg/567.8 L of water of N.P.K (20.20.20). Field and bed management was carried out both mechanically and by hand. Melon diseases and pests were managed conventionally. Bravo Weather Stik® (chlorothalonil 720 SC;1.5 lbs Active ingredient (a.i.)/acre), Quintec® (0.098 Quinoxyfen lbs a.i./acre) and Procure® 50WS (8 oz a.i./acre) fungicides (Syngenta, Wilmington, DE), Fontelis® (DuPontTM (Penthiopyrad; 16 fl oz/acre) were used for management of melon foliar diseases. Fruit were harvested at the ¾ slip stage and kept under refrigeration in a walk-in cooler (4^{o}C) before processing.

Survival and colonization of *S.* Newport on melon fruit was evaluated for the five melon cultivars infected separately with *Fusarium* spp. (*F. armeniacum*-F2015007, *F. oxysporum*-F2015002, *F. fujikuroi*-F2015003, and *F. proliferatum*-F2016V016A). Fruit infected with the three *Fusarium* spp., but not *S.* Newport were evaluated as controls.

6.2. Microbial Material *Fusarium* spp.

Isolates for these experiments were obtained from a field survey that was carried out on muskmelon grown in an organic field at UM-LESREC, during the summer 2015 and in Delaware at a farmer's field during summer 2016. Melons with visible white or pinkish mycelia were collected, kept in a cooler and transported to the lab for processing. Isolations were made within 24 h of collection and a total of eight monoconidial *Fusarium* isolates were obtained. These were later molecularly identified based on their translocation elongation alpha factors (1&2) region and RNA polymerase II gene (RPB2) primers (O'Donnell et al., 2013; LeBlanc, et al., 2015; Table 3). From the eight total *Fusarium* spp. isolates, four species were identified (*F. armeniacum, F. oxysporum, F. fujikuroi,* and *F. proliferatum*; Table 8). An isolate identified as *F. proliferatum,* isolated in 2016 from a production field in Laurel, Delaware, was chosen to replace *F. armeniacum* during the fourth experiment replication (Table 9). The strain of *S.* Newport used in this study was isolated from an irrigation pond in Virginia and was identical to an outbreak strain traced back to that same farm (Green et al., 2008). The isolate was adapted for 80 µg/ml rifampicin resistance. We have compared growth of this isolate under various conditions to other *S.* Newport isolates in our collection and note no differences in growth rate either in culture or on produce surfaces.

6.3. Inoculation Experiments.

The experiments were conducted as a factorial design with five cultivar treatments and eight inoculation treatments for a total of forty experimental units. The inoculation treatments were one of four *Fusarium* spp. with *S.* Newport. Controls

received no *Fusarium* inoculum (water only). Another control with no *Fusarium* or *Salmonella* inoculum was also conducted to ensure no background *Salmonella* was present on melons. The treatments were replicated six times in the first, third and fourth experiment replications, and three times in the second experiment. A biscuit cutter was used to cut melon rind discs (surface area=158.5 cm^2). Eight melon rind discs cut from an individual melon were distributed uniformly to each of the eight treatments except when the fruit were too small, and two fruit were used to obtain the required number of discs.

6.4. *Salmonella* Newport Inoculum Preparation.

The *S*. Newport strain was revived from frozen stock by growing at 37^{o}C in tryptic soy agar (TSA; Fisher Scientific, Fair Lawn, NJ) supplemented with rifampicin (50 µg $mL^{-1)}$ (Sigma Aldrich, USA). Isolated *S*. Newport colonies were then picked and transferred into a 25 mL tube containing TSB with rifampicin (TSB+rif) and incubated with continuous shaking at 35°C for 16-18 h. *S*. Newport was surface plated on TSA+rif supplemented with rifampicin (50 µg mL-1) and incubated at 37^{o}C for 24 h. Colonies from this culture were used to make a suspension of *S*. Newport, suspended in sterile 0.1% Peptone Water (PW), to an OD600 0.5 read on a spectrophotometer (United Products & Instruments, Inc. Model 1100Rs, Unico), equivalent to ~10^9 cfu mL-1 and diluted to ~10^4 cfu mL-1in 0.1% PW for melon rind disc inoculation. The culture was then vortexed spread plated onto TSB+rif and incubated overnight for confirmation of the initial number of cells present.

6.5. *Fusarium* spp. Inoculum Preparation.

Fusarium spp. isolates were preserved in 15% glycerol (Fisher scientific) at -80^{o}C. To prepare the isolates for melon inoculation, frozen isolates were streaked onto fresh Potato Dextrose Agar (PDA, Fisher scientific) and incubated at 25^{o}C for 10 days. To produce inoculum, conidial suspensions were prepared by scrapping a piece of sporulating mycelium using an inoculating loop and inserting into a sterile 10 mL test tube containing 0.1% PDA. The suspension was vortexed to allow mycelia to release conidia. Conidia were harvested by filtering through four-layered cheese cloth and rinsed with sterilized deionized water (dDI H_2O) into a sterile beaker. The conidial concentration (spore mL^{-1}) was determined by using Hemocytometer (Fisher scientific-Hausser Scientific Partnership 1475). Concentrations were adjusted to ca. 106 spores mL-1 by preparing serial dilutions in sterile deionized water (dDI H_2O) containing 0.1% PDA.

6.6. Sample Processing, *Fusarium* spp. and *Salmonella* Newport, and Inoculation and Enumeration of *Salmonella* Newport.

Melon fruit were washed with dDI H2O and a soft brush to remove dirt and any foreign material on the rind surface, transferred to a plastic container with 1.12% Chlorine (NaOCl) for five minutes for surface sterilization. After five minutes, melons were transferred into another plastic container with dDI H2O and kept for another five minutes to rinse off traces of NaOCl. Thereafter, melon fruit were removed and placed on a sterile countertop, blotted dried with a paper towel, and left to air dry. A total of 6-10 melon rind discs from each melon fruit were then cut using a sterile biscuit cutter. Rind discs were placed in a sterile tray in preparation for

inoculation. Discs from each fruit were equally distributed among the eight treatments tested during four experiment replications as follows: *F. oxysporum*-F2015002 for experiment runs 1-4, *F. fujikuroi*-F2015003 for experiment runs 1-4, *F. armeanicum*-F2015007 for experiment runs 1-3, *F. proliferatum*-F2016V016A for experiment run 4 (Table 9), and their controls, no *Fusarium* with *S.* Newport control, and no *Fusarium*, no *S.* Newport control, with a minimum of six discs (replicates) per treatment. The second experiment replication was an exception when only three replicates were used. Five circles (0.5 cm diameter) were drawn with a marker on each disc equally distributed across the rind disc surface. Melon discs were separately placed in labeled whirl Pak bags (Fisher Scientific) for inoculation and kept under BSL-2 conditions. Melon discs were inoculated with a 100 µl (20 µl in each circle), pre-prepared *Fusarium* spp. inoculum (10^6 spore mL^{-1}) and water as controls accordingly. Samples were then incubated at 25^{o}C under 12 h fluorescent light and 12 h darkness for four days.

After four days of incubation, each *Fusarium* inoculation site received with 10^4 cfu mL^{-1} *S.* Newport (20 µl per circle- a total of 100 µl total per disc) inoculum and was incubated at 25^{o}C for an additional 24 h. After 24 h following *S.* Newport inoculation, samples were removed from the incubator and washed in 1:1 w/v 0.1% PW in the same bag. Samples were then shaken, hand-rubbed, and massaged for 2 mins and a 10-ml aliquot of sample was pipetted into a 10 mL sterile test tube. Serial dilutions were plated onto TSA+rif and cycloheximide (50µg/ml; Sigma-Aldrich, USA). Inoculated plates were incubated at 37^{o}C for 24 h and colonies were counted for cfu mL^{-1} calculations.

6.7. Data Analysis.

Analysis of Variance (ANOVA) was conducted to evaluate the differences in survival of *S.* Newport in the presence of *Fusarium* spp.; *F. oxysporum, F. fujikuroi* and *F. armeniacum* or *F. proliferatum* on: 'Arava', 'Athena', 'Dulce Nectar', 'Jaune', and 'Sivan' melon cultivars. A mixed model was constructed with response being bacterial counts and model effects being *Fusarium* treatment, cultivar and the interaction between *Fusarium* treatment and cultivar. The numbers of CFUs counted were transformed to $\log_{10}$ cfu mL^{-1} prior to analysis. An alpha level of 0.05 was used for determining statistical significance. Tukey-Kramer HSD was used to compare the mean differences within an effect when the overall effect was significant. All calculations were conducted using JMP Pro 11.1.1. (SAS Institute).

Results

7.1. Impact of *Fusarium* Species on *Salmonella enterica* Newport Survival on Melon Rinds.

Generally, melon rinds infected with *Fusarium* spp. supported the survival of *S.* Newport. During the first (field-grown melon) and third (high tunnel-grown melon) experimental runs, *Fusarium* spp. (*F. armeniacum*, *F. fujikuroi*, and *F. oxysporum*), did not impact a significant number of *S.* Newport recovered compared to the water control (p=0.84 and p=0.47, respectively; Table 9; Fig. 1a; Fig. 1c). However, in the second experiment (melons grown in the field); *S.* Newport counts recovered from *F. oxysporum* treated melon were significantly lower (5.66 log cfu mL^{-1}) compared to *F. fujikuroi* (8.64 log cfu mL^{-1}; p<0.0001; Table 9). Counts from the *F. armeniacum* and

control treatments were equivalent and were also significantly different from the two other *Fusarium* treatments (p=0.01; Fig. 1b). By contrast, in the fourth experiment conducted on melons grown in the high tunnel, lower counts of *S.* Newport were recovered from melons infected with *F. fujikuroi* (3.60 log CFU mL^{-1}) compared to the no *Fusarium* control (4.74 log CFU mL^{-1}; p<0.05; Fig. 1d). *Salmonella* Newport was not recovered from melons infected with *Fusarium* or water but not *S.* Newport (data not shown).

7.2. Impact of Melon Cultivars on *Salmonella enterica* Newport Survival and Growth on Melon.

Melon cultivar (type) consistently impacted the survival and growth of *S.* Newport. Growth of *Salmonella* Newport was detected in several melon samples, and on all samples of 'Athena', Arava' and 'Sivan' (i.e. retrieved counts where higher than inoculum). Overall, 'Athena' and 'Arava' (mean counts of 5.88 and 5.77 log cfu mL^{-1}, respectively) supported significantly higher counts of *S.* Newport and colonized better compared to 'Jaune' and 'Dulce Nectar' (mean counts of 4.08 and 3.91 log cfu mL^{-1}, respectively; p<0.001; Table 9). The cultivar effect varied slightly among experiments, but this trend was consistent. 'Arava' supported significantly higher counts than 'Dulce Nectar' in the first experiment conducted on melons grown in the field (5.78 and 3.69 log cfu mL^{-1}, respectively; p<0.01; Table 9; Fig. 1a). In the first high tunnel melon-grown experiment, *S.* Newport counts from 'Athena' were significantly higher than from all other cultivars, with a ~3 log cfu mL-1 difference from 'Jaune' and 'Dulce Nectar' (p<0.001; Table 4; Fig. 1a). The second-high tunnel grown-melon experiment yielded similar results, with counts from 'Arava' (highest at

5.54 log cfu mL^{-1}), 'Sivan' and 'Athena' significantly exceeding (by 1.4-2.5 logs) counts from 'Jaune' and 'Dulce Nectar' (lowest at 3.03 log CFU mL^{-1}; $p<0.001$; Table 9; Fig. 1c). The second experiment carried out on melon grown in the field, and late summer season, gave the most varying results. Melons of the 'Dulce Nectar' cultivar grown in the field yielded counts of *S.* Newport comparable to 'Arava', 'Sivan' and 'Athena', but 'Jaune' still yielded only 3.80 log cfu mL^{-1} and was significantly different from all other cultivars ($p<0.001$; Table 9; Fig. 1b).

