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Title: Survival and growth of *Listeria monocytogenes* on whole cantaloupes is dependent on site of contamination and storage temperature

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Abstract

Whole cantaloupes (*Cucumis melo* L), marketed as ‘Rocky Ford’, were implicated in a large multi-state outbreak of listeriosis in the United States in 2011; however, survival and growth of *Listeria monocytogenes* on whole cantaloupes remains relatively unexplored. The research presented here evaluated three different storage temperatures, two sites of contamination of cantaloupes, and two cantaloupe varieties to determine their effect on the survival of *L. monocytogenes*. ‘Athena’ and ‘Rocky Ford’ cantaloupe cultivars were grown in soil and harvested, and individual melons subsequently received a multi-strain inoculum of *L. monocytogenes* (6 log CFU/melon), which were then stored at 4°C, 10°C, and 25°C. Changes in *L. monocytogenes* populations on the rinds and stem scars of cantaloupes stored at each temperature were determined at selected times for up to 15 days. An analysis of variance revealed that inoculation site and storage temperature significantly affected survival of *L. monocytogenes* on cantaloupes during storage ($P<0.05$), but cultivar did not influence *L. monocytogenes* ($P>0.05$). Populations of *L. monocytogenes* on stem scars of cantaloupes stored at 25°C increased by 1 – 2 log CFU/melon on day 1, and were significantly greater than those on cantaloupes stored at 4°C or 10°C ($P<0.05$), which remained constant or increased by approximately 0.3 log CFU/melon, respectively, over the same time period. A decrease of 2 – 5 log CFU/melon of *L. monocytogenes* occurred on the rinds of cantaloupes during storage by day 7, and were not significantly different at the three different storage temperatures ($P>0.05$). In trials performed in rind juice extracts, populations of *L. monocytogenes* decreased by 3 log CFU/mL when stored at 25°C by day 3, but grew by 3 – 4 log CFU/mL when stored at 4°C over 7 days. Overall, site of contamination and storage temperature influenced the survival of *L. monocytogenes* on cantaloupes more than cantaloupe cultivar type.

43 **Keywords:** *Listeria monocytogenes*, listeriosis, United States (U.S.), cantaloupes, multistate,
44 outbreak.

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49 1.0 Introduction

50 The U.S. Centers for Disease Control and Prevention (CDC) estimate that foodborne pathogens
51 cause approximately 48 million illnesses each year in the United States, of which 9.4 million
52 may be caused by known pathogens (CDC, 2011b). It is estimated that 46% of foodborne illness
53 cases are related to contaminated produce (Painter et al., 2013). Contaminated fresh produce
54 were responsible for approximately 2% of the total number of foodborne disease outbreaks
55 between 1973 and 1987, but increased to 23% between 2009 and 2010 (Walsh et al., 2014).
56 Increased consumption of fresh produce may have contributed to increased incidence of
57 foodborne disease outbreaks, but processing and distribution of fresh and fresh-cut fruit and
58 vegetable commodities can also be a factor (Lynch et al., 2009). The complexity and potential
59 for breaks in preventive controls in the farm-to-fork supply chain of fresh produce presents
60 multiple opportunities for contamination and proliferation of pathogens that could lead to large-
61 scale outbreaks of foodborne diseases. The lack of a ‘kill step’ to destroy pathogens on fresh
62 produce prior to consumption accentuates the potential risk and challenge to maintain safe
63 production, handling, and storage conditions.

64 Between 1973 and 2011, the CDC identified 34 foodborne disease outbreaks that were associated
65 with the consumption of contaminated melons, and 19 of which involved cantaloupes (Walsh et
66 al., 2014). *Salmonella* spp. were implicated in nineteen (56%) of the previous outbreaks
67 associated with melons in the U.S. However, in 2011, the deadliest foodborne disease outbreak
68 in the U.S. in nearly 90 years was associated with consumption of cantaloupes (*Cucumis melo*
69 L), marketed as ‘Rocky Ford’, contaminated with *Listeria monocytogenes* (CDC, 2011a). This

