PROJECT TITLE: *Salmonella* and *Listeria monocytogenes* Growth and Potential Transmission in Cantaloupe Plants and Fruit

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PROJECT SUMMARY:

Overall Project Goals and Objectives: This project was designed to better understand the potential interactions *Salmonella* and *L. monocytogenes* have with cantaloupe plants. These interactions, including how well these pathogens can survive and potentially move to the mature fruit following contamination of the plant, may have contributed to the contamination of cantaloupes implicated in recent outbreaks.

SUMMARY OF RESULTS:

Objective 1: Transmission of *Salmonella* Typhimurium to fruit following seed, soil, and leaf contamination. This objective examined the ability of *Salmonella* Typhimurium (implicated in 2012 Indiana outbreak) to persist and move to cantaloupe fruit using PCR based techniques for final detection of the bacterium. *Salmonella* Typhimurium was introduced to plant tissue by various routes (i.e., seed, soil, leaf) to simulate contamination events that could occur during normal field conditions. Green Fluorescent Protein (GFP) produced by this strain of *Salmonella* Typhimurium was used as a marker to differentiate strains introduced experimentally from any potentially naturally occurring *Salmonella*.

Salmonella Typhimurium was detected in fruit from the contaminated plants, however, a total of 2 fruits in the control plants (out of 227 sampled) were also positive (Table 1). This indicates that cross-contamination must have occurred during some point in the growing process; this experiment is currently being repeated.

Table 1: A total of 227 cantaloupe fruit were sampled from 27 different contaminated or control plants. *Salmonella* Typhimurium was detected in the contaminated fruit (CONTAM) from each of the three different routes of contamination (seed, leaf, soil). However, positive samples were also detected in the control (CTRL) leaf and soil samples indicating that cross-contamination occurred at some point during the growing process.

TREATMENT	No. PLANTS TESTED	No. FRUITS SAMPLED	TOTAL GFP POSITIVE	TOTAL GFP NEGATIVE	% GFP POSITIVE
CONTAM SEED	5	68	15	53	22.06
CONTAM LEAF	5	46	9	37	19.57
CONTAM SOIL	2	13	2	11	15.38
CTRL SEED	5	35	0	35	0.00
CTRL LEAF	5	34	1	33	2.94
CTRL SOIL	5	31	1	30	3.23
TOTAL SAMPLED	**27**	**227**	**28**	**199**	**12.33**

Objective 2: Growth of Jensen farm *L. monocytogenes* isolates (from 2011 outbreak) on cantaloupe plant tissues. This objective examined if *L. monocytogenes* could grow and persist on cantaloupe plant tissue. This would indicate that the bacterium is ale to use the plant as a carbon source as would a known plant bacterial pathogen. In addition, the same plant cultivars and *L. monocytogenes* isolates that were implicated in the 2011 Jensen Farms outbreak were used to determine if growth/persistence could have influenced the severity of the outbreak. Following seed contamination, *L. monocytogenes* grew to high levels and persisted through 30 days (Figure 1-4). *L. monocytogenes* 10403S is a laboratory control strain.