7.3. Interaction between *Fusarium* spp. and *Salmonella* Newport.

No significant interaction was detected between the two factors being tested (*Fusarium* treatment x cultivar) in the first field experiment and the two high tunnel experiments. A significant interaction however was seen in the second field experiment ($p<0.001$; Table 9; Fig.1b). This was attributed to the cultivar 'Jaune' which, when infected with *F. oxysporum* and *F. armeniacum*, as well as the control, was significantly different than other cultivars ($p<0.001$), but not when infected with *F. fujikuroi* (Fig. 1b). In the latter case, *S.* Newport counts (7.85 log cfu mL^{-1}) were equivalent to other cultivars under any treatment. Similarly, an interaction was also seen with 'Sivan', whereby *Salmonella* counts from melons infected with *F. oxysporum* were significantly lower (3.92 log cfu mL^{-1}) than other 'Sivan' treatments and other cultivars, excluding 'Jaune' (Fig. 1b).

Discussion.

Overall, *Salmonella* Newport cells (log CFU mL^{-1}) recovered from the netted rind melon ('Arava', 'Athena' and 'Sivan') were significantly higher than those recovered

from the smooth rind melon cultivars (‘Dulce Nectar’ and ‘Jaune’). Our study demonstrated that *S.* Newport can grow in the presence of *Fusarium* spp. on melon fruit but did not provide strong evidence that presence of *Fusarium* spp. on melon affected growth of *S.* Newport compared to uninfected melon rind. In other studies, *Salmonella* Newport co-inoculated with a plant pathogenic bacterium, *Erwinia tracheiphila*, on cantaloupe fruit rind increased the number of *S.* Newport recovered compared to the controls (Gautan et al., 2012). Additionally, Richards and Beuchat (2005) revealed that *S.* Poona was recovered on the wounded and inoculated rind surfaces of cantaloupe fruits stored at 20^{o}C. Richards and Beuchat (2005), further reported that the presence of *Cladosporium cladosporioides* and *Penicillium expansum* also facilitated the migration of *S.* Poona 3-4 cm below the cantaloupe rind wound surface into underlying mesocarp tissues. Both *C. cladosporoides*, and *P. expansum* were recovered from the inoculated rind and underlying tissues throughout storage at 20^{o}C for 10 days (Richards and Beuchat, 2005a). Potnis et al. (2014) found that activation of effector-triggered immunity by avirulent *Xanthomonas perforans* resulted in a dramatic reduction in S. enterica populations. The *S. enterica* populations persisted at ∼10 times higher levels in leaves co-inoculated with virulent *X. perforans* than in those where S. enterica was applied alone. In contrast, *S. enterica* populations were ∼5 times smaller in leaves coinoculated with avirulent *X. perforans* than in leaves inoculated with *S. enterica* alone. Co-inoculation with virulent *X. perforans* increased *S. enterica* aggregate formation (Potnis et al., 2014). Aruscavage et al. (2006) reported that *Salmonella* Thompson was found in fungal lesions on

cilantro plants, and further revealed enterobacterial pathogens may be more likely to be found in the presence of fungal pathogens than on non-infected produce.

Our findings show that cultivar was a significant factor in *Salmonella* colonization efficiency. One major difference among cultivars is the topography of the rind. Netted rind surfaces of 'Arava', 'Athena', and 'Sivan' supported better growth of *S.* Newport cells compared to smooth surface rinds of both 'Dulce Nectar' and 'Jaune'. This confirms previous research that topographical features of fruit influence the attachment of *S.* Newport (Simko et al., 2015, Wells and Butterfield, 1999). Simko et al. (2015), found that when a cocktail of *Salmonella*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* were inoculated onto melon rind surfaces, the *Salmonella* strains exhibited the highest surface hydrophobicity. Fissures in the cantaloupe netting have also been reported to provide attachment sites for cells of *Salmonella* and aid in survival when fruit were in contact with aqueous sanitizers (Annous et al., 2005 & 2004). Bacterial attachment to melon rind surfaces has been reported to be influenced by the cell surface charge and hydrophobicity and to some extent by the presence of flagella and fimbriae as well as extracellular polysaccharides (Fletcher and Loeb, 1979, and Fernandes et al., 2014). Moreover, *E. coli*, *Salmonella* and *Listeria* have also been reported to adhere more effectively to peach fruit than plum surface which is attributed to the increased surface area of the peach due to the presence of trichomes (Collignon and Korsten, 2010). The surface type or size of produce have been linked to enhance attachment of human pathogenic bacteria. For example, Patel and Sharma (2010) found that the affinity of *Salmonella* serovar attachment to lettuce is two to three-fold higher than to cabbage, which may help

explain the greater association of lettuce to foodborne outbreaks, whereas outbreaks associated with cabbage have been rare (Patel and Sharma, 2010).

Topography and architecture of the surface of the plant are also important factors in bacterial adhesion (Patel and Sharma, 2010). In our study, topography seemed related to greater *S*. Newport recovery from netted melons ('Arava', 'Athena', 'Sivan') than from smooth surface melon ('Dulce Nectar', 'Jaune'). It is likely that the netted surface of melon can aid microbe-surface adherence, increasing survival and may protect against the harsh environment on the plant tissue.

Salmonella Newport can grow in the presence of three *Fusarium* spp. (*F. oxysporum*, *F. fujikuroi*, *F. armeniacum*, and *F. proliferatum*) on melon, and the *Fusarium* species present on melon may influence the survival and growth of *S*. Newport. Unexpectedly, *Fusarium* spp. did not significantly affect the growth of *S*. Newport compared to uninfected melon. However, similar results to our finding that *F. fujikuroi* supported higher number of *S*. Newport on riper 'Jaune' cultivar (full slip) have been previously reported on tomatoes (Marvasi et al., 2013), where significant differences were observed in the proliferation of *Salmonella* in tomatoes harvested at different maturity stages.

Marvasi et al. (2013), further revealed that final cells numbers of *Salmonella* were, on average 1 log higher in ripe tomatoes compared to the unripe tomatoes under the same conditions. Our finding that riper Jaune melon in the presence of *F. fujikuroi* supported higher number of *S*. Newport compared to other cultivars and *Fusarium* species could be attributed to production of cell wall-degrading enzymes like pectinases (Bateman and Basham, 1976 and Cleveland and Cotty, 1991). In many

immature fruits, the mechanisms limiting pathogen aggression are associated with either preformed antimicrobial substances or with phytoalexins, enzymes, or physical resistance structures (Jarvis, 1994).

When the fruit tissue discs representing cantaloupe fruit of varying ripeness were inoculated with *P. cucurbitae*, immature fruit (20 and 30 days old; Zhang et al., 1997) exhibited a much lower percentage of maceration compared with mature fruit (40 and 50 days old). This suggests that the immature fruit have biochemical or physical factors that restrict fungal growth or production of the cell wall-degrading enzymes by the fungus. These inhibiting factors may contribute to the fungal dormancy during the early stages of fruit development and become ineffective at fruit maturity (Zhang et al., 1997).

During this study, *Fusarium* spp. (*F. oxysporum, F. fujikuroi, F. armeniacum, F. proliferatum*) were identified and determined to be non-pathogenic on honeydew melon and to be less aggressive melon pathogens and could have attributed to their relationship with and influence on the survival of *Salmonella* Newport on melon. More studies should be conducted to evaluate adhesion factors of various plant pathogenic fungi causing rot on melon, and their interaction with *S.* Newport. Also, antagonistic activities of both *S.* Newport and *Fusarium* spp. against each other need to be evaluated. To broaden the fungal-bacterial knowledge on produce, the behavior of other human pathogenic bacteria should be tested on the presence of prevalent *Fusarium* spp. of melon. Apparently, *Salmonella* Newport colonization on melon was not enhanced by post-harvest infestation with local *Fusarium* spp. used during this study. Melon cultivar type, however, influenced survival of *Salmonella* may be an

effective strategy in reducing *Salmonella* colonization risk in areas where *Salmonella* may be endemic in the environment and the inherent risk is deemed higher. Assessing risk of a growing region should be part of a farm food safety plan, and production of smooth rind melons such as honeydew and Canary types over netted types should be considered as a mitigating strategy in higher risk areas.

Chapter 5: Internalization of *Salmonella enterica* Newport on Smooth Surface Melon (Honeydew- Type) and Netted Melon (Cantaloupe-Type), Alone or in the Presence of *Fusarium proliferatum*.

Abstract

The presence of plant pathogens on fruit can influence the survival of human pathogens. This study investigated the impact of *Fusarium proliferatum* on internalization and subsequent survival of the human pathogen, *Salmonella enterica* subsp. serovar Newport on two melon types; smooth (honeydew-type) and netted (cantaloupe- type). Melon fruit were inoculated with (1) either *F. proliferatum* alone followed by nonsimultaneous inoculation with *S.* Newport four days later (F0S4), or water followed by *S.* Newport four days later (H0S4), and (2) *F. proliferatum* plus *S.* Newport simultaneously (F0S0), or, *S.* Newport alone (H0S0). On days 5 and 10, melons were first subdivided into four transverse segments (rind/surface, exocarp, mesocarp, and endocarp/seed cavity) and processed separately. The experiment was performed in three experiment replications (n=20). Direct plate counts of *Salmonella* Newport were transformed to Log_{10} *S.* Newport per melon and analyzed using a mixed model with Tukey's HSD post-hoc test (JMP). *Salmonella* Newport internalization occurred in all treatments and both melon types, but variation in population levels were observed. In general, internalization of *Salmonella* occurred in both melon types all the way to the seed cavity. *Salmonell*a persisted for up to 10 days in the internal fruit tissues of melon. The incubation period (24 hrs, 5 or 10 days) was a major factor, *Salmonella* Newport gradually reduced over time post

inoculation. *S.* Newport retrieved at 24 hr. post inoculation was significantly higher than the ones retrieved after 5 and 10 days, regardless of inoculation treatment type. Also, *Fusarium proliferatum* did not impact internalization of *S.* Newport in either cantaloupe or honeydew type melons. However, a longer infection period of *Fusarium proliferatum* on honeydew type melon, impacted significantly higher counts of *S.* Newport compared to cantaloupe. In some experiments, it appeared that *F. proliferatum* was antagonistic to *S.* Newport. We could not conclude from this study that *S.* Newport survival on or in fruit was enhanced by the presence of *Fusarium proliferatum* infection. However, *Salmonella* did internalize and survive for up to 10 days in both cantaloupe and honeydew melon types.

Keywords: *Salmonella enterica* subsp. serovar Newport, *Fusarium* Fruit Rot, *Fusarium proliferatum*, Melon, Cultivars, Cantaloupe, Honeydew, Survival, Internalization.

Introduction

8.1. Microbes in Plants.

A wide range of microorganisms grow on fruit, which is due to high levels of nutrients on the fruit surface (Watt and Merrill, 1950). Human pathogens contaminate and become internalized in fruits, including commensal microbes, as well as pathogenic ones (Penteado et al., 2004). *Fusarium* species are one of the most important plant pathogenic fungal groups infecting economically important plant crops in the U.S. (Abu Bakar et al., 2013; Biles et al., 2000 & Champaco et al., 1993). Some of these *Fusarium* species exist and colonize plants as saprophytes, which may

become opportunistic pathogens (Pereyra and Dill-Macky, 2008). Human pathogenic bacteria like *Salmonella* and *Escherichia coli* O157:H7 have also been associated with plants, and reported to survive and internalize into plant tissues, including fruit (Deering et al., 2012; Burnett et al., 2000; Gautam et al., 2014, and JeongA et al., 2014).