outbreak was the first documented listeriosis outbreak linked with the consumption of contaminated cantaloupes, and it resulted in 147 illnesses in 28 states, 33 fatalities, and 1 miscarriage (McCollum et al., 2013). Five distinct pulsed-field gel electrophoresis (PFGE) patterns of *L. monocytogenes*, consisting of three serotype 1/2a and two serotype 1/2b strains, were identified from patients, cantaloupes, and surfaces of equipment and floors from the packing shed where cantaloupes were washed and boxed (Garner and Kathariou, 2016; Lomonaco et al., 2013). *L. monocytogenes* is a gram-positive bacterium that primarily affects older adults, infants, immunocompromised individuals, and pregnant women (Lund and O'Brien, 2011). Listeriosis can be an invasive illness associated with a high hospitalization rate (91% of cases), with more severe clinical symptoms, such as meningitis, encephalitis, bacteremia, neonatal sepsis, and preterm labor; while a noninvasive form of listeriosis is characterized by febrile gastroenteritis and flu-like symptoms that can occur in immunocompetent individuals (Silk et al., 2012). The CDC estimates that 1600 cases of listeriosis and 260 fatalities can occur every year in the U.S. *L. monocytogenes* is the third leading cause of deaths due to foodborne infections in the U.S. (19%) after *Salmonella* spp. (28%) and *Toxoplasma gondii* (24%) (Scallan et al., 2011).

L. monocytogenes is a psychrotroph that can proliferate at refrigeration temperatures, and can withstand diverse stresses in different foods and food processing environments (Swaminathan and Gerner-Smidt, 2007). Although the intact rind and complex netting structure of a cantaloupe surface provides a physical barrier that prevents entry of bacteria into the fruit tissue, it harbors non-pathogenic and pathogenic microorganisms (Ukuku et al., 2012). The objective of this study is to evaluate the effects different cantaloupe varieties, storage temperatures, and sites of

pathogen contamination have on the survival and proliferation of *L. monocytogenes* on field-grown cantaloupes.

2.0 Materials and Methods

2.1 Bacterial strains and cultures

Listeria monocytogenes strains LI0072 (PFGE ID: 713431 10-B; a serotype 1/2a), LIS0077 (PFGE ID: 713432-13C; a serotype 1/2a), and LIS0094 (PFGE ID: 643701-6; a serotype 1/2b), all isolates from the 2011 listeriosis outbreak associated with cantaloupes in the U.S., were obtained from the U.S. Food and Drug Administration (U.S. FDA). Frozen stock cultures for each *L. monocytogenes* strain was maintained in trypticase soy broth (TSB; BD, Franklin Lakes, New Jersey, USA) in sterile vials containing 15% glycerol and stored at -80°C . Active cultures were prepared for each *L. monocytogenes* strain by streaking from the frozen stock cultures onto separate plates of trypticase soy agar (TSA) supplemented with 0.06% yeast extract (TSAYE; Neogen, Lansing, Michigan, USA), and incubated at 37°C for 24 h and stored at 4°C . Prior to each experiment, each *L. monocytogenes* strain was isolated on fresh TSAYE medium and incubated at 37°C for 24 h. A single colony of each strain was transferred to a separate 10 mL aliquot of TSBYE (TSB plus 0.06% yeast extract) and incubated at 37°C for 24 h. Cultures were centrifuged (Allegra 25R Centrifuge, Beckman Coulter, Danvers, Massachusetts, USA) at 5,000 g for 10 min, and the cell pellets resuspended in 10 mL of 0.1% peptone water (PW; BD Difco). The prepared cell suspensions containing 8.7 ± 0.4 , 8.1 ± 0.5 , and 8.9 ± 0.4 log CFU/mL of *L. monocytogenes* strains LI0072, LIS0077, and LIS0094, respectively, were thoroughly vortexed. A multi-strain inoculum of *L. monocytogenes* was prepared by mixing 1 mL of cell suspensions of the three strains in a 1:1:1 ratio, and then diluted in PW to 7 log CFU/mL. Serial dilutions

were used to enumerate *L. monocytogenes* populations. Each dilution (100 µL) was plated in duplicate onto Rapid'L.mono medium (Bio-Rad, Hercules, California, USA) using a spiral plater (WASP2, Don Whitley, Microbiology International, Frederick, Maryland, USA). Plates were incubated at 37°C for 24 h and colonies were counted.

