CENTER FOR PRODUCE SAFETY
CPS 2009 RFP
FINAL PROJECT REPORT, DUE NOVEMBER 30, 2010

Project Title: Reducing tomato contamination with *Salmonella* through cultivar selection and maturity at harvest

Project Period: October 1, 2009 through October 31, 2010

Principal Investigator
Max Teplitski
Dept. of Soil and Water Sciences
University of Florida
352.273.8189
maxtep@ufl.edu

Co-Principal Investigator
Jason Noel
Dept. of Soil and Water Science
University of Florida
352.273.8195

Objectives
Objective 1: To characterize cultivar-dependent differences in persistence on or inside fruits.

Objective 2: To identify ripeness-dependent differences in the ability of Salmonella to persist in tomatoes.

Project Title
Reducing tomato contamination with *Salmonella* through cultivar selection and maturity at harvest

FINAL REPORT

Abstract
Contamination of vegetables with human enteric pathogens most likely occurs both pre- and post-harvest (even though routes of infection and sources of pathogens in the production environment are still a matter of discussion). The goal of this project was to contribute to the development of strategies for improving produce safety without imposing further regulatory burdens or additional costs on producers. We aimed to test the possibility that there already exist commercial tomato varieties that might differ in their "susceptibility" to contamination with *Salmonella*. If such cultivars or genotypes already exist, future efforts could be made to develop more resistant varieties so as to minimize the contamination of produce with *S. enterica*, much as breeders select for disease-resistant crops varieties. Such future breeding efforts require an easy screen. However, because *Salmonella* contaminates plants without causing visible symptoms or damage during colonization and spread, the selection of "*Salmonella* resistant" plant genotypes is less than straightforward. The first step to really solving this problem was to identify those bacterial genes that are crucial to the ability of *Salmonella* to contaminate and persist in tomatoes and then test whether the corresponding *Salmonella* gene reporters could be used for a direct and straightforward screen of the existing tomato cultivars or maturity stages for those that may be less susceptible to contamination with *Salmonella enterica*. The *overall objective* of this proposal was to test whether it is possible to identify a cultivar and fruit ripeness stage (or their combination) that may be less susceptible to contamination with *Salmonella* or less conducive to the growth of the pathogen. We screened 21 tomato varieties (field and greenhouse grown, at two maturity stages) for their "susceptibility" to the type strain of *Salmonella* and outbreak strains. We discovered that green tomatoes were on average less conducive to proliferation of *Salmonella* within fruits. We also report that some heirloom and commercial tomato varieties are more and/or less conducive to proliferation of *Salmonella*. Quantitative data supporting these findings are presented below.

Background
Based on the USDA ERS data, tomato is the most consumed fresh vegetable: in 2009, Americans purchased 18.7 lbs of fresh tomatoes per capita. Throughout this decade, according to USDA ERS data, California and Florida led the Nation in vegetable and melon farm cash receipts, highlighting the importance of these crops in our states. Florida ranks first (or second, depending on the metric) nationally in the acreage, production, and value of fresh market tomato. In 2004, for example, Florida produced about 1.5 billion pounds of fresh market tomatoes valued at more than $500 million. In the 2007-2008 growing season, 31,500 acres were under cultivation for the fresh tomato market. Fresh market tomatoes comprise about 40% of Florida's fresh market vegetable cash receipts. In Florida, ~33,000 workers are directly involved in tomato production and harvest each year. The issues of food safety represent the greatest threat to sustainability and profitability of Florida tomato industry. Based on USDA ERS data, following the June-August 2008 outbreak of salmonellosis caused by *S. enterica* sv. St. Paul (which was initially wrongly blamed on tomatoes from Florida) the price of tomatoes at

the point of first sale dropped from 56.8 cents/lb in June to 25.6 cents/lb in August. This nearly obliterated tomato industry in the state.