8.2. Human Pathogenic Bacteria in the Plant Environment.

Human pathogenic bacteria are known to survive harsh environmental conditions, outside their primary host, especially during produce production, handling, and processing (Barak et al., 2008). The life cycle of *Salmonella* comprises an infection and persistence phase within the host, and survival, and persistence for a long period in the external environment while transitioning to a new host (Schikora, 2012; Cucak et al., 2018). *Salmonella enterica* has low epiphytic fitness on plants and it is reported to have lower survival compared other common epiphytes, such as *Pseudomonas syringae, Pseudomonas chlororaphis,* and *Pantoea agglomerans* (Potnis et al., 2014; Barak and Liang, 2008). However, survival of *Salmonella* strains on and in healthy and non-infected produce demonstrates that some *Salmonella* spp. can survive and persist in harsh environmental conditions (Cooley et al., 2003; Deering et al., 2012 & Meng et al., 2013 & Han and Micallef, 2016). The ability of human pathogenic bacteria to survive in the environment may be due in part to conducive conditions created by prevailing microbial communities (Wells and Batterfield, 1997; Richards and Beuchat, 2005b; Potnis et al., 2015). *Listeria monocytogenes* can internalize on avocado fruit (Chen et al., 2016). Tomatoes can support internalization of *Salmonella* (Barak et al., 2011; Turner et al., 2016). Furthermore, internalization of *Salmonella*

has been reported on baby spinach (Gomez-Lopez et al., 2013). Within tomato there is variation in ability to support survival, and tomato variety can influence internalization of *S.* Thompson (Xia et al., 2012).

There is some evidence that damaged plant tissue can support greater survival and persistence of human pathogens (Aruscavage et al., 2008; Riordan et al., 2000; Richards and Beuchat, 2005b). Human pathogenic *enterobacteria* may attach and proliferate better on damaged tissue due nutrient leakage (Potnis et al., 2015; Wells and Batterfield, 1997). Wounds also may act as sites of coinfection with other microorganisms that can alter microenvironment, favoring growth of human bacteria (Richards and Beuchat, 2005a). Richards and Beuchat (2005a), revealed that *Cladosporium cladosporioides* and *Penicillium expansum* on cantaloupe rinds enhanced migration of *S.* Poona into mesocarp tissues. Evidently, fluid leakages from plant tissue resulting from produce processing, may provide enough nutrients to support the growth of human pathogenic bacteria (Beuchat, 1999; Riordan et al., 2000; Aruscavage et al., 2006). Moreover, infection of produce by plant pathogenic fungi can create water-soaked lesions that can create conducive microenvironment for human bacteria (Riordan et al., 2000). Plant pathogenic fungi that infect either leaves or fruit contribute to the proliferation of human *enterobacteria* (Riordan et al., 2000 & Simko et al., 2015). For example, downy mildew lesions on lettuce caused by *Bremia lactucae,* was reported to promote growth of *E. coli* O157:H7 and *Salmonella enterica* lettuce (Simko et al., 2015). Additionally, apples with lesions caused by *Glomerella cingulata* promoted the growth of *E. coli* O157 and *E. coli* O157:H7

(Riordan et al., 2000). Cilantro plants with fungal lesions were associated with *S.* Thompson (Brandl and Mandrell, 2002).

8.3. Melon Fruit and Potential Human Pathogenic Bacteria Internalization.

Melon fruit is susceptible to contamination by human pathogens (Richards and Beuchat, 2005b), however, how contamination occurs in melon is not well documented. Muskmelon fruit usually develop on the soil surface during crop production, and at this period, a fruit is in contact with saprophytes, soilborne plant or human pathogens (Lopez-Velasco et al., 2012). Modern and powerful detection tools like scanning electron microscopy (SEM) has been used to show the movement and internalization of, and the common sites for microbial aggregation on produce (Deering et al., 2012). These sites can be the veins (Barak et al., 2008, 2011); and the cell wall junctions (Romantschuk et al., 1996). Microbial aggregation also facilitates production of enough polysaccharide material to protect microorganisms from dehydration (Romantschuk et al., 1996).

In other produce, plant leachate has been identified as a source of nutrition for the bacteria (Beuchat, 1999). The presence of bacteria can also affect fungal development and spore production, as for the plant-pathogenic oomycete *Phytophthora alni*, the saprophytic cheese-associated fungus *Penicillium roqueforti*, and several fungal symbionts (Adams et al., 2009; Chandelier et al., 2006). The presence of plant pathogenic infections has been implicated in the survival and increase of co-inhabiting human pathogens (Deering et al., 2012). Moreover, infected, and rotten fruits aid the survival and replication of *Salmonella* bacteria compared to uninfected healthy fruits (Wells and Butterfield, 1999). Human pathogens, including *Salmonella*

may benefit from the presence of plant associated bacterial and fungal organisms on produce (U.S. Food and Drug Administration, 1999). The potential internalization of human pathogens is a concern in food safety because these pathogens are less likely to be removed during the washing steps after harvest (Meneley and Stanghellini, 1974, Watt and Merrill, 1950 and Brandl and Mandrell, 2002).

8.4. *Fusarium* Fruit Rot of Melon.

Fusarium fruit rot caused by *Fusarium* spp. is common in honeydew melons, occurring most frequently on the stem end (Bruton and Duthie, 1996). Natural infection of melon fruit by *Fusarium* spp. appears to be related to net development where *Fusarium* inoculum is incorporated into the rind (Bruton and Duthie, 1996). Large numbers of conidia are produced on field-culled and unharvested melons (Bruton and Duthie, 1996).

We hypothesized that *Fusarium* infected melon rind tissue enhance *Salmonella* in two ways: first, the infected tissue can be readily colonized by *Salmonella* because of *Fusarium* mediated changes in microenvironment. Secondly, as the fungal infection progress, the fruit rind rot caused by *Fusarium* spp. can facilitate *S.* Newport penetration into inner melon tissues. Thirdly, this penetration may vary depending on rind type such as smooth versus netted melons, as *Fusarium* infection progresses differently on these melon types (Webster and Craig, 1976).

Materials and Methods

9.1. Bacterial Strains, Labeling, Storage and Inoculum Preparation.

One strain of *Salmonella enterica* Newport isolated from a tomato fruit (Green et al.,

2008) was used to inoculate melon fruit. The *Salmonella enterica* Newport strain was adapted to grow at 37°C in tryptic soy agar (TSA; Fisher Scientific, NC) supplemented with rifampicin (50 µg mL^{-1}). *Salmonella* was surface plated on TSA supplemented with rifampicin (50 µg mL^{-1}) and incubated at 37°C for 24 h and held at 4°C until used to prepare inoculum. Suspensions of *S*. Newport were made in sterile 0.1% Peptone water and the optical density of the suspensions measured at OD600 spectrophotometer (United Products & Instruments, Inc. Model 1100Rs, Unico) and adjusted by serially diluting in Peptone Water (0.1%) to 10^5 colony forming unit per mL (cfu mL^{-1}).

9.2. Procedures for Growing *Fusarium* spp., and Preparation of Inoculum.

Fusarium proliferatum (F2016V016A), isolated from melon fruit which were collected from a grower field in Delaware were used for inoculation. To prepare *Fusarium* spp. inoculum, frozen isolates were inoculated and cultured on 100 mL mineral salts media (Fisher Scientific) and incubated with constant shaking (150 RPM) at 25°C for five days. After the five days, the *Fusarium* spp. culture was strained through a 4-layered sterile cheese cloth. Conidia number were determined using Hemocytometer and counted under a light microscope, the concentration of spores was adjusted to 10^6 mL^{-1} for inoculation.

9.3. Experimental Design.

Two melon types (netted-cantaloupe and smooth-honeydew; cultivar unknown) were purchased from a local retail store in Salisbury, MD. To evaluate the impact of *Fusarium proliferatum* infection time on *Salmonella enterica* Newport survival and

internalization, each melon fruit type was divided into two groups, groups I and II melons. The inoculation and sampling schemes were as follows: group II melons were inoculated with either *Fusarium proliferatum* alone followed by non-simultaneous inoculation with *S.* Newport four days later, or water followed by *S.* Newport four days later, and group I melons, were inoculated with *Fusarium proliferatum* plus *S.* Newport simultaneously, or *S.* Newport alone. On days 5 and 10, melons were subdivided into four transverse segments (rind/surface, exocarp-4mm, mesocarp-6mm and endocarp/seed cavity-10mm) and processed separately.

9.4. Enumeration of *Salmonella enterica* Newport.

Core samples from inoculated fruit were transverse-cut into segments of rind and inner fruit pieces; rind/surface- 7mm depth, exocarp-10mm depth, mesocarp-5mm depth, and endocarp or seed cavity were excised during sampling 2 h post inoculation (HPI) of *Salmonella* and on days 5, and 10. The four-melon rind and inner segments were processed separately for *S.* Newport cells. To process, the transverse-cut melon pieces were separately cut with sterile scalpel, each time changing the scalpels, and placed into sterile whirl-pak bags (7oz., Fisher sci.) containing Peptone water (0.1%; 1:1 w/v; Fisher sci.) and hand massaged from the outside for 2 min followed by 1 min of vigorous hand shaking and homogenizing. The aliquots (10 ml) were collected in sterile glass tubes and serially diluted in Peptone water (0.1%) and plated onto TSA supplemented with rifampicin and cycloheximide (50μg mL^{-1}) for *S.* Newport counts.

9.5. Data Analysis.

The experiment was performed three times, each conducted independently, with a total of 20 fruit of either honeydew or cantaloupe type per replicate. The mean and standard of log base 10 transformed colony count of *Salmonella* were analyzed using analysis of variance procedures with Mixed model-JMP (SAS Institute, Cary, NC). A three-factor factorial model was utilized to compare melon type, *Fusarium proliferatum* vs no *F. proliferatum*, melon segments, and sampling time. Simple effects mean for sampling time given *Fusarium proliferatum* inoculation and melon type given sampling time were reported and analyzed with planned contrasts. Additionally, significant differences in *Fusarium proliferatum* infestation on the two different melon types as affected by sampling time (day 5 and 10), and distance from rind surface were determined using Tukey-Kramer tests. All statistical tests were considered significant at $p \leq 0.05$.

Results

10.1. Impact of *Fusarium proliferatum* Inoculated on Cantaloupe Type Melon on *Salmonella enterica* Newport Survival.

Salmonella enterica Newport was enumerated after 24 hrs, 5, or 10 days post inoculation. During days 5 and 10 *Salmonella* Newport was lower when inoculated alone (H0S0), without *F. proliferatum* was significantly lower than *S.* Newport inoculated four days later (H0S4), but not to simultaneous or nonsimultaneous *Fusarium proliferatum* plus S. Newport (F0S0 or F0S4) (Table 10 & Figures 2a and 2b; $p<0.01$, $p<0.03$), for day 5 and 10, respectively. In general, *Fusarium* did not

appear to have a positive impact on *Salmonella* survival. *Salmonella* Newport incubation time was a factor, however. Counts at 5 days post *Salmonella* inoculation (the simultaneous inoculations F0S0 and H0S0 treatments) tended to be lower than counts at 24 hpi *Salmonella* (nonsimultaneous inoculations F0S4 and H0S4). Any differences were generally not detected by day 10, but when they were, *Salmonella* levels were higher on nonsimultaneous inoculations (Table 10; Figures 2a and 2b).

10.2. Impact of *Fusarium proliferatum* on *S.* Newport Survival on Honeydew Type Melon.

Overall, *F. proliferatum* did not influence the survival of *S.* Newport, however as observed in cantaloupe melon type, at day 5, for the honeydew inoculations, *S.* Newport retrieved from melon inoculated with simultaneous *F. proliferatum* plus *S.* Newport (F0S0) and *S.* Newport alone (H0S0) were significantly lower than nonsimultaneous inoculations (H0S4 and F0S4; Fig 2a, $p<0.03$), but not at day 10 ($p<0.08$; Table 10).