2.1 Cantaloupes

Cantaloupes (*Cucumis melo* L.) marketed as 'Rocky Ford' caused the 2011 listeriosis outbreak in the U.S. while 'Athena' is a widely consumed cantaloupe in the U.S. due to its outstanding flavor, aroma, and firm flesh. Seeds of 'Improved Rocky Ford' and 'Athena' cultivars of cantaloupe were obtained from Southern States, Fort Collins, Colorado, USA. Seeds of the two cultivars of cantaloupe were planted in the fields and high tunnels at the U.S. Department of Agriculture, Agricultural Research Service (USDA ARS), Beltsville Agricultural Research Center (Beltsville, Maryland), and the University of Delaware Carvel Research and Education Center (Georgetown, Delaware). The Athena and Rocky Ford cantaloupes were grown in separate plots in the same field, and the melons were harvested when stalks showed half or full separation/slip from the melons. At the time of harvest, the field temperatures of rind surfaces and internal fruit tissues (measured by inserting needle of the thermometer into fruit tissue) were recorded for both Athena and Rocky Ford melons using an Infrared LED illumination thermometer (ZyTemp TCT303F, Radiant Innovation Inc., HsinChu, Taiwan). Multiple, random measurements revealed temperature differences at different locations on the rind surface as well as the internal fruit tissue of the same melon. At harvest, the mean rind-surface temperatures for Athena and Rocky Ford were $30.0 \pm 3.0^{\circ}\text{C}$ and $31.1 \pm 2.0^{\circ}\text{C}$, respectively, while the internal fruit tissue temperatures were $28.4 \pm 2.0^{\circ}\text{C}$ and $28.9 \pm 1.0^{\circ}\text{C}$, respectively. Wounded and/or rotten melons were removed. The melons were packed into separate, new, fresh produce boxes (Uline,

Pleasant Prairie, Wisconsin, USA) and transported to the Environmental Microbial and Food Safety Laboratory (EMFSL, USDA ARS, Beltsville, Maryland) and stored at 4°C until inoculation.

2.2 Inoculation of cantaloupe

Nineteen cantaloupes of each cultivar were removed from storage at 4°C and equilibrated to ambient temperature. A permanent marker was used to draw circles (11 cm² areas) on the rind and stem scar of each melon. The marked areas on each melon were spot-inoculated with 50 µL (10 drops x 5 µL; approximately 6 log CFU per 11 cm²) of *L. monocytogenes*. The spots of inocula were allowed to dry at room temperature for approximately 1 h.

2.3 Effect of storage temperatures (4°C, 10°C, and 25°C) on populations of *L. monocytogenes* on the whole cantaloupe

The recommended optimal temperature for storage of ripe cantaloupes is 3°C, with the acceptable temperature range of 2°C – 7°C (Cantwell et al., 2002). Three different storage temperatures (4°C, 10°C, and 25°C) were selected to simulate storage of cantaloupes at refrigeration temperature, temperature abuse in a refrigerator, and room temperature, respectively. Inoculated Athena and Rocky Ford cantaloupes were packed separately into three separate fresh produce boxes (6 melons per box), and one box of each cultivar stored at 4°C, 10°C, and 25°C, respectively. On days 0 (before storage), 1, 3, 5, 7, 9, and 15 of storage, one melon of each cultivar, held at each storage temperature, was analyzed to determine the changes in the numbers of *L. monocytogenes* on the rinds and stem scars. Separate sterile knives were used to excise (approximately 1 mm into the mesocarp) the marked/inoculated areas on each melon. The excised samples from each melon were placed into separate Whirlpak filter bags

(Nasco, Fort Atkinson, Wisconsin, USA) containing 50 mL of PW, and stomached (Bagmixer MiniMix Stomacher, Interscience, Woburn, Massachusetts, USA) at maximum speed for 2 min. Aliquots (1 mL) from each sample were serially diluted in PW, and the numbers of *L. monocytogenes* enumerated as described in section 2.1. The samples from the above step were stored at 4°C overnight. When plate counts were too few to count (TFTC), the rind or stem scar samples were enriched in 100 mL demi-Fraser broth (base, AES Chemunex, Cranberry, New Jersey, USA; supplement, BD Difco) at 37°C for 24 h. Enriched broth (10 µL) was then spread onto Rapid'L.mono plates to determine the presence or absence of *L. monocytogenes*.