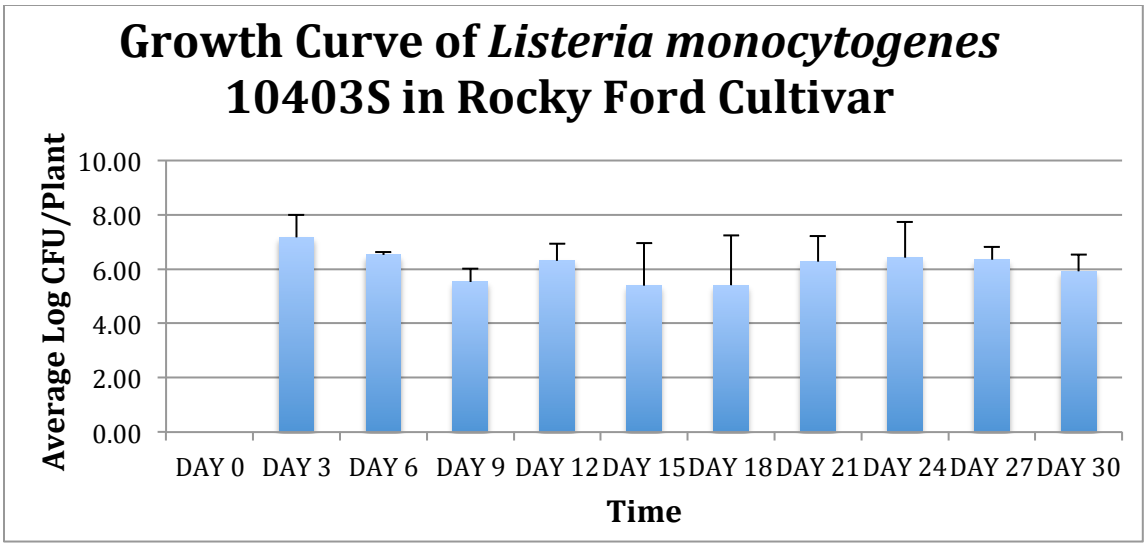

Figure 1: Growth of *Listeria monocytogenes* 10403S laboratory control strain in Azure Dandelion Rocky Ford Green Cantaloupe over a 30-day period. Day 0 was not tested.

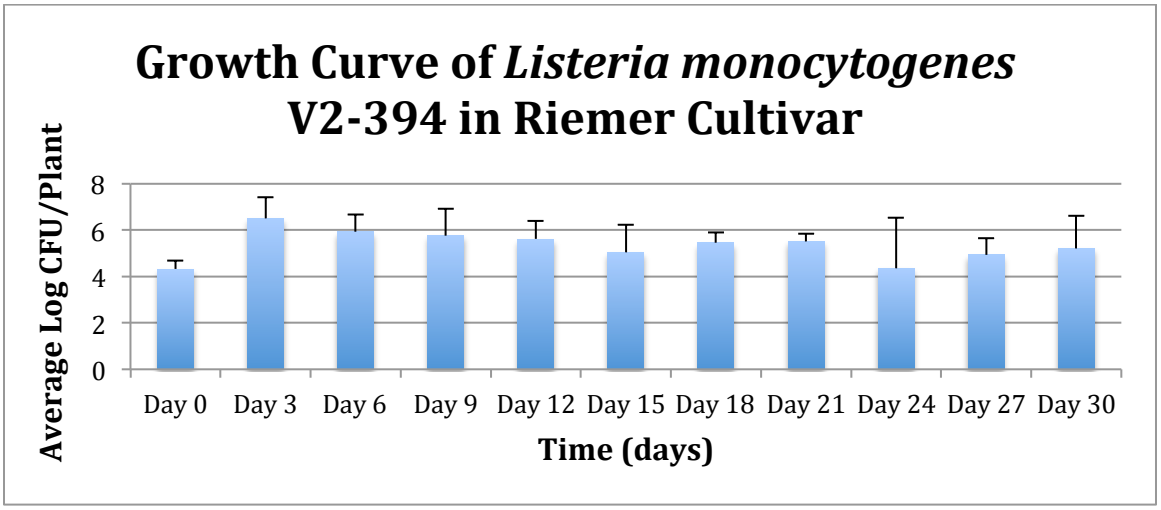

Figure 2: Growth of *Listeria monocytogenes* V2-394 (Jensen Farm isolate) in Riemer Seeds Rocky Ford Cantaloupe over a 30-day period.