10.3. Impact of *Fusarium proliferatum* on *S.* Newport Internalization on Cantaloupe and Honeydew Type Melon.

Overall, there were no significant differences among the four melon segments (surface, exocarp, mesocarp, and endocarp) at both days 5, and 10 for either cantaloupe or honeydew melon types, and similar trends was observed in all the four *Fusarium* inoculation treatments (data not shown). A similar trend between the two melon types and across all four inoculation treatments was observed where the

surface supported survival of lower number of *S.* Newport compared to inner sites of the melon (mesocarp and endocarp).

Internalization of *Salmonella* occurred in both melon types all the way to the seed cavity. *Salmonella* persisted for up to 10 days in the internal fruit tissues of melon. Incubation period (24 hrs vs Day 5 vs Day 10) was a major factor. *Salmonella* Newport gradually reduced over time post inoculation. *S.* Newport counts retrieved at day 5 was significantly higher than the ones retrieved at day 10, irrespective of inoculation treatment type. Also, *Fusarium proliferatum* did not impact internalization of *S.* Newport in both cantaloupe and honeydew type melons. However, longer infection period of *Fusarium proliferatum* on honeydew type melon increased counts of *S.* Newport compared to cantaloupe in one experimental repeat.

Discussion

We found that *S.* Newport can internalized in the presence or absence of *Fusarium proliferatum* and it occurred in both cantaloupe and honeydew types of melon. Other studies have reported that *Salmonella* spp. can internalize in plant tissues using natural openings (Dong et al., 2003; Gomez et al., 2013; Itoh et al., 1998; Kroupitski et al., 2009; Shaw et al., 2008 & 2011). *Salmonella* internalization was observed in all *Fusarium* treatments (H0S0, F0S0, H0S4, and F0S4). Incubation period of *F. proliferatum* on honeydew type melon impacted survival of *S.* Newport but not its internalization. Additionally, *F. proliferatum* enhanced *S.* Newport growth in some experimental replications, however, that trend was not consistent across all the three experimental replications. *Salmonella* persisted in all melon segments for 10 days,

however, their number gradually declined. The *Salmonella* decline was consistent in both melon types, also across the *Fusarium* treatments.

During some experimental replications, *Fusarium proliferatum* simultaneously inoculated with *S*. Newport, influenced the growth of *S*. Newport for 5 days but the same was not seen after 10 days. This phenomenon demonstrates that *F. proliferatum* infection on melon initially created a suitable microenvironment for *S*. Newport to thrive. However, the nutrients may decrease with time and *F. proliferatum* may have provided competition and decline of *S*. Newport at Day 10.

The different impacts of *F. proliferatum* on *S*. Newport survival and growth observed among the experiment replications, may have occurred in part because we purchased our melon samples from a retail store, and they may have had different ripening levels. The ripening level or storage time of melon may have affected the ability of *F. proliferatum* to infect. Our earlier findings on *Fusarium* and *Salmonella* interactions on various melon cultivars were that *Fusarium fujikuroi* significantly impacted *S*. Newport only when inoculated on riper 'Jaune'-canary melon type compared to other *Fusarium* species (Figure 1b). More ripe melon fruit may have weaker defense mechanism against plant pathogens compared to the freshly harvested melon. Also, more ripe melon may provide more readily available sugars on the rind for *S*. Newport survival compared to less ripe melon. Other factors that may have attributed to this inconsistent survival of *S*. Newport is due to the microbiota on melon. Our melon samples came from different production fields at different times and these two factors may had major impacts on types of microbes present on or in the melon.

Even though, generally *S.* Newport declined over time following the inoculation, there were some instances when simultaneous inoculation of *F. proliferatum* plus *S.* Newport treatments were observed to enhance counts of *S.* Newport on the surface at day 10 compared to the ones recovered during the Day 5. Possibly, there was more cell wall/rind degradation after 10 days of incubation compared to 5 days, and *S.* Newport may have accessed more nutrients then.

Bacterial reduction over time on produce was also reported by Simko et al. (2015) who co-inoculated downy mildew with either *Escherichia coli* O157 or *S.* Typhimurium on lettuce leaves. They reported that population sizes of *E. coli* O157 multiplied within 24 h post-inoculation however, reduction between 24 and 48 h was observed. Simko et al. (2015) suggested the possibility of leaves drying up between 24 and 48 post-inoculation altered multiplication of bacteria. Competition between bacteria and *Fusarium* has been evaluated by Dijksterhuis et al. (1999) who reported that soil bacterium, *Paenibacillus polymaxy* was antagonistic towards *Fusarium oxysporum* when grown on a liquid medium.

Fusarium proliferatum inoculated alone or together with *S.* Newport caused lesions on melon fruit. Gautam et al. (2014) also found similar results when they inoculated *S. enterica* together with plant pathogen *Erwinia tracheiphila*. Bacterial internalization on plant tissues has been associated with artificially induced lesions (Burnett et al., 2000).

Additionally, physical damage of plant tissue caused by plant pathogens can enhance human pathogen internalization (Wade and Beuchat, 2003). Chemotropic interaction has also been reported to promote *Salmonella* and *E. coli* attachment near the plant

stomata (Kroupistski et al., 2009). Ukuku and Fett (2002) showed that *Salmonella* had a stronger attachment to cantaloupe surface compared to *E. coli* and *Listeria* monocytogenes. The above study also demonstrated that both surface hydrophobicity and surface charge play major roles in bacterial attachment to cantaloupe surfaces.

Conclusion

Salmonella Newport internalization occurred in all treatments and both melon types, but variation in population levels were observed. *Fusarium proliferatum* infection period played a role in survival of *S.* Newport on honeydew melon type (smooth) but not on cantaloupe type melon (netted). In some experiments, it appeared that *F. proliferatum* was antagonistic to *S.* Newport. Therefore, we could not conclude in this study that *S.* Newport survival on or in fruit was enhanced by the presence of *Fusarium proliferatum* infestation. Future studies should focus on evaluating the microbiome of melon fruit before interactions of *Fusarium* and *Salmonella*. The small impact of *F. proliferatum* on *S.* Newport internalization could be due to its opportunistic pathogenicity on melon fruit (Table 6).

Physiological changes on melon fruit can be evaluated to assess the infection characteristics of *Fusarium* at initial and in advance stages of inoculation and when *Salmonella* is introduced. Additionally, defense response/mechanisms of melon fruit when *Salmonella* is introduced to the surface should be evaluated. Experiments can be replicated to evaluate impact of other prevalent fungal pathogens of melon on *Salmonella* colonization. Lastly, *Fusarium* impact can be evaluated on other common human pathogenic bacteria like *Escherichia coli* and *Listeria*.

Chapter 6: General Conclusion.

The *Fusarium* survey that we carried out in Maryland and Delaware, revealed that five species of *Fusarium* are common in melon infestation in the fields surveyed. *Fusarium fujikuroi* and *F. proliferatum* were the two most prevalent species. *Fusarium graminearum* and *F. verticillioides* were the least common *Fusarium* species identified. Additionally, the diversity of *Fusarium* species was consistent during production seasons of 2016 and 2017. Most of the *Fusarium* spp. collected and isolated from the five sampling locations in Maryland and Delaware were not pathogenic on honeydew type melon, which indicated they may be saprophytes or opportunistic pathogens. *Fusarium* species impacted survival of *Salmonella enterica* Newport differently.

Fusarium fujikuroi significantly supported higher number of *S.* Newport compared to *F. oxysporum* and *F. armeniacum* when inoculated on riper melon fruits. *Fusarium fujikuroi* presence significantly increased *S.* Newport survival on riper '*Jaune de Canaris*' melon compared to when the same melon cultivar was harvested at the three-quarter slip. '*Jaune de Canaris*' melon has smooth and tougher rinds compared to Arava, Athena, and Sivan melons. Riper '*Jaune de Canaris*' melon may have provided easy access for infection by *F. fujikuroi* because of softened rind therefore creating an easier pathway for *S.* Newport attachment and acquisition of nutrients for survival. Generally, *Salmonella* Newport was not impacted by infestation of *Fusarium* species. The presence of *F. proliferatum* on melon did not have significant influence on *S.* Newport internalization. The melon type (smooth vs netted) however, did not have a significant impact on *S.* Newport internalization to melon. *Salmonella*

Newport internalized was within 24 hrs post inoculation on both honeydew and cantaloupe types and could be detected all the way in the seed cavity. It was demonstrated in this study that *S*. Newport population although gradually declining, can persist for ten days post inoculation in all four melon segments (surface, exocarp, mesocarp, and endocarp).

Choice of melon cultivar type may be an effective strategy in reducing *Salmonella* colonization risk in areas where *Salmonella* may be common in the production fields. Assessing risk of a growing region should be part of a farm food safety plan, and production of smooth rind melons such as honeydew and Canary types over netted types should be considered as a way forward in reducing contaminations in higher risk areas.

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Table 1. *Fusarium* isolates' lesion sizes (mm) measured at five locations on melon fruit (3-center, stem scar, blossom end). Molecular identification of the selected *Fusarium* isolates collected from Baltimore, Maryland during the 2016 growing seasons.

		Center lesion (mm)		Stem lesion (mm)		Blossom end (mm)				Base Pairs	TEF/RPB2-*UFID% match
Isolate	Cultivar	Depth	Width	Depth	Width	Depth	Width	TEF1*Y-spp. ID	RPB2*Z-spp.ID		
F2016B019	Athena	21.83ab*T	21.33a	14.50abcd	17.00abc	23.5a	17.3a	*F. fujikuroi*	*F. fujikuroi*	550	96.1/99.8
F2016B018-1	Athena	22.28ab	21.06a	13.17abcd	16.50abc	20.3ab	16.5a	*F. proliferatum*	*F. proliferatum*	560	99.7/99
F2016B021-2	Athena	22.67ab	19.78ab	15.17abcd	15.83abc	19.3abc	19.2a	*F. proliferatum*	-	550	99.3/-
F2016B028	Athena	20.89ab	17.78ab	18.67ab	14.83abc	14.0abc	19.3a	*F. fujikuroi*	-	560	99.5/-
F063-2*X	NA	17.83abc	17.50abc	20a	13.50abc	14.0abc	12.5a	NA	NA	NA	NA
F2016B006	Athena	17.61abc	16.72abc	14.33abcd	13.83abc	13.33abc	15.7a	*F. proliferatum*	-	550	99.4/-
F2016B003-2	Athena	18.78ab	16.11abc	16.5ab	11.67bc	10.7bc	18.0a	*F. fujikuroi*	*F. fujikuroi*	570	99.4/99.7
F2016B024	Athena	17.78abc	16.00abc	12.3bcd	12.17abc	15.17abc	18.0a	*F. fujikuroi*	*F. fujikuroi*	550	99.7/100
F2016B021-1	Athena	20.94ab	15.22abc	13.17abcd	17.16abc	13.0abc	19.3a	*F. fujikuroi*	*F. fujikuroi*	530	100/99.5
F2016B027	Athena	16.39abc	15.11abc	12.0bcd	13.83abc	15.17abc	19.83a	*F. proliferatum*	-	560	99.5/-
F2016B013-2	Athena	15.17bc	14.72abc	12.67bcd	11.17bc	10.2bc	15.0a	-	-	560	-
H2O	NA	7.33c	7.17c	7.0d	7.00c	7.0c	7.0a	NA	NA	NA	NA

TABLES

Table 2. *Fusarium* isolates' lesion sizes measured on five melon locations (3-center, stem scar, blossom end), for pathogenicity test. Molecular identification of the selected *Fusarium* isolates collected from WYEREC, Maryland during the growing seasons, 2016 and 2017.