2.4 Effect of background aerobic microorganisms on the numbers of *L. monocytogenes* over time in cantaloupe rind juice

The rinds of uninoculated cantaloupes were removed (approximately 1 mm into the mesocarp) using separate sterile knives and placed in separate Whirlpak bags, then weighed and transferred into blender containers (Blender 700G, Waring, Torrington, Connecticut, USA). Equivalent volumes of sterile water were added and the rind was blended for 5 min at room temperature. The contents of each blender were transferred into separate Whirlpak filter bags and the pulp separated from the juice to obtain cantaloupe rind juice. The pH of rind juice for cantaloupes were determined using an Orion bench-top pH meter with an Orion 915BNH triode probe (Thermo Electron Corporation, Madison, Wisconsin, U.S.) at room temperature. Rind juice for each cultivar (25 mL) were transferred into separate sterile vials (n=3 per cultivar), and the vials were inoculated with approximately 3 log CFU/mL *L. monocytogenes*. Inoculated rind juice from each cantaloupe cultivar was thoroughly vortexed and stored at 4°C and 25°C, and 1 mL was analyzed on days 0, 1, 3, and 7 to obtain the numbers of *L. monocytogenes* and total aerobic

bacteria as described in section 2.1. For total aerobic counts, serial dilutions were plated onto TSA and incubated at 37°C for 24 h.

2.5 Statistical analysis

JMP Pro 11 statistical software (SAS Institute Inc., Cary, North Carolina, USA) was used for data analysis. Three replicates of each experiment were conducted and reported values are means of the numbers of *L. monocytogenes* and the standard deviations (SD). Data were analyzed using the fit model (linear models) for analysis of variance (ANOVA) with cantaloupe cultivar (Athena and Rocky Ford), inoculation site (rind and stem scar), and storage temperature (4°C, 10°C, and 25°C) as independent variables, and means of the numbers of *L. monocytogenes* (log CFU/melon) at the various time points as the dependent variable. A factorial mode was selected in the model to ascertain whether each of the individual treatments (cultivar, site, and temperature), as well as the interactions of treatments had significant effects on the numbers of *L. monocytogenes* over time ($\alpha=0.05$). The Tukey's HSD (honest significant difference) test was then used for multiple pairwise comparisons of population means to determine if they were significantly different ($P<0.05$).

3.0 Results

3.1 Factors that affected *L. monocytogenes* survival on cantaloupes

The statistical analyses revealed that interactions (factorial) between cultivar, temperature, and inoculation site were significantly affected the numbers of *L. monocytogenes* on melons during storage ($P<0.05$). The interaction between inoculation site (rind and stem scar) and temperature (4°C, 10°C, and 25°C) significantly affected the numbers of *L. monocytogenes* on melons during storage ($P<0.05$); however, interaction between cultivar and temperature, or cultivar and

inoculation site was not statistically significant ($P>0.05$). A comparison using Tukey's HSD test showed that the numbers of *L. monocytogenes* on stem scars over time were significantly greater than those on the rind regardless of cultivar or storage temperature ($P<0.05$). Similarly, populations of *L. monocytogenes* were significantly ($P<0.05$) greater on melons stored at 25°C compared to those at 4°C or 10°C. However, the effect of cultivar did not significantly affect *L. monocytogenes* populations on melons during storage ($P>0.05$).