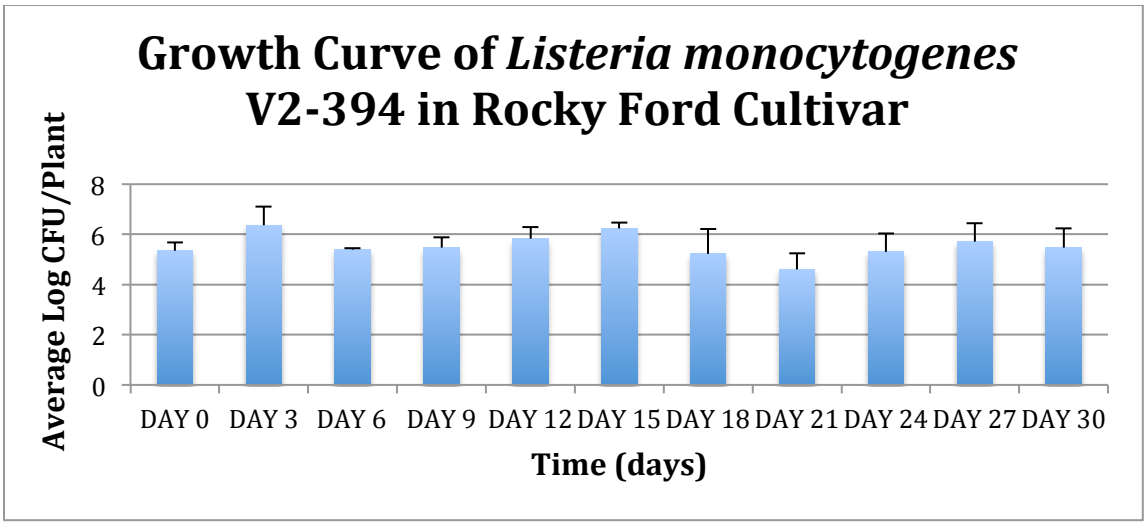

Figure 3: Growth of *Listeria monocytogenes* V2-394 (Jensen Farm isolate) in Azure Dandelion Rocky Ford Green Cantaloupe over a 30-day period.

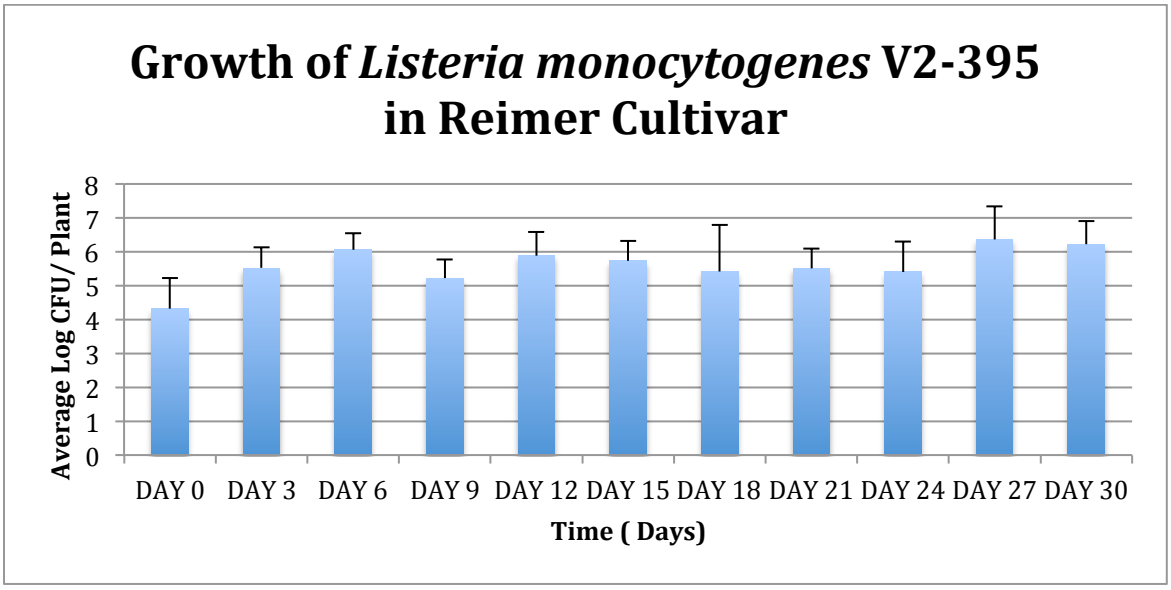

Figure 4: Growth of *Listeria monocytogenes* V2-395 (Jensen Farm isolate) in Reimer Seeds Rocky Ford Cantaloupe over a 30-day period.