The number of produce-associated outbreaks of salmonellosis is on the rise and is comparable to the outbreaks associated with the consumption of meats and poultry. Furthermore, incidents of salmonellosis caused by *Salmonella* serovars that are commonly isolated from fresh produce have increased by ~40%, based on the CDC data. Despite improvements in GAPs and BMPs, contamination of tomatoes and other produce with non-typhoidal *Salmonella* has resulted in several multi-state and international outbreaks, each causing multi-million dollar damages to the tomato and food industries. This is, perhaps, not surprising considering that in tissues of contaminated tomato fruits, *Salmonella* is capable of building up to high cell numbers, easily reaching 10^5 cells/gram of tissue, levels that are well above those known to cause infections in humans. Furthermore, fruit tissues protect the pathogen from surface sanitation (such as chlorine washes), making it difficult to implement effective fruit wash procedures. Promoting safety of fresh domestic produce will help avoid future attribution errors. Ensuring microbiological safety of tomatoes will benefit millions of consumers, tens of thousands farmers, packers and retailers.

Research Methods and Results

We screened 21 tomato cultivars (at two maturity stages, field and greenhouse-grown) for their "susceptibility" to *Salmonella*. Because *Salmonella* does not cause any obvious symptoms in tomato fruits, "susceptibility" was defined as multiplication of the pathogen in fruits to a level that is above average across cultivars tested. Tomato fruits were inoculated with the type strain of *Salmonella enterica* sv. Typhimurium 14028 and with the six isolates of *Salmonella* (sv. Braenderup, Javiana, Newport) that were isolated either from tomatoes linked to outbreaks of human salmonellosis, or from humans with salmonellosis linked to the consumption of tomatoes or from tomato fields on the Eastern Shore of Virginia. Several thousands tomato infections were performed to obtain statistically significant data.

Results

We identified seven potentially interesting varieties, which were more or less "susceptible" to *Salmonella*. To begin understanding the basis of this phenotype, expression of four *Salmonella* genes was tested in each of these varieties at two maturity stages using *in vivo* expression technology. We pursued both the "resistant" and "susceptible" candidate varieties in order to gain a better understanding of this phenotype. *In vivo* expression technology allows to document and quantify bacterial gene expression within a single cell, however it also requires that bacteria are actively dividing.

1. Screen of tomato cultivars for "susceptibility" to the type strain of *Salmonella enterica* sv Typhimurium 14028.

Project Title

Reducing tomato contamination with *Salmonella* through cultivar selection and maturity at harvest

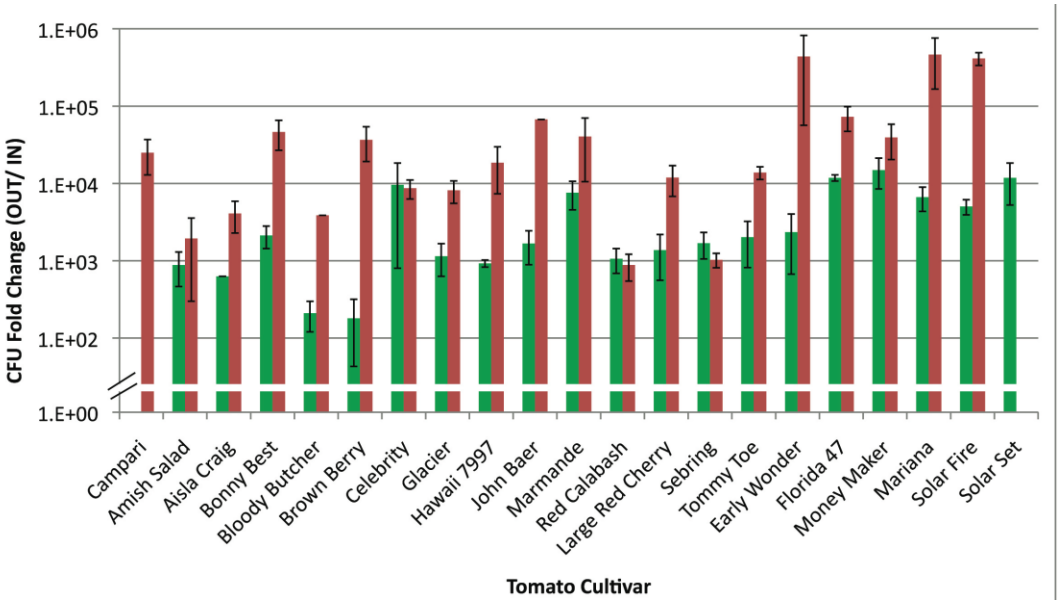

Fig. 1. Proliferation of *Salmonella* 14028 in red ripe and mature green fruits of tomatoes.