		Center lesion (mm)		Stem lesion (mm)		Blossom end lesion (mm)		TEF1[*Y]-spp. ID	RPB2[*Z]-spp.ID	BP	TEF/RPB2-FID% match
Isolate	**Cultivar**	**Depth**	**Width**	**Depth**	**Width**	**Depth**	**Width**				
F2016WYE003	Athena	27.0a***T**	29.0a	28.5a	33.0a	34.0a	40.0a	*F. fujikuroi*	*F. fujikuroi*	550	99.7/99.7
F2016WYE004	Spanish Sun	21.3b	26.2ab	25.0a	27.5b	30.0a	38.0a	*F. fujikuroi*	*F. fujikuroi*	550	99.8/99.7
F063-2[*x]	NA	20.7b	21.2b	13.0b	8.5bc	27.5a	NA	NA	NA	NA	NA
H2O	NA	7.0c	7.0c	7.0b	7.0c	7.0b	NA	NA	NA	NA	NA
F2017WYE025-2	Athena	28.3a	30.2abc	50.0a	56.0a	35.0ab	52.5a	*F. fujikuroi*	*F. fujikuroi*	550	99.8/99.7
F063-2[*x]	NA	24.7abc	27.7abcde	17.5bc	16.5cde	18.5cdefg	27.0cdef	NA	NA	NA	NA
F2017WYE012	Athena	20.3abcd	27.5abcde	17.5bc	17.5cde	36.5a	57.5a	*F. proliferatum*	*F. proliferatum*	550	98.9/99
F2017WYE004	Spanish Sun	23.0abcd	27.0abcde	20.0bc	35.0a	32.5abc	35.0bcde	*F. proliferatum*	*F. proliferatum*	550	100/99
F2017WYE007	Athena	19.7abcd	22.0abcde	17.0bc	12.0cde	31.5abcd	33.5cde	*F. proliferatum*	*F. proliferatum*	550	100/99
F2017WYE025-1	Athena	20.3abcd	19.2bcdef	18.5bc	16.0cde	36.0a	26.0cdefg	*F. proliferatum*	*F. proliferatum*	550	98.9/99
H20	NA	7.0e	7.0f	7.0c	7.0e	8.0g	7.8g	*NA*	*NA*	NA	NA

TABLES

Table 3. *Fusarium* isolates' lesion sizes measured on five melon locations (3-center, stem scar, blossom end), for pathogenicity test. Molecular identification of the selected *Fusarium* isolates collected from LESREC-A, Maryland during the growing seasons, 2016 and 2017.

LESREC-A 2016		Center lesion (mm)		Stem lesion (mm)		Blossom end (mm)		TEF1[*Y]-spp. ID	RPB2[*Z]-spp.ID	BP	TEF/RPB2-FID% match
Isolate	**Cultivar**	**Depth**	**Width**	**Depth**	**Width**	**Depth**	**Width**				
H2O	NA	7.0g[*T]	7.0d	7.0e	7.0d	7.0c	7.0c	NA	NA	NA	NA
F2016LA022	Sivan	7.0g	7.0d	7.5e	7.5c	7.0b	7.0c	*F. oxysporum*	-	540	98.4/-
F063-2[*X]	NA	18.5f	14.5cd	16.0cde	13.5cd	18.0bc	18.0bc	NA	NA	NA	
F2017LA001	Athena	23.7de	26.5b	12.0de	14.0cd	24.5abc	29.5ab	*F. oxysporum*	*F. armeniacum*	550	98.8/98.8
F2017LA003	Arava	28.2b	29.5b	22.0bcd	16.0cd	22.5abc	24.0abc	-	-	-	-
F2017LA008	Arava	32.5b	27.5b	7.0e	7.0d	40.0a	40.0a	*F. oxysporum*	*F. oxysporum*	550	100/99.5
F2017LA010	Sivan	33.0b	32.5ab	29.0b	28.5b	24.5abc	31.5ab	*F. fujikuroi*	*F. fujikuroi*	550	99.2/97.9
F2017LA011	Jaune	32.0b	26.7b	8.5e	11d	28.0ab	29.5ab	-	-	550	-
F2017LA012	Sivan	28.2b	28.5b	26.0bc	23.5bc	35.0ab	23.5abc	*F. oxysporum*	*F. oxysporum*	550	99.3/87.9

TABLES

Table 4. *Fusarium* isolates lesion sizes measured on five melon locations (3-center, stem scar, blossom end), for pathogenicity test. Molecular identification of the selected *Fusarium* isolates collected from LESREC-B, Maryland during the growing seasons, 2016 and 2017.

		Center lesion (mm)		**Stem lesion (mm)**		**Blossom end (mm)**		**TEF1[*Y]-spp. ID**	**RPB2[*Z]-spp.ID**	**Base Pairs**	TEF/RPB2-FID% match
Isolate	Cultivar	**Depth**	**Width**	**Depth**	**Width**	**Depth**	**Width**				
F2016LB017	Sivan	22.7b[*T]	33.0a	18.5cde	18.0abc	29.5ab	36.5ab	*F. fujikuroi*	*F. fujikuroi*	550	99.7/99.4
F2016LB025	Athena	19.3bc	30.8ab	18.0cde	16.5abc	25.0abc	24.5ab	*F. proliferatum*	*F. proliferatum*	550	100/99
F2016LB013	Arava	18.2bcd	12.5def	19.0cd	17.5abc	27.5ab	54.0a	*F. proliferatum*	NA	550	97.9/-
F2016LB015	Arava	16.8bc	16.7cde	17.5cde	29.0a	20.0abc	20.0c	*F. oxysporum*	*F. oxysporum*	550	99.3/99.5
F2016LB010	Sivan	7.0e	13.0def	7.5d	8.0c	23.5abc	23.5c	*F. fujikuroi*	*F. fujikuroi*	550	99.2/97.9
F2017LB003	Dulce	25.8a	27.8b	30.0bc	19.5abcd	30.0bc	30.0bc	*F. oxysporum*	*F. oxysporum*	550	99.5/99.5
F2017LB004	Sivan	23.8a	27.5b	34.5ab	30.5a	34.5ab	30.0bc	*F. oxysporum*	*F. oxysporum*	500	99.8/99.5
F2017LB002	Sivan	18.8bc	20.0bc	53.5a	19.5abcd	53.5a	53.5a	*F. oxysporum*	*F. oxysporum*	550	100/99.5
F2017LB005	Arava	18.0bcd	19.0bc	28.0bc	15.5bcd	28.0ab	27.5bc	*F. fujikuroi*	NA	550	99.6/-
F063-2[*X]	NA	17.8cd	15.8bcd	14.0cd	13.5bcd	14.0cd	12.5cd	NA	NA	NA	NA
H20	NA	7.2e	7.1e	7.0d	7.0d	7.0d	7.0d	NA	NA	NA	NA

TABLES

Table 5. *Fusarium* isolates lesion sizes measured on five melon locations (3-center, stem scar, blossom end), for pathogenicity test. Molecular identification of the selected *Fusarium* isolates collected from Laurel, Delaware during the growing seasons, 2016 and 2017.

		Center lesion (mm)		Stem lesion (mm)		Blossom end (mm)		TEF1[*Y]-spp. ID	RPB2[*Z]-spp.ID	BP	TEF/RPB2-FID% match
Isolate	**Cultivar**	**Depth**	**width**	**depth**	**width**	**Depth**	**width**				
F2016V008	Athena	26.3ab[*T]	26.3ab	7.0b	7.0c	7.0b	7.0b	*F. proliferatum*	*F. proliferatum*	550	98.9/100
F2016V010-2	Athena	26.5ab	25.3abc	8.5b	7.0c	53.5a	32.5ab	*F. oxysporum*	*F. oxysporum*	550	100/87.7
F2016V024-B	Athena	22.2abc	19.3abcde	11.0b	7.0c	32.5bcd	29.5ab	*F. proliferatum*	*F. proliferatum*	550	98.3/99
F2016V024-A	Athena	19.2bcde	16.5def	20.0ab	20.0a	34.0bcd	22.0abc	*F. armeniacum*	*F. oxysporum*	550	95.8/86.4
F2016V016A	Ariel	20.8bcd	16.3def	9.5b	8.0c	9.5b	15.0bc	*F. proliferatum*	*F. proliferatum*	540	99.67/99
F063-2[*X]	NA	18.7bcde	15.3defg	11.0b	7.0c	11.0b	16.5bc	-	-	-	-
F2016V011-2	Athena	17.2cde	11.8ef	26.0a	15.0ab	26.0a	32.5ab	*F. oxysporum*	*F. oxysporum*	550	99.7/99
F2016V011-2	Athena	17.2cde	11.8ef	26.0a	15.0ab	26.0a	32.5ab	*F. oxysporum*	*F. oxysporum*	550	99.7/99
H2O	NA	7.0f	7.0g	7.0b	7.0c	7.0b	7.0c	NA	NA	NA	NA
F2017V008-2	Ariel	25.7a	21.2a	27.5a	37.0a	14.0bc	21.5bc	*F. proliferatum*	*F. proliferatum*	530	98.9/86
F2017V005	Ariel	19.3ab	25.7a	19.5b	15.5bc	60.0a	67.0a	*F. fujikuroi*	F. *fujikuroi*	530	100/99.2
F2017V007	Athena	11.2bc	11.2bc	25.0ab	21.0b	23.5bc	19.0bc	-	-	550	-
H20	-	7.0cd	7.0cd	7.0c	7.0c	7.0c	7.0c	-	-	-	-
F2017V006	Ariel	1.2d	1.2d	7.0c	7.0c	11.0bc	13.5bc	*F. fujikuroi*	*F. graminearum*	530	86.4/100

TABLES

Table 6. *Fusarium* isolates' lesion sizes measured on five melon locations (3-center, stem scar, blossom end), for pathogenicity test. Molecular identification of the selected *Fusarium* isolates collected from CMREC, Maryland during the growing seasons, 2016 and 2017.

		Center lesion (mm)		Stem lesion (mm)		Blossom end (mm)					
Isolate	**Cultivar**	**Depth**	**Width**	**depth**	**Width**	**depth**	**width**	**TEF1[*Y]-spp. ID**	**RPB2[*Z]-spp.ID**	**BP**	**TEF/RPB2-FID% match**
F063-2[*X]	NA	18.67**cde**[*T]	15.33**de**	11.00bc	7.00c	19.50cd	16.50bc	Na	**NA**	NA	NA
H2O	NA	7.00**f**	7.00**e**	7.00c	7.00c	7.00d	7.00c	NA	NA	NA	NA
CMREC001	Athena	12.00**ef**	16.67**cde**	11.00bc	11.00c	25.00bc	23.00abc	NA	NA	NA	NA
CMREC002	Athena	25.83**bc**	31.83**a**	26.00a	25.00abc	32.50bc	27.50ab	*F. proliferatum*	*F. proliferatum*	550	98.9/99
CMREC003	Athena	27.50**ab**	29.33**ab**	20.00abc	20.00abc	35.00b	27.00ab	NA	NA	550	NA
CMREC005	Athena	19.50b**cde**	18.67**bcde**	15.00abc	15.50bc	25.00bc	26.00ab	NA	NA	NA	NA
CMREC006	Athena	17.50**de**	16.00**de**	22.50abc	35.00a	35.00b	40.00a	NA	NA	NA	NA
CMREC007	Athena	26.83**ab**	28.50**abc**	17.50abc	17.50abc	33.50b	25.00abc	*F. oxysporum*	*F. oxysporum*	550	99.8/99
CMREC008	Athena	25.17b**cd**	22.83**abcd**	25.00ab	32.50ab	55.00a	35.00ab	*F. oxysporum*	NA	540	100/-
CMREC009	Athena	20.83**bcd**	22.67**abcd**	25.00ab	22.50abc	22.50bc	29.50ab	NA	NA	NA	NA
CMREC10	Athena	20.67**bcd**	19.50**bcd**	20.00abc	25.00abc	27.50bc	25.00abc	NA	NA	NA	NA
CMREC011	Athena	34.33**a**	30.17**ab**	22.50ab	30.00ab	25.00bc	32.50ab	*F. oxysporum*	*F. oxysporum*	550	100/99.7
CMREC012	Athena	25.83**bc**	29.33**ab**	22.50ab	20.50abc	32.50bc	32.00ab	NA	NA	NA	NA

Notes: Tables 1-6. Notes: Base Pairs-Gel-Electrophoresis band sizes, **LESREC-LA**-University of Maryland Lower Eastern Shore Research and Education Center organic field, Salisbury, MD, **LESREC-LB**- University of Maryland Lower Eastern Shore Research and Education Center conventional field, Salisbury, MD, **WYEREC-UMD** -WYE Research and education center, **CMREC**- UMD- Upper Marlboro Research and Education Center. **Table 1. Notes**: Base Pairs-Gel-Electrophoresis band sizes, **LESREC-LA**-University of Maryland Lower Eastern Shore Research and Education Center organic field, Salisbury, MD, **LESREC-LB-** University of Maryland Lower Eastern Shore Research and Education Center conventional field, Salisbury, MD, **WYEREC-UMD** -WYE Research and education center, **CMREC**- UMD- Upper Marlboro Research and Education Center.[*X]Control isolate (F063-2), used. [*T] Same letters within the same column are not statistically different ($p \leq 0.05$), according to Student's t-test least significant differences (Mixed-model-JMP).