3.2 The numbers of *L. monocytogenes* decreased on the rinds of cantaloupes during storage

The numbers of *L. monocytogenes* decreased on the rinds of cantaloupes over storage time at all three temperatures. Populations of *L. monocytogenes* decreased from 5.8 ± 0.1 log CFU/melon on Athena cantaloupes on day 0 to 1.5 ± 2.1 log CFU/melon after 15 days of storage at 4°C (Figure 1), to 2.7 ± 1.4 log CFU/melon after 9 days of storage at 10°C (Figure 2), and to 3.2 ± 2.5 log CFU/melon after 7 days of storage at 25°C (Figure 3). Similarly, the numbers of *L. monocytogenes* on Rocky Ford cantaloupes decreased from 5.7 ± 0.3 log CFU/melon to 3.1 ± 0.5 log CFU/melon after 15 days of storage at 4°C (Figure 1), to 4.0 ± 0.8 log CFU/melon after 9 days of storage at 10°C (Figure 2), and to 4.0 ± 2.1 log CFU/melon after 7 days of storage at 25°C (Figure 3). However, the numbers of *L. monocytogenes* on the rinds of Athena and Rocky Ford melons showed 0.7 log CFU and 1 log CFU increase, respectively, from day 9 to day 15 during storage at 4°C (Figure 1). The presence of *L. monocytogenes* on the rind of some melons was presumptively confirmed following enrichment. The numbers of *L. monocytogenes* on the rinds of melons were not significantly different at 4°C, 10°C, and 25°C ($P>0.05$). The quality (visual appearance) of cantaloupes that were stored at 10°C and 25°C deteriorated more rapidly than those stored at 4°C during the study.

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226 **3.3 *L. monocytogenes* proliferated on the stem scars of cantaloupes during storage**

227 *L. monocytogenes* proliferated on the stem scars in contrast to the decline of populations on rinds
228 of cantaloupes. On stem scars of Athena cantaloupes, the initial (day 0) *L. monocytogenes*
229 populations of 5.8 ± 0.1 log CFU/melon increased to 7.6 ± 0.4 log CFU/melon after 15 days of
230 storage at 4°C (Figure 1), and to 6.4 ± 0.7 log CFU/melon after 9 days of storage at 10°C (Figure
231 2); A rapid increase in populations from 5.8 ± 0.1 to 7.2 ± 0.6 log CFU/melon occurred after
232 just one day of storage at 25°C (Figure 3). On stem scars of Rocky Ford cantaloupes, *L.*
233 *monocytogenes* increased from an initial population of 5.8 ± 0.1 log CFU/melon to 6.7 ± 1.2 log
234 CFU/melon after 15 days of storage at 4°C (Figure 1), to 6.4 ± 0.4 log CFU/melon after 9 days of
235 storage at 10°C (Figure 2). and pathogen to 7.6 ± 0.7 log CFU/melon after one day of storage at
236 25°C (Figure 3).. Based on Tukey's HSD test, populations of *L. monocytogenes* on stem scars of
237 cantaloupes stored at 25°C were significantly greater than those stored at 10°C and 4°C over 15
238 days ($P < 0.05$), but the numbers of *L. monocytogenes* on stem scars stored at 10°C were not
239 significantly greater than those stored at 4°C over 15 days ($P > 0.05$).

240 **3.4 *L. monocytogenes* in the presence of total aerobic bacteria in rind juice**

241 The pH of Athena rind juice was approximately 6.0 while that of Rocky Ford rind juice was 5.7
242 at room temperature. The numbers of total aerobic bacteria originally present in rind juice of
243 both Athena and Rocky Ford melons were 5.5 – 6 log CFU/mL, but increased to 6.5 – 7.5 log
244 CFU/mL by day 7 of storage at 4°C, whereas the numbers of *L. monocytogenes* remained
245 constant at 3 log CFU/mL for the first 3 days, and then increased to approximately 7 log
246 CFU/mL over the same period. Conversely, storage of rind juice of both Athena and Rocky Ford

melons at 25°C resulted in an unrestricted proliferation of the total aerobic bacteria from 5.5 – 6 log CFU/mL to approximately 10 log CFU/mL. However the numbers of *L. monocytogenes* decreased quickly by 3 log CFU/mL and the numbers were not quantifiable by day 3. *L. monocytogenes* was presumptively present by enrichment of rind juice after 7 days of storage at 25°C.