Green bars represent data for green tomatoes; red bars represent data for red tomatoes. Error bars are averages of at least 3 technical replications and 3 biological repeats (each cultivar was tested at least 9 times). Tomatoes were harvested from the field in Quincy, FL and from the biocontainment roof-top greenhouse on UF's Gainesville campus. Fruits of Campari tomatoes were purchased at a local supermarket. *Salmonella* was inoculated onto shallow surface puncture wounds made in harvested fruit. Tomatoes were incubated at 20oC (40-60% RH) for a week. Fruits were then stomached in an equal volume of Phosphate-buffered saline, and aliquots were plated onto a selective medium (XLD). *Salmonella* colonies (which appear black on XLD medium) were counted. To account for the differences in tomato sizes, data are presented as increase in *Salmonella* numbers within the fruit.

These results are important because they represent the first systematic screen of tomato commercial and heirloom varieties for their susceptibility to *Salmonella*. As shown in Fig. 1, green tomatoes were on average much less conducive to multiplication of this human pathogen (in green tomatoes, numbers of *Salmonella* increased by 100-1,000 fold; in red ripe tomatoes, *Salmonella* increased by 1,000-1,000,000 fold). Green fruits of heirloom varieties Bloody Butcher and Brown Berry were the least conducive to proliferation of the type strain of *Salmonella*. 100-fold cultivar-dependent differences in proliferation of *Salmonella* within green tomato fruits were observed (Fig. 1). Of the commercial cultivars, green fruits of cv. Sebring and Early Wonder were least conducive to growth of the type strain of *Salmonella*. The type strain of *Salmonella enterica* proliferated the least in the red fruits of heirloom varieties Amish Salad and Bloody Butcher, equally low proliferation was observed in red ripe fruits of cv. Sebring. Red ripe fruits of tomatoes Early Wonder, Mariana and Solar Fire were the most conducive to multiplication of the type strain of *Salmonella enterica* sv. Typhimurium 14028.

1,000-fold differences in the proliferation of the type strain of *Salmonella* in red ripe fruits were observed.

Multiplication of the outbreak strains of *Salmonella enterica* in tomatoes.

To test whether strains of *Salmonella*, which were linked to or recovered from the actual outbreaks of salmonellosis associated with the consumption of tomatoes, the ability of a cocktail of six outbreak strains of *Salmonella* to multiply within green or ripe fruits of tomatoes was measured as above.