[*Y]TEF1-Translation Elongation Factor alphar-1, [*Z]RPB2- RNA polymerase II second largest subunit. [*U] FID%- Online *Fusarium* ID database percentage match.

TABLES

Table 7. *Fusarium* isolates' lesion sizes measured on five melon locations (3-center, stem scar, blossom end). Molecular identification of the selected *Fusarium* isolates for the pathogenicity test, after sequencing and identification (TEF1& RPB2). These isolates represented isolates collected from LESREC-A, LESREC-B, and Laurel, DE.

		Center lesion (mm)		Stem lesion (mm)		Blossom end lesion (mm)			
Isolate	**Cultivar**	**depth**	**width**	**depth**	**Width**	**depth**	**Width**	**TEF1[*Y]-spp. ID**	**RPB2[*Z]-spp.ID**
F063_2[*X]	NA	25.50ab[*T]	21.82abcd	23.95abc	20.17abcd	21.22bcd	22.69bc	NA	NA
F2015002	Sivan	23.56a	21.56abcd	14.67cdefg	9.00d	33.33b	32.00ab	*F. oxysporum*	*F. oxysporum*
F2015003	Sivan	27.67ab	31.56ab	11.33efg	13.67abcd	35.00b	45.33a	*F. fujikuroi*	F. fujikuroi
F2015007	Sivan	28.33a	27.78abc	18.33bcde	25.00a	35b	37.33ab	*F. armeniacum*	*F. armeniacum*
LA16015	Sivan	22.56ab	18.89cde	15.67cdefg	22.67abc	35.67b	34.00ab	*F. proliferatum*	*F. proliferatum*
LA17011	NA	31.00a	33.00a	29.67a	24.00ab	36.00b	44.33a	-	-
LA17012	NA	27.33a	28.32abc	27.45ab	12.17abcd	57.22a	39.19ab	*F. oxysporum*	*F. oxysporum*
LB17011	Na	10.33cd	8.64de	7.03g	7.84d	9.39d	8.25c	*F. verticilloides*	*F. verticilloides*
V16016A	Ariel	15.00bcd	11.00de	12.67defg	10.00d	31.00bc	24.33bc	*F. proliferatum*	*F. proliferatum*
V16020	Athena	21.83abc	18.49bcde	19.45abcde	15.67abcd	32.22bc	26.19abc	*F. proliferatum*	*F. proliferatum*
V16024	Athena	22.56ab	18.22cde	17.67bcdef	12.67bcd	34.67b	21.67bc	*F. oxysporum*	*F. oxysporum*
V17005	Ariel	24.11ab	21.22bcd	21.00abcd	20.00abcd	35.00b	36.00ab	*F. fujikuroi*	*F. fujikuroi*
V17007	Athena	8.78d	8.67e	8.33fg	11.33cd	14.67cd	21.33bc	-	-

[*X]Control isolate (F063-2), used. [*T] Same letters within the same column are not statistically different ($p \leq 0.05$), according to Student's t-test least significant differences (Mixed-model-JMP).

[*Y]TEF1-Translation Elongation Factor alphar-1, [*Z]RPB2- RNA polymerase II second largest subunit.

TABLES

Table 8. *Fusarium* spp. isolates obtained from 'Sivan' F1 certified organic cantaloupe fruits during the summer season of 2015 (*Fusarium* ID and NCBI sequence comparison) and Ariel.

Species	Isolate	Cultivar Origin	Most-related accession-NCBI	Sequence similarity* (%)
Fusarium armeniacum	F2015-007	Sivan F1	KJ737376.1	99/99
Fusarium fujikuroi	F2015-003	Sivan F1	LC055826.1	98/99
Fusarium oxysporum	F2015-001	Sivan F1	BCHB01000001.1	99/100
Fusarium oxysporum	F2015-002	Sivan F1	BCHB01000001.1	99/99
Fusarium oxysporum	F2015-005	Sivan F1	BCHB01000001.1	99/99
Fusarium oxysporum	F2015-006	Sivan F1	BCHB01000001.1	99/99
Fusarium oxysporum	F2015-008	Sivan F1	BCHB01000001.1	99/98
Fusarium proliferatum	F2016V016A	Ariel	FJ895272.1	99.67/100

*Sequence percentage similarity: Percentage similarity to *Fusarium* strains stored in either NCBI or *Fusarium* ID online library database.

TABLES

Table 9. ANOVA table of *p*-values of interaction between *Fusarium* spp. (*F. armeniacum*, *F. oxysporum*, *F. fujikuroi*, *F. proliferatum*) and *Salmonella enterica* Newport on five different melon cultivars.

	Experiment Replication 1	Experiment Replication 2	Experiment Replication 3	Experiment Replication 4
Source	Prob > F	Prob > F	Prob > F	Prob > F
Fusarium	0.84	<0.0001***	0.47	0.028*
Cultivar	0.004**	<0.0001***	<0.0001*	<0.0001*
*Fusarium**Cultivar	0.53	<0.004**	0.53	0.72

Notes: Prob-Probability, DF-Degrees of freedom,

* Prob-Probability, DF-Degrees of freedom, *statistically significant ($p \leq 0.05$), ** highly statistically significant different ($p \leq 0.001$), ***Very highly significant different, compared to the control isolate (F-063-2) according to Tukey-Kramer-HSD least significant differences (Mixed-model-JMP).

TABLES

Table 10. ANOVA table of p-values for the impact of *Fusarium proliferatum* on *Salmonella enterica* Newport inoculated on smooth (honeydew) and netted (cantaloupe) surface type melon.

H0S4					**H0S0**				
Source	**24 hrs**		**5 days**			**5 days**		**10 days**	
	DF	Prob*[Y] > F	DF*[Z]	Prob > F	Source	DF	Prob > F	DF	Prob > F
Melon	1	0.11	1	0.05*[X]	Melon	1	0.74	1	0.30
Site	3	0.79	3	0.94	Site	3	0.80	3	0.53
Melon*Site	3	0.63	3	0.60	Melon*Site	3	0.39	3	0.22
F0S4					**F0S0**				
	24 hrs		**5 days**			**5 days**		**10 days**	
Source	DF	Prob > F	DF	Prob > F	Source	DF	Prob > F	DF	Prob > F
Melon	1	0.03	1	0.95	Melon	1	0.75	1	0.04
Site	3	0.12	3	0.82	Site	3	0.95	3	0.28
Melon*Site	3	0.92	3	0.76	Melon*Site	3	0.89	3	0.55

Notes: *[X] Statistically significant ($p \leq 0.05$), *[Y]Prob-Probability, *[Z]DF-Degrees of freedom.

FIGURE LEGENDS

Figure 1.a, b, c & d. Population levels of *Salmonella* Newport $\log_{10\ CFU}\ mL^{-1}$ retrieved from melon rinds of five different cultivars infected with *Fusarium oxysporum, F. fujikuroi, F. armeniacum, F. proliferatum,* or uninfected (water control).

Figure 1a. Box plots display the population of *S.* Newport $\log_{10\ CFU}\ mL^{-1}$ (Tukey-Kramer HSD-Mixed model-JMP), differences recovered among the five melon cultivars (Arava, Athena, Dulce, Jaune, Sivan) during the Run 1. Box plots with the same letters are not significantly different ($p \leq 0.05$).

Figure 1b. Box plots display the population of *S.* Newport $\log_{10\ CFU}\ mL^{-1}$ (Tukey-Kramer HSD-Mixed model-JMP), differences recovered among the five melon cultivars (Arava, Athena, Dulce, Jaune, Sivan) during the Run 2. Box plots with the same letters are not significantly different ($p \leq 0.05$).

Figure 1c. Box plots show median and interquartile range. Small letters denote significant differences among cultivars and *Fusarium* treatments (B) ($p \leq 0.05$) during the Run 3. Box plots display the population of *S.* Newport $\log_{10}$/melon rind (Tukey-Kramer HSD-Mixed model-JMP), differences recovered among the five melon cultivars (Arava, Athena, Dulce, Jaune, Sivan). Box plots with the same letters are not significantly different.

Figure 1d. Population levels of *S.* Newport $\log_{10\ CFU}\ mL^{-1}$ retrieved from melon rind of five different cultivars infected with *F. fujikuroi*, *Fusarium oxysporum*, *F. armeniacum* or uninfected (water control) during the Run 4. Box plots display the population of *S.* Newport $\log_{10}$/melon rind (Tukey-Kramer HSD-Mixed model-JMP), differences recovered among the five melon cultivars (Arava, Athena, Dulce, Jaune, Sivan). Box plots with the same letters are not significantly different ($p \leq 0.05$).

Figure 2a. Impact of incubation time (24 hrs.) on *Salmonella* Newport survival on cantaloupe and honeydew type melon ($p \leq 0.05$), when inoculated alone or in the presence of *Fusarium proliferatum*.

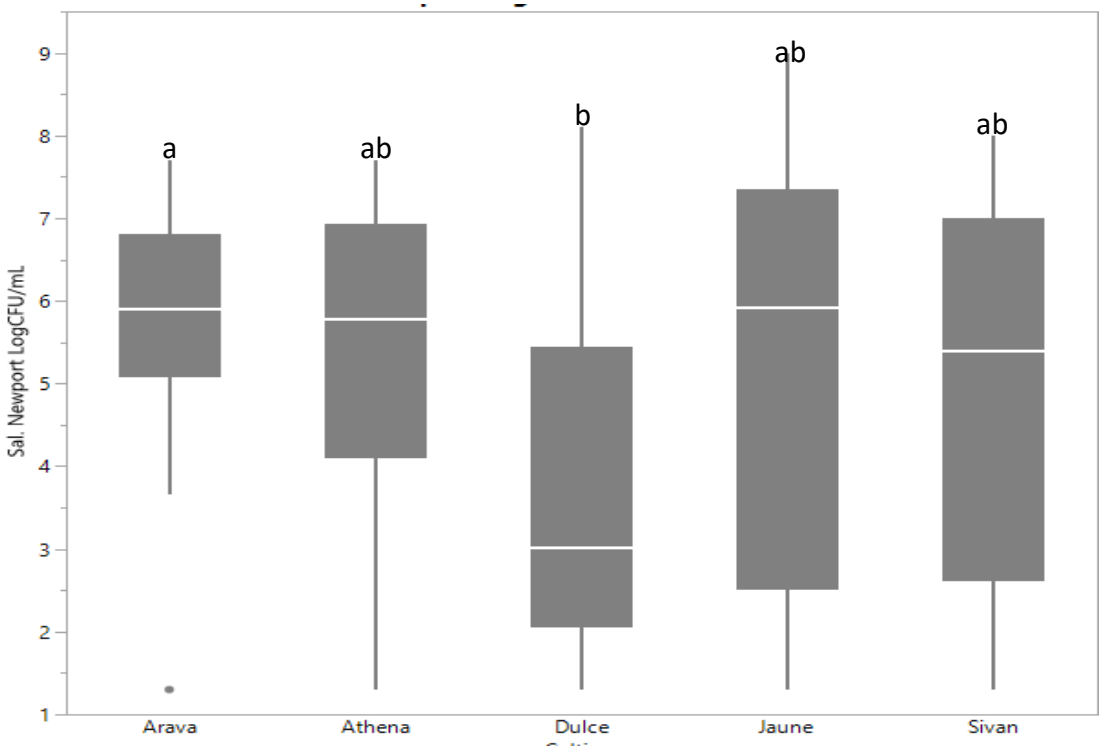
Box plots displaying population of *S.* Newport $\log_{10\ CFU}\ mL^{-1}$ / melon segment (Tukey-Kramer HSD-Mixed model-JMP), differences recovered among the four melon segments (Surface, Exocarp, Mesocarp, and Endocarp), after 24 hr. post inoculation when *Fusarium proliferatum* plus *S.* Newport were simultaneously inoculated, or nonsimultaneously, at 4 days later, and retrieved at Day 5 ($p \leq 0.05$).