4.0 Discussion

In our study, the numbers of *L. monocytogenes* decreased by 2 – 5 log CFU/melon on the rinds of both Athena and Rocky Ford cantaloupe cultivars at all three storage temperatures, but either increased by 1 – 2 log CFU/melon or remained constant on stem scar areas of melons at all three storage temperatures over 15 days.. The numbers of *L. monocytogenes* on the stem scar areas were significantly greater than those on the rind portions of cantaloupes. The results from our study are in agreement with previous work showing declines in the numbers of *L. monocytogenes* on cantaloupe rinds (Ukuku and Fett, 2002). In that study, the numbers of *L. monocytogenes* on cantaloupe rinds stored at 4°C and 20°C for 15 days declined by 1.5 – 2 log CFU/cm² (Ukuku and Fett, 2002). However, the approximately 1 log CFU increase in the numbers of *L. monocytogenes* on the rinds of both Athena and Rocky Ford cantaloupes from day 9 to day 15 during storage at 4°C in our study could be due to observed cracks in the rinds of cantaloupes as a result of prolonged storage, and the exposure of the surviving pathogens to the exocarp layer which provided access to nutrients which potentially supported growth of the pathogen. Storage temperature significantly affected the survival of *L. monocytogenes* on stem scars.

268 Increases in storage temperature correlated with increases in the numbers of *L. monocytogenes*
269 on stem scars of cantaloupes over time.. The numbers of *L. monocytogenes* on stem scars of
270 cantaloupes stored at 25°C were significantly greater than those stored at 10°C and 4°C, but
271 there were no statistical differences between *L. monocytogenes* populations on stem scars of
272 melons stored at 10°C and 4°C. . Unlike populations of *L. monocytogenes* on stem scars of
273 whole cantaloupes stored at 10°C and 25°C, which increased between day 0 and 1, those on stem
274 scars stored at 4°C did not increase until after day 5, indicating a prolonged lag phase of five to
275 seven days at 4°C. Regardless of storage temperature, *L. monocytogenes* contamination of stem
276 scar areas of cantaloupes may present a greater risk to consumers than contamination of rind
277 areas due to the ability of the pathogen to grow on stem scars of melons at various temperatures.

278 Cantaloupes have been implicated in more outbreaks of foodborne illness diseases than the other
279 types of melons (watermelons, honeydew melons, and other musk melons) in the United States
280 (Walsh et al., 2014). Fields where melons are grown can be a potential source of contamination
281 of whole melons by *L. monocytogenes*, because natural habitats for this pathogen include
282 sewage, soil, sludge, and decaying vegetation (Kerouanton et al., 2010). Surface topography and
283 wettability are important physicochemical properties that can influence bacterial attachment to
284 surfaces, with rough and corrugated surfaces that are overlaid with liquids being conducive to
285 bacterial attachment (Hsu et al., 2013). Rough ridges on the rind surfaces of cantaloupes may
286 facilitate especially strong microbial attachment, making cleaning and sanitizing of cantaloupe
287 surfaces difficult. *L. monocytogenes* has the capacity to form biofilms on the rinds of
288 cantaloupes, which can make their removal or disinfection difficult (Annous et al., 2005).
289 Bacteria attached in cracks and crevices would be less likely affected by desiccation stress and
290 may exhibit retarded decline, depending on storage temperature. *L. monocytogenes* populations

which decline slowly on the rinds of melons may still present a risk of contamination to the flesh of melons, and under temperature abuse condition, may be sufficient to result in illness to some individuals.

Postharvest treatments of melons include removing field heat to preserve quality aspects by forced air- or hydro-cooling to a final temperature of 2 – 7°C. Chlorinated and/or unchlorinated water used to remove soil debris and microbial contaminants from surfaces of cantaloupes may serve as a source of contamination of cantaloupes with *L. monocytogenes*. Sanitizers are applied to inactivate bacteria and fungi present on cantaloupe rinds, but incomplete inactivation of microbial pathogens may result in contamination of stem-scars as well as infiltration of water with pathogens into flesh of cantaloupes. A secondary treatment of melons with chlorinated water was found to be ineffective to completely inactivate background bacteria and fungi on the surfaces of melons (Gagliardi et al., 2003). The use of wash tanks for cleaning and hydro-cooling could be potential sources of bacterial and fungal contamination of cantaloupes and honeydew melons with pathogens, especially if the water is recycled in the system and chlorine concentration of the wash water is not sufficient to inactivate these microbial populations on melons (Gagliardi et al., 2003). Hot water surface treatment of cantaloupes, as well as application of different types of chemical sanitizing agents, have all been found to be ineffective at inactivating bacteria and fungi present on cantaloupe rind surfaces (Fan et al., 2008; Fan et al., 2009).