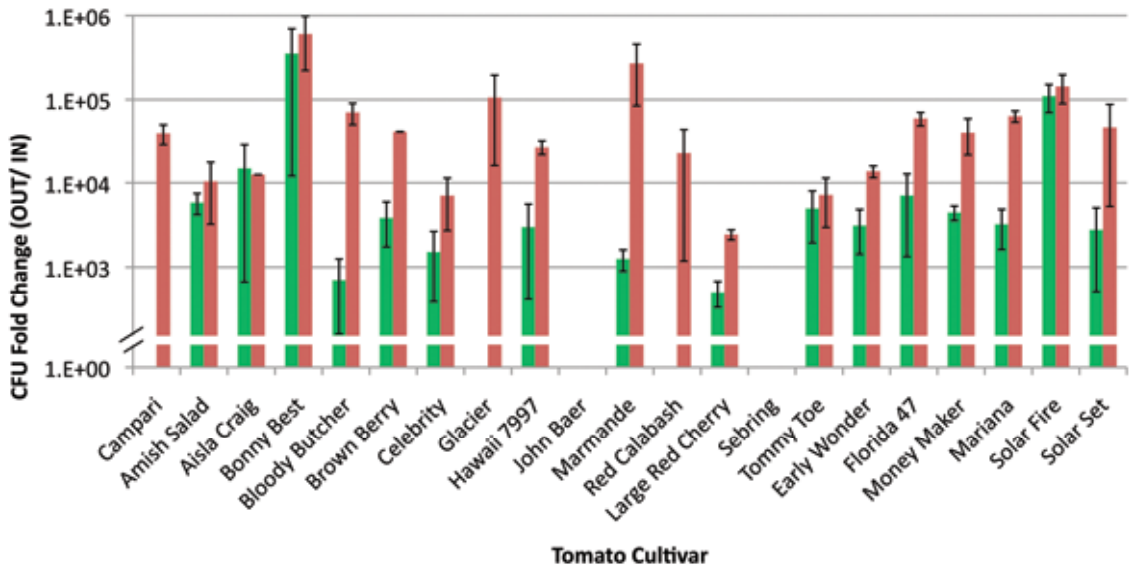

Fig. 2. Proliferation of the outbreak strains of *Salmonella* in greenhouse-grown tomatoes. Data was collected and assembled exactly as in Fig.1. Tomatoes were only harvested from the biocontainment facility. Fruits of Campari tomatoes were purchased at a local supermarket. Analysis of data for varieties John Baer and Sebring is still in progress.

On average, we observed less dramatic (compared to the type strain) difference in the ability of the outbreak strains to proliferate in green tomatoes, compared to red tomatoes. Even though outbreak strains of *Salmonella*, on average, grew somewhat better in tomatoes, the overall final numbers of the pathogen in tomatoes was not higher for the outbreak strains. As with the type strain 14028, less proliferation was observed in green fruit of the heirloom tomato Bloody Butcher. Green fruit of Large Red Cherry tomato were also not very conducive to proliferation of the outbreak strains. With the exception of Solar Fire, all green fruit of commercial tomato varieties had a similar ability to sustain proliferation of the type stain of *Salmonella*. The outbreak strains of *Salmonella* proliferated the least in the red fruit of Large Brown Cherry. Proliferation within red ripe fruits of commercial varieties Celebrity, Early Wonder and Solar Set was similar. Red ripe fruits of tomatoes Solar Fire, Bonny Best, Glacier and Marmande were the most conducive to proliferation of the pathogen.

Salmonella gene expression tomatoes of "resistant" and "susceptible" varieties.

Previously, we have observed that *Salmonella* gene expression differed in tomatoes of different varieties, and also depended on the maturity of the fruit (and the accumulation of specific compounds that depend on fruit ripeness) (Noel et al., 2010). Therefore, we tested whether the observed "susceptibility" or "resistance" of tomatoes to *Salmonella* would also correlate with differences in gene expression in tomato-specific *Salmonella* genes. Using *In Vivo* Expression Technology, we tested regulation of two representative *Salmonella* tomato-specific genes (*cysB, fadH*).

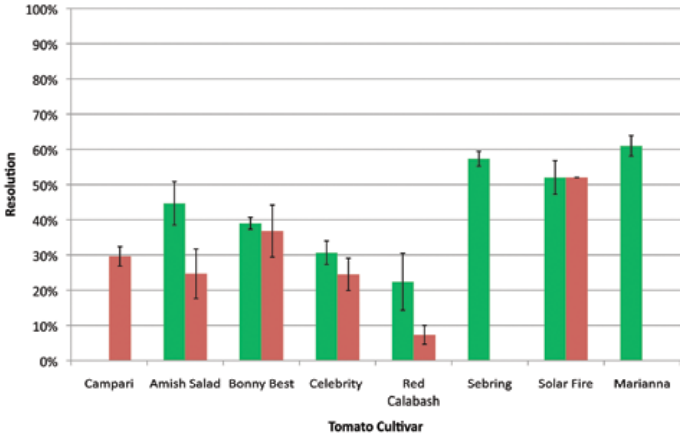

Fig. 3. Expression of the *Salmonella* reporter *cysB* inside green and red tomatoes.

We have previously reported that the expression of the *Salmonella cysB* gene depended on the tomato variety (Noel et al., 2010), even though deletion of *cysB* did not affect the ability of *Salmonella* to proliferate in tomato fruit (Noel et al., 2010). As shown in Fig. 3, we have again observed differences in the regulation of the *Salmonella cysB* gene in tomatoes of different varieties, however differences in *cysB* gene regulation did not correlate with the ability of *Salmonella* to proliferate in tomato fruits (e.g. in fruits of Sebring and Red Calabash *Salmonella* proliferated the same way (Fig.1), however expression of *cysB* was dramatically different.