FIGURE LEGENDS

Figure 2b. Impact of *Fusarium proliferatum* and incubation time of *Salmonella* Newport (5 and 10 days), inoculated on cantaloupe type melon ($p \leq 0.05$).
Box plots display the population of *S.* Newport $\log_{10\ CFU}\ mL^{-1}$ /melon segment (Tukey-Kramer HSD-Mixed model-JMP), differences recovered among the four *Salmonella* Newport treatments (H0S0, F0S0, H0S4, F0S4), after 5 post inoculation when *Salmonella* Newport was inoculated alone (H0S0), *Fusarium proliferatum* plus *S.* Newport simultaneously (F0S0), or nonsimultaneously at day 0 or 4 days later (H0S4, F0S4), and retrieved at Day 5 and 10 ($p \leq 0.05$).

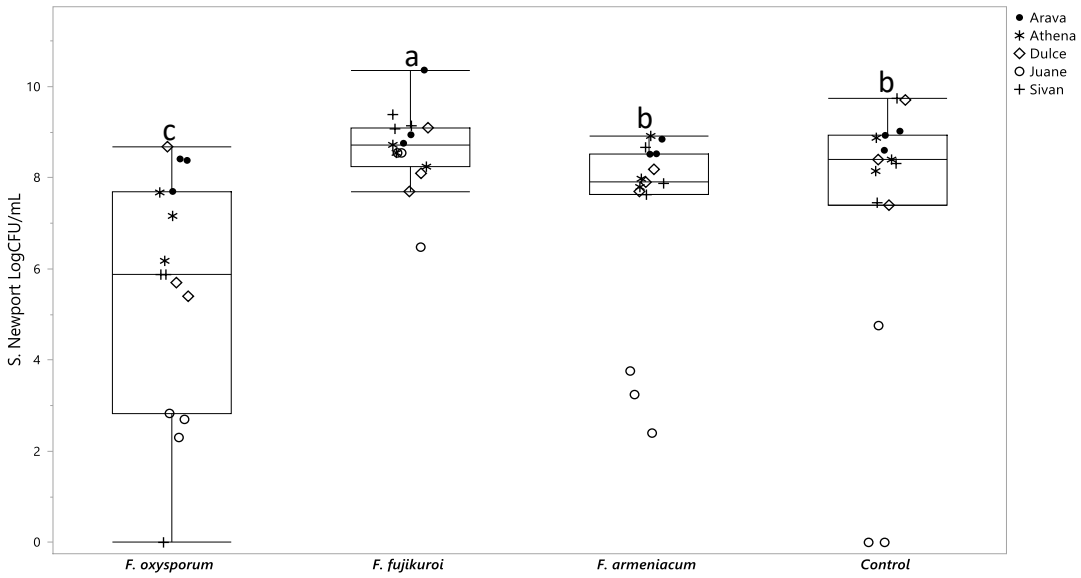
FIGURES

Fig. 1a.



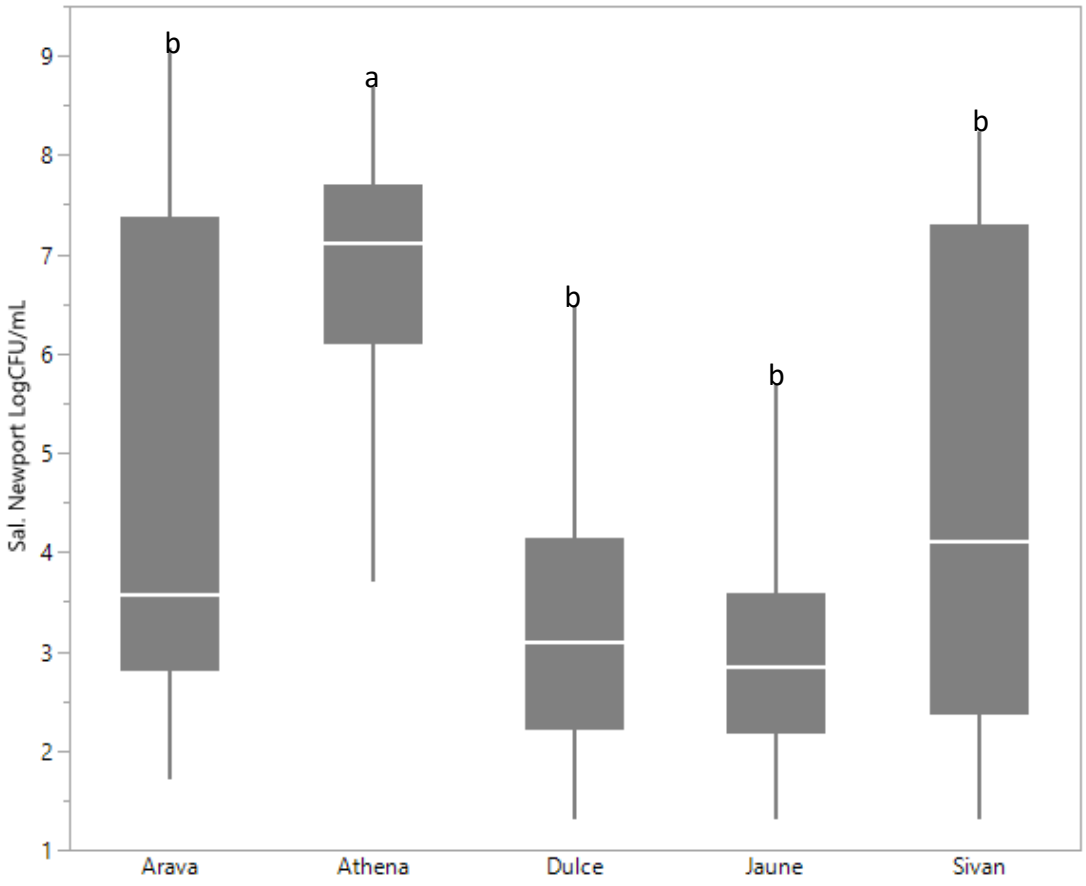
FIGURES

Fig. 1b.



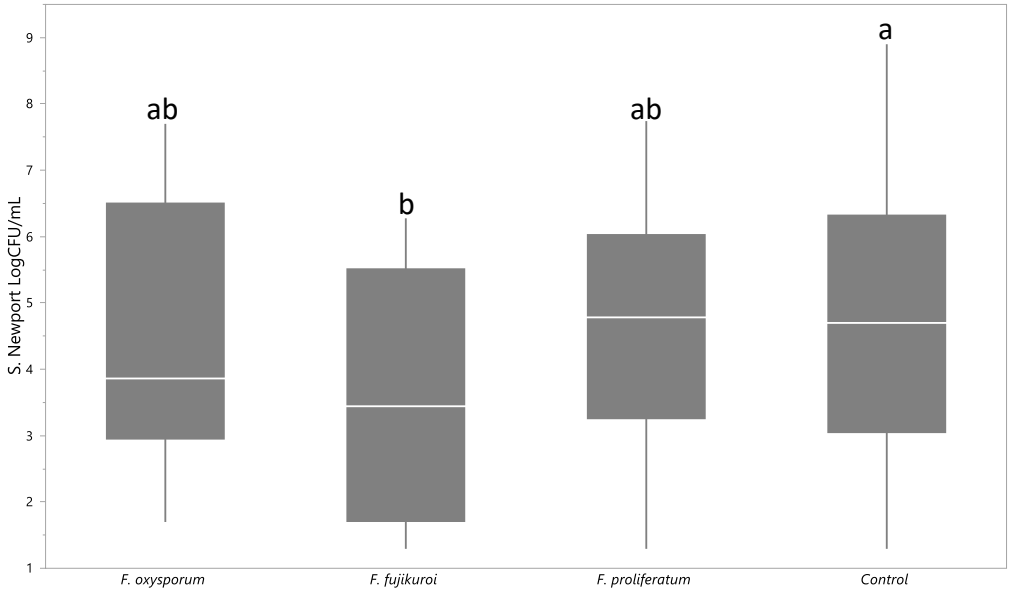
FIGURES

Fig. 1c.



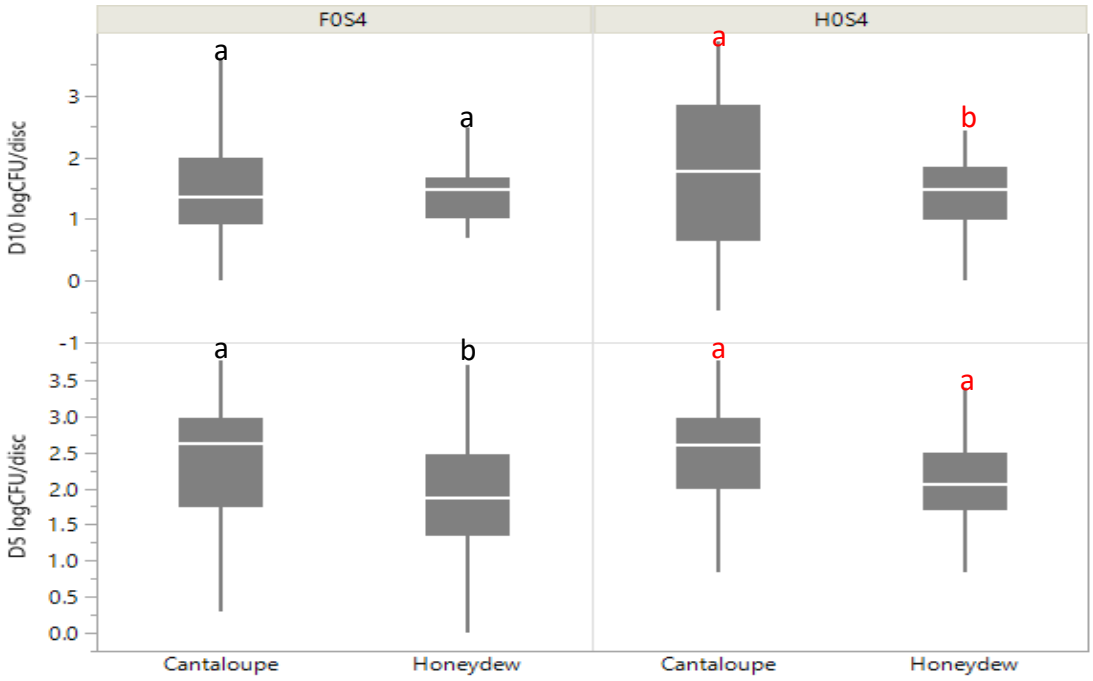
FIGURES

Fig. 1d.