In the 2011 U.S. multi-state outbreak of listeriosis associated with contaminated Rocky Ford cantaloupes, *L. monocytogenes* was recovered from equipment in the packing shed where cantaloupes were processed (FDA, 2015). The field heat was not removed which may have allowed them to remain relatively warm (above 2 – 7°C) during and after packing (spraying,

washing). The use of wash water and lack of removal of field heat from melons might have created a scenario that allowed *L. monocytogenes* to transfer from contaminated equipment surfaces or wash water to the stem scar areas of warm melons, which then promoted unrestricted proliferation of the pathogen on the surfaces of melons, and potentially contaminated the flesh of melons during cutting. Our study showed that *L. monocytogenes* decreased on the rind areas of melons during storage and were not significantly affected by the different storage temperatures, but populations on the stem scar areas increased when melons were stored at 25°C..

The results from the *L. monocytogenes* survival and growth in rind juice may explain some of the limiting factors of growth on the rinds of cantaloupes during storage. In inoculated rind juice stored at 4°C, the numbers of *L. monocytogenes* increased by 3 – 4 log CFU/mL by day 7, whereas the numbers of mesophilic aerobic bacteria, which were initially much greater, increased by 1 – 1.5 log CFU/mL during the same time interval. These results are not in agreement with our observed results from the whole cantaloupe study, where the numbers of *L. monocytogenes* decreased on the rinds of whole cantaloupes stored at 4°C. The difference in the observed decrease in the numbers of *L. monocytogenes* on the rinds of whole cantaloupes and the growth of these populations in rind juice at 4°C suggests a lack of moisture and/or nutrient source might have contributed to the decrease in the numbers of *L. monocytogenes* on the rinds of whole cantaloupe during storage. Conversely, at 25°C, *L. monocytogenes* populations decreased by 3 log CFU/mL by day 3, and populations of mesophilic aerobic bacteria increased by 4 log CFU/mL in rind juice. On whole cantaloupes, the numbers of *L. monocytogenes* decreased by 2.5 log CFU/mL on the rinds of cantaloupes stored at 25°C. These results from the rind juice studies show that at 4°C, *L. monocytogenes* can tolerate and grow in the presence of microbial competition encountered on the cantaloupe rind. However, the harsh physical

conditions on the surface of cantaloupe rinds and the microbial growth of aerobic mesophiles contribute to the decline of *L. monocytogenes* populations in both the juice and surfaces of cantaloupe rinds at 25°C.

In conclusion, *L. monocytogenes* was able to grow on the stem scar areas of harvested cantaloupes at 25°C, and did not decline at 4 or 10°C. The numbers of *L. monocytogenes* on the rinds of whole cantaloupes declined regardless of the storage temperature evaluated. The numbers of aerobic bacteria and harsh physical conditions affected the decline of *L. monocytogenes* populations on the rinds of cantaloupes. Cultivar did not affect the survival or growth of *L. monocytogenes* on the stem scar or rind of cantaloupes. These results reinforce the need for good agricultural practices, hygienic maintenance of melon processing equipment and surfaces, and proper temperature storage to limit the survival and growth of *L. monocytogenes* on cantaloupes.

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429 **Figure Legends**

430 Figure 1: Change in populations of *L. monocytogenes* over time on the rinds and stem scars of
431 cantaloupes stored at 4°C; values are means (n=3) plus standard deviations.

432 Figure 2: Change in populations of *L. monocytogenes* over time on the rinds and stem scars of
433 cantaloupes stored at 10°C; values are means (n=3) plus standard deviations.

434 Figure 3: Change in populations of *L. monocytogenes* over time on the rinds and stem scars of
435 cantaloupes stored at 25°C; values are means (n=3) plus standard deviations.

436 Figure 4: Change in populations of *L. monocytogenes* and aerobic microbial populations over
437 time in Athena and Rocky Ford cantaloupes rind juice stored at 4°C; values are means (n=3) plus
438 standard deviations.

439 Figure 5: Change in populations of *L. monocytogenes* and aerobic microbial populations over
440 time in Athena and Rocky Ford cantaloupes rind juice stored at 25°C; values are means (n=3)
441 plus standard deviations.

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Figure 1

Figure 2

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