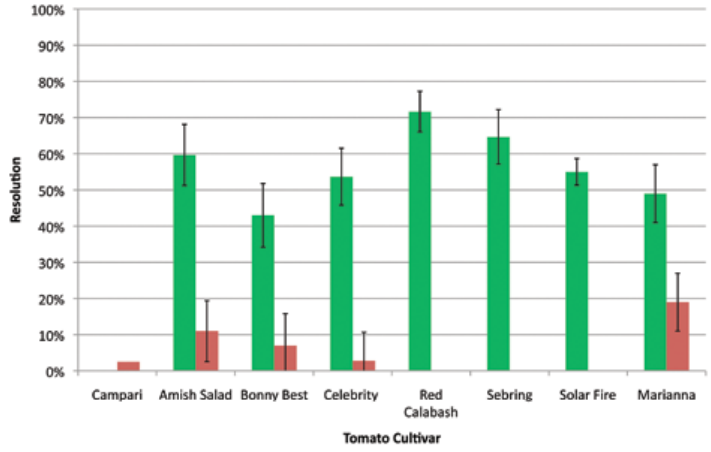

Fig. 4. Expression of the *Salmonella fadH* gene reporter in tomato fruits. We have previously reported (Noel et al., 2010) that expression of *fadH* depended most strongly on the ripeness of the fruit, and was driven by the availability of linoleic acid (which is high in green fruit). Consistently with this previous finding, we note here that the expression of *fadH* depended strongly on the maturity of the fruit. Cultivar-depended difference were statistically insignificant (with the exception of Bonny Best vs Red Calabash). Even though Bonny Best and Red Calabash supported different levels of *Salmonella* proliferation within fruits, no similar trends were observed for other cultivars.

We attempted to "match" a resistant/susceptible tomato cultivar with changes in gene expression of specific *Salmonella* genes. If such a correlation exists, it would make future breeding programs much easier. So far, it does not appear that such a correlation exists. However, we are testing two more *Salmonella* genes for their differential regulation in "susceptible" and/or "resistant" tomatoes.

Outcomes and Accomplishments

This was the first systematic study which surveyed existing commercial and heirloom tomato varieties for their "susceptibility" to *Salmonella*. In this study, we used a prick-inoculation method to mimic the most likely route of contamination of tomatoes under the production conditions. Up until now, no such data existed. When conceiving this project, we aimed to provide producers with the data on which of the already existing tomato varieties may be more or less susceptible to *Salmonella*. This was first such a screen, and was a fairly risky project. We also reasoned that if we were able to find differences in "resistance" to *Salmonella* in tomatoes, this would provide breeders with a list of tomato varieties that could be used in future breeding programs to develop a more *Salmonella*-resistant variety.

Summary of Findings and Recommendations

While these results are not by themselves sufficient to dictate the choice of a cultivar that a producer will plant in a given production season, knowing that fruits of some tomato varieties are more conducive to *Salmonella* proliferation gives the producers the knowledge to make educated risk management decisions. For example, if a more "susceptible" variety is planted, additional care should be taken to cull damaged tomatoes in the field. Alternatively, fruits of the "susceptible" varieties can be harvested at the mature green stage. Producers can also choose to sample smaller batches of "susceptible" tomatoes and larger batches of "resistant" tomatoes during their microbiological surveys. These are not recommendations, rather examples of potential applications of these discoveries.

Even though our screen was limited, it revealed 10-1,000 –fold differences in susceptibility of tomato varieties to *Salmonella*. These differences could now be exploited in tomato breeding programs. We have initiated collaborations with a tomato molecular biologist Dr. J. Giovannoni and tomato breeder, Dr. J. Scott, to more systematically approach this question and learn more about the genetic basis of this phenotype.