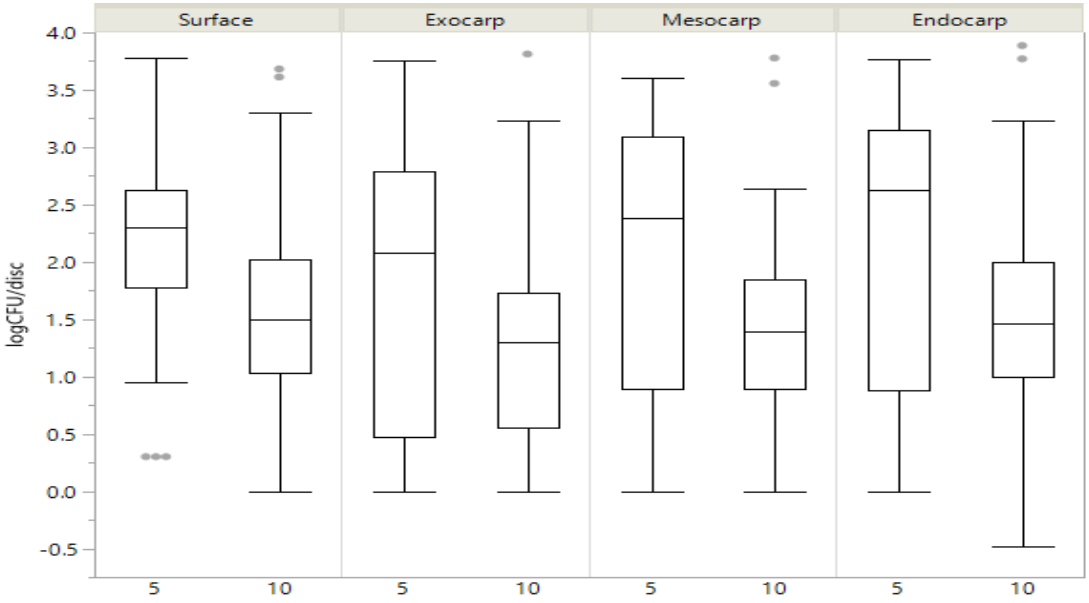
FIGURES

Fig. 2a.



FIGURES

Fig. 2b.



Appendices

A1. A list of *Fusarium* species identified morphologically. These *Fusarium* species were isolated from melon grown in Baltimore, MD. U. S. during the growing season of 2016.

A2. A list of *Fusarium* species identified morphologically. These *Fusarium* species were isolated from melon grown in LESREC, organic field in 2016, Salisbury, MD. U. S.

A3. A list of *Fusarium* species identified morphologically. These *Fusarium* species were isolated from melon grown in LESREC, conventional field in 2016, Salisbury, MD. U. S.

A4. A list of *Fusarium* species identified morphologically. These *Fusarium* species were isolated from melon grown in Laurel, DE.

A5. A list of *Fusarium* species identified morphologically. These *Fusarium* species were isolated from melon grown in WYEREC, Queen's Anne, MD. U. S.

A6. Overall percentage of *Fusarium* species isolated from the melon and morphologically identified in five locations during the growing season of 2016, MD during the production season, 2016 (*n*=71).

A7. i. Melon in the field infected by presumptive *Fusarium* spp**. ii.** *Fusarium* strain cultures after 7 days of inoculation on PDA plates. **iii.** 'Honeydew' melon with lesions on five different locations (3-circumference, stem scar, and blossom end) after being inoculated with the isolate#WYE003 (*F. fujikuroi*) for ten days. **iv.** *Fusarium* strains DNA PCR products' Gel bands observed under a UV-light.

APPENDIX A1. *Fusarium* spp. identified morphologically from the isolates obtained from melon grown in Baltimore, MD during 2016.

Isolate ID	**Origin**	**Cultivar**	***Fusarium* spp.**
F2016B003	Baltimore	Athena	*F. semitectum*
F2016B006	Baltimore	Athena	*F. nygamai*
F2016B013A	Baltimore	Athena	*F. nygamai*
F2016B016	Baltimore	Athena	*F. scirpi*
F2016B017	Baltimore	Athena	*F. semitectum, scirpi, solani*
F2016B018	Baltimore	Athena	*F. equiseti*
F2016B019	Baltimore	Athena	*F. equiseti*
F2016B021	Baltimore	Athena	*F. crookwellense*
F2016B024B	Baltimore	Athena	*F. oxysporum*
F2016B024A	Baltimore	Athena	*F. sumbucinum, solani*
F2016B027B	Baltimore	Athena	*F. polyphialidicum, F. semitectum*
F2016B028B	Baltimore	Athena	*F. scirpi*
F2016B029B	Baltimore	Athena	*F. semitectum, scirpi*
F2016B025A	Baltimore	Athena	*F. semitectum, F. nygamai*
F2016B025B	Baltimore	Athena	*F. solani*
F2016B029A	Baltimore	Athena	*F. equiseti, scirpi*
F2016B030A	Baltimore	Athena	*F. longipes, F. aveneceum*
F2016B030B	Baltimore	Athena	*F. oxysporum*

A2. ***Fusarium*** **spp. identified morphologically from the isolates obtained from melon grown in LESREC-A, MD during 2016.**

Isolate ID	**Origin**	**Cultivar**	***Fusarium*** **spp.**
F2016LA001	LESREC-A	Sivan	*F. crookwellense*
F2016LA002A	LESREC-A	Sivan	*F. semitectum*
F2016LA005A	LESREC-A	Sivan	*F. semitectum*
F2016LA008A	LESREC-A	Sivan	*F. scirpi*
F2016LA009	LESREC-A	Sivan	*F. semitectum*
F2016LA010	LESREC-A	Sivan	*F. solani*
F2016LA011	LESREC-A	Sivan	*F-scirpi*
F2016LA014	LESREC-A	Sivan	*F. semitectum*
F2016LA015	LESREC-A	Sivan	*F. solani*
F2016LA016	LESREC-A	Sivan	*F. oxysporum*
F2016LA017	LESREC-A	Sivan	*F. solani*
F2016LA019	LESREC-A	Sivan	*F. culmorun*
F2016LA020	LESREC -A	Sivan	*F. scirpi*
F2016LA021	LESREC-A	Sivan	*F. campactum*

A3. ***Fusarium*** **spp. identified morphologically from the isolates obtained from melon grown in LESREC-B, MD during 2016.**

Isolate ID	Origin	Cultivar	*Fusarium* spp.
F2016LB002	LESREC-B	Arava	*F. solani*
F2016LB002	LESREC-B	Arava	*F. compactum, semitectum*
F2016LB003	LESREC-B	Sivan	*F. semitectum*
F2016LB004	LESREC-B	Jaune	*F. crookwellense, solani*
F2016LB006	LESREC-B	Arava	*F. solani*
F2016LB007	LESREC-B	Sivan	*F. scirpi*
F2016LB009	LESREC-B	Jaune	*F. compactum, nygamai, solani*
F2016LB010	LESREC-B	Sivan	*F. scirpi*
F2016LB013	LESREC-B	Arava	*F. compactum, vericilloides*
F2016LB014	LESREC-B	Arava	*F. solani, avenaceum*
F2016LB017	LESREC-B	Sivan	*F. solani*
F2016LB018	LESREC-B	Arava	*F. semitectum*
F2016LB020	LESREC-B	Athena	*F. avenaceum*
F2016LB023	LESREC-B	Athena	*F. solani*
F2016LB024	LESREC-B	Athena	*F. solani*

A4. ***Fusarium*** **spp. identified morphologically from the isolates obtained from melon grown in Laurel, DE during 2016.**

Isolate ID	Origin	Cultivar	*Fusarium* spp.
F2016V006B	Laurel, DE	Athena	*F. solani*
F2016V008A	Laurel, DE	Athena	*F. scirpi*
F2016V008A	Laurel, DE	Athena	*F. scirpi*
F2016V008B	Laurel, DE	Athena	*F. compactum, F. nygamai, F. solani*
F2016V010B	Laurel, DE	Athena	*F. compactum*
F2016V011B	Laurel, DE	Athena	*F. compactum, F. verticilloides*
F2016V013A	Laurel, DE	Ariel	*F. solani, F. avenaceum*
F2016V016A	Laurel, DE	Ariel	*F. solani*
F2016V022A	Laurel, DE	Athena	*F. polyphialidicum, F. semitectum*
F2016V024A	Laurel, DE	Athena	*F. semitectum*
F2016V024B	Laurel, DE	Athena	*F. solani*
F2016V024B2	Laurel, DE	Athena	*F. semitectum*
F2016V027B	Laurel, DE	Athena	*F. avenaceum*
F2016V027B2	Laurel, DE	Athena	*F. solani*

A5. ***Fusarium*** **spp. identified morphologically from the isolates obtained from melon grown in WYEREC, Queen's Anne, MD during 2016.**

Isolate ID	**Origin**	**Cultivar**	***Fusarium*** **spp.**
F2016WYE001	WYEREC	Sivan	*F. equiseti*
F2016WYE002A	WYEREC	Jaune	*F. solani*
F2016WYE003A	WYEREC	Athena	*F. scirpi*
F2016WYE004A	WYEREC	Spanish Sun	*F. solani*
F2016WYE009A	WYEREC	Jaune	*F. solani*
F2016WYE009B	WYEREC	Jaune	*F. equiseti*
F2016WYE011A	WYEREC	Athena	*F. semitectum*
F2016WYE018B	WYEREC	Eden Gem	*F. compactum*
F2016WYE020A	WYEREC	Jaune	*F. scirpi*

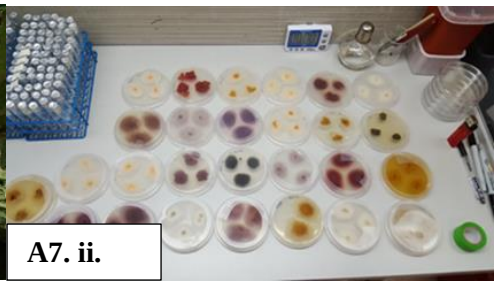
A6. Overall percentage of *Fusarium* spp. identified morphologically identified from the isolates obtained from melon grown in the five different locations in Maryland and Delaware during 2016.

***Fusarium* spp.**	**Location A**	**B**	**C**	**D**	**E**	**Total#**	**%**
F. avenaceum	0	0	1	2	1	4	5.63
F. culmorum	0	0	1	0	0	1	1.41
F. equiseti	3	2	0	0	0	5	7.04
F. oxysporum	0	0	2	0	0	2	2.82
F. polyphialidicum	0	0	0	0	1	1	1.41
F. scirpi	4	2	3	2	2	13	18.31
F. semitectum	5	1	1	3	2	12	16.90
F. solani	3	3	2	7	6	21	29.58
F. compactum	0	1	4	1	2	8	11.27
F. crookwellense	1	0	0	1	0	2	2.82
F. nygamai	2	0	0	0	0	2	2.82
	18	9	14	16	14	71	100.00
Total#%	**25.35**	**12.68**	**19.72**	**22.54**	**19.72**	**100**	

Notes: A= Baltimore, MD, B= WYEREC, C= LESREC-A, D=LESREC-B, E=Laurel, DE.

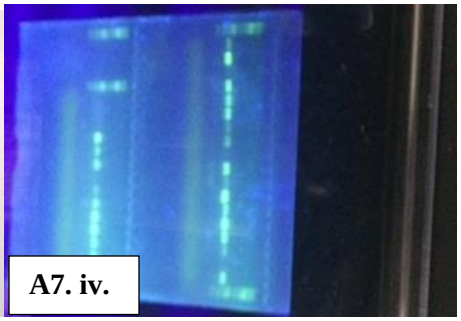
A7. ***Fusarium*** **on melon, Potato Dextrose plates, and Gel-electrophoresis.**

A7. i



A7. ii.

A7.iii.



A7. iv.

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