Objective 3: Determining the presence of *L. monocytogenes* naturally present on commercial cantaloupe seeds. Control seeds were screened for the presence of *L. monocytogenes* using PCR. The primer pair for the targeted 23S ribosomal RNA gene was selected because it is commonly found in all *Listeria* spp. as it is essential to protein assembly. Listeriolysin O is gene found in *L. monocytogenes* and is responsible, in part, for the pathogenicity of the bacterium in humans. Finally, the sigma B primer set was selected because all *L. monocytogenes* contain this protein as it is necessary for adaptation in stressful environments, as well as contributes to the invasion of human intestinal epithelial cells during pathogenesis.

Table 2: Presence of *Listeria monocytogenes* on negative control Azure Dandelion Rocky Ford Cantaloupe Seeds. Seeds were first screened on modified oxford medium (MOX) to determine presence of *Listeria* spp. and then positive colonies were transferred to *Listeria monocytogenes* plating medium (LMPM). PCR was preformed on all samples that were *Listeria* spp. presumptive positive on MOX. + = growth and color change indicating positive on plate, + / - = growth without any color change, and - = no growth or color change observed.

Seeds	MOX	LMPM	23S	LLO	SIG B
ADPB012	+	+	+	+	**N/A**
AD1	+	+ / -	+	-	-
AD2	+ /-	+ / -	-	-	-
AD3	+ / -	+ / -	+	-	-
AD4	+	+ / -	+	-	-
AD5	+	+ / -	+	-	-
AD6	+ / -	+ / -	-	-	-
AD7	+ / -	+ / -	-	-	-
AD8	+	+ / -	-	-	-
AD9	+	+ / -	-	-	-
AD10	+	+ / -	-	-	-
AD11	+ / -	+ / -	-	-	-
AD12	+ / -	+ / -	-	-	-
AD13	+ / -	+ / -	-	-	-
AD14	+ / -	+ / -	-	-	-
AD15	+ / -	+ / -	-	-	-

Table 3: Presence of *Listeria monocytogenes* on negative control Reimer Seeds Rocky Ford Cantaloupe Seeds. Seeds were first screen on modified oxford medium (MOX) to determine presence of *Listeria* spp. and then positive colonies were transferred to *Listeria monocytogenes* plating medium (LMPM). PCR was preformed on all samples that were *Listeria* spp. presumptive positive on MOX. + = growth and color change indicating positive on plate, + / - = growth without any color change, and - = no growth or color change observed.

Seeds	MOX	LMPM	23S	LLO	SIG B
RSPB102	+	+	+	+	**N/A**
RSPB114	+	+	+	+	**N/A**
RS1	+	+ / -	-	-	-
RS2	+ / -	+ / -	-	-	-
RS3	+ / -	+ / -	-	-	-
RS4	+ / -	+ / -	-	-	-
RS5	+ / -	+ / -	-	-	-
RS6	+	+ / -	-	-	-
RS7	+	+ / -	-	-	-
RS8	+ / -	+ / -	-	-	-
RS9	+ / -	+ / -	-	-	-
RS10	+	+ / -	-	-	-
RS11	+ / -	+ / -	-	-	-
RS12	+ / -	+ / -	-	-	-
RS13	+ / -	+ / -	-	-	-
RS14	+ / -	+ / -	-	-	-
RS15	+ / -	+ / -	-	-	-