Project Title
Reducing tomato contamination with *Salmonella* through cultivar selection and maturity at harvest

APPENDICES

Publications and Presentations

Publications:

1. Noel, J.T., Arrach, N., Alagely, A., McClelland, M., Teplitski, M. 2010. Specific responses of *Salmonella enterica* to tomato varieties and fruit ripeness identified by *in vivo* expression technology. *PLoS One* **5**: e12406
2. Noel, J.T., Joy, J., Smith, J.N., Ahmer, B.M., Schneider, K.R., and Teplitski, M. 2010. *Salmonella* SdiA recognizes *N*-acyl homoserine lactone signals from *Pectobacterium carotovorum in vitro*, but not inside a tomato soft rot. *Mol Plant Microbe Interact* **23**:273-282
3. Noel, J., Alagely, A., McClelland, M., and Teplitski, M. 2010. Insight into the functional genomics of *Salmonella*-tomato interactions. *Abst Gen ASM Mtng* **110**: p. 56, N-329

Presentations:

1. Teplitski, M. (Oral Presentation) Cultivar selection, maturity at harvest and susceptibility of tomatoes to contamination with *Salmonella*. Produce Marketing Association Fresh Summit 2010 (trade show). October 15-18, 2010. Orlando, FL
2. Teplitski, M. (Oral Presentation) Tomatoes and *Salmonella*: How realistic is breeding for "resistance" to human enteric pathogens. 25th Annual Tomato Disease Workshop. November 17, 2010. Wimauma, FL
3. Ericson, K.J., Noel, J.T., and Teplitski, M. The role of acid stress response in *Salmonella enterica* sv Typhimurium during interactions with tomato fruits. Presented at 2010 Florida Association for Food Protection Annual Education Conference, May 4-6, 2010. Sunny Isles Beach (Miami), FL.
4. Zaragoza, W., and Teplitski, M. The role of cellulose in attachment to tomato surfaces by *Salmonella enterica.* Presented at 2010 Florida Association for Food Protection Annual Education Conference, May 4-6, 2010. Sunny Isles Beach (Miami), FL.
5. Teplitski, M. (Seminar). Plant-bacterial interactions: from rhizosphere biology to food safety. North Florida Research and Education Center, Quincy, FL. October 26, 2010.
6. Teplitski, M. (Seminar) Functional genomics of *Salmonella*-tomato interactions: from microbial ecology to food safety. Georgetown University, Washington, DC. Feb 4, 2010
7. Teplitski, M. (Seminar) *Salmonella*-tomato interactions and food safety. Food and Drug Administration, Office of Regulatory Science, Division of Microbiology, College Park, MD, Oct 8, 2009
8. Ericson, K., Noel, J., Teplitski, M. 2010. The role of acid stress response pathways in *Salmonella* persistence in tomatoes. 1st Annual Microbiology and Cell Science Undergraduate Research Symposium. Gainesville, FL. April 22, 2010.

Project Title
Reducing tomato contamination with *Salmonella* through cultivar selection and maturity at harvest

News Releases:

UF-IFAS News Release (Sept 21, 2010)
http://news.ufl.edu/2010/09/21/salmonella/
Mutlimedia
http://news.ufl.edu/2010/09/21/salmonella-multimedia/uf-research-finds-salmonella-responds-differently-to-tomato-varieties-ripeness/
Orlando Sentinel (Sept 29, 2010)
http://articles.orlandosentinel.com/2010-09-29/features/os-heather-salmonella-resistant-tomat20100929_1_salmonella-contamination-florida-tomato-committee-tomato-variety
Fresh Plaza News
http://www.freshplaza.com/news_detail.asp?id=69164
Gator Country (an ESPN Affiliate)
https://www.gatorcountry.com/gatorbeat/University/UF_research_finds_salmonella_responds_differently_to_tomato_varieties_ripeness/related_links
GrowingProduce.Com
http://www.growingproduce.com/news/?storyid=4366
Southeast Farm Press
http://southeastfarmpress.com/vegetables/tomato-variety-influences-salmonella-problems?page=2
Florida Magazine
http://staging.ufalumnimagazines.com/florida/features/winter-2010/out-of-date/

Budget Summary (required)

Tables and Figures (optional)

Suggestions to CPS (optional)
None