CPS-WCFS 2013 RFP
FINAL PROJECT REPORT

Project Title

Does splash from overhead sprinkler irrigation systems contaminate produce with *Salmonella* in the southeastern United States?

Project Period

July 1, 2013 – June 30, 2014

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Objectives

1.) Quantify the levels of Salmonella*, soil, and organic matter in splash resulting from solid set impact sprinkler systems and center pivot irrigations systems.*

2.) Determine if Salmonella *persists on the crop until harvest in greater numbers in the presence of organic matter and soil that is deposited on the produce by irrigation water splash.*

Funding for this project provided by the Center for Produce Safety through:
The Western Center for Food Safety

George Vellidis, University of Georgia
Does splash from overhead sprinkler irrigation systems contaminate produce with Salmonella in the southeastern United States?

FINAL REPORT

Abstract

The overall goal of this proposal was to develop knowledge which will allow vegetable producers who rely on untreated surface sources of irrigation water coupled with overhead sprinkler irrigation to effectively address recently proposed FDA rules. In January 2013, the FDA proposed that all *agricultural water* be *safe* for its intended use (proposed § 112.41.) FDA's definition of *agricultural water* is water that comes into *direct contact* with produce and includes irrigation water that is applied using direct water application methods such as overhead sprinkler irrigation. Companion CPS-funded studies conducted in the southeastern United States by members of our project team have consistently found measurable concentrations of *Salmonella* and other pathogens in ponds used to irrigate fruits and vegetables. Members of the project team also conducted a pilot study to assess the presence of *Salmonella* in irrigation water in four different irrigation systems on three farms. *Salmonella* and generic *E. coli* were found in samples collected from all three irrigation systems fed by pond water. These findings are intriguing and a more comprehensive study is underway to draw defensible conclusions. However, that project does not address potential contamination by irrigation-generated splash. In the southeastern Coastal Plain, overhead sprinkler irrigation is most frequently used with leafy greens, cabbage, cantaloupe, and watermelons, some of which are highly susceptible to contamination from splash. During our pilot irrigation water study, we observed that overhead sprinkler irrigation systems apply irrigation water at very high rates and this quickly overwhelms the infiltration capacity of the soils. As a result, irrigation water ponds on the soil surface and begins to run off. As more irrigation water is applied, the ponded water and saturated surface soils begin to splash.

With that in mind, the objective of this study was to better understand whether *Salmonella* contamination can stem from splash associated with farm soils, directly-applied irrigation water, or both. We measured *Salmonella,* generic *E. coli*, and total suspended solids concentrations in overhead sprinkler irrigation water reaching the crop as well as in splash generated by the irrigation water. We also measured *Salmonella* concentrations on produce and associated it with measured organic matter and soil residues deposited on the produce by splash.

Based on field testing, we selected 2, 4, 8, 16, and 32 inches above the soil surface as the 5 heights at which to install the samplers during sampling. The vertical increments were designed to allow us to discriminate between the quality of the irrigation water alone (top sampler, 32 in), the quality of irrigation water + associated splash (ground-level sampler, 2 in) and the height to which splash has an effect (intermediate heights). The sampling methodology we developed successfully provided us with a vertical gradient of splash contamination. This is evidenced by the clear trend of decreasing total suspended solids concentrations with height. It is not clear if our tallest sampling height (32in) was high enough to eliminate the possibility of splash contamination because the mean total suspended solids concentration at this height is slightly higher than that of pond water. If a follow-up study is conducted, an additional sampler should be installed at 64in. The *Salmonella* data are inconclusive. However, because three of the four positive samples were from the sampling pans at 2 and 4 inches, the results indicate that irrigation water splash may be more frequently positive than irrigation water itself. A more comprehensive study that includes the entire summer growing season is needed to answer this question conclusively.

Background

Irrigation is an essential component of fruit and vegetable production. Yet irrigation water has been shown to be a vector for the contamination of fresh produce by pathogenic bacteria and has been implicated in outbreaks of foodborne human disease and deaths (CDC, 2008; Greene et al., 2008). The FDA recognizes this and in its recently released "Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption", it proposes to establish the requirement that all *agricultural water* must be *safe* and of adequate sanitary quality for its intended use (proposed § 112.41.) FDA's definition of *agricultural water* is water that comes into *direct contact* with produce and includes irrigation water that is applied using direct water application methods such as overhead sprinkler irrigation. It does not include indirect water application methods such as drip or furrow irrigation. However, the proposed rules do acknowledge that use of contaminated water in drip or furrow irrigation may still serve as a vehicle for bringing contaminants into the growing environment, which may potentially be transferred to produce by rain splash, workers, or equipment. Proposed § 112.42 and § 112.43 would require producers to ensure that their water sources (including ponds) are safe, that the sources' watersheds are protected, and that their water distribution systems (such as irrigation systems) are free of pathogens. Proposed §112.45(b)(1) requires weekly sampling during the growing season of any untreated surface water (for example, a river or pond) which receives precipitation-driven surface runoff and is used as agricultural water. FDA also proposes that agricultural water be considered *safe* if test results do not exceeded 235 CFU per 100 mL generic *E. coli.* FDA seeks comment on these proposed rules.

Although data may be available with which to respond to the FDA proposals on *safe agricultural water* for some parts of the United States, there are still significant knowledge gaps in the southeastern United States that prevent us from doing so. Before a science-based response can be formulated, this knowledge gap must be closed. This project addresses this knowledge gap on *safe agricultural water* and specifically addresses irrigation water used by vegetable producers in the southeastern United States. The information from this region will also be relevant to other agricultural regions of the United States that rely on untreated surface water and on-farm reservoirs as sources of irrigation water.

The southeastern Coastal Plain (SECP) is an ecoregion which spans portions of Louisiana, Mississippi, Tennessee, Alabama, Florida, Georgia, South Carolina, North Carolina, and Virginia. It is an important vegetable production area of the United States with a long growing season that allows for at least two vegetable crops per year in most areas. Southern Georgia is in the heart of SECP and has been identified by federal agencies and researchers as being representative of the agricultural practices, climate, and water resources of the SECP (Jang et al., 2013; Cho et al., 2010; Sheridan et al. 1992).

In the SECP, a variety of irrigation sources are used by vegetable producers with the most common source a constructed farm pond. These ponds are typically created by damming a 2nd or 3rd order stream. During the growing season, the ponds serve as source waters for on-farm irrigation systems. They are replenished by the stream, direct surface runoff during precipitation events, and sometimes by ground water from nearby wells. Even when a ground water well is available, vegetable producers frequently irrigate directly from the pond because they can withdraw water from the pond at a much higher capacity than from the well. If the water is withdrawn for use in drip irrigation, it passes through a series of sand filters before entering the piping of the irrigation system. If the water is withdrawn for use in overhead sprinkler irrigation systems (center pivot or solid set), then it enters the piping of the irrigation system directly without any filtration.

Two recently completed CPS-funded studies which were conducted in the SECP consistently found measurable concentrations of *Salmonella*, shiga toxin-producing *E. coli* (STEC) (Gu et al., 2013a), and *Campylobacter jejuni* (Gu et al., 2013b) in water samples collected from ponds used for irrigation. During the 2012 spring/summer and the summer/autumn vegetable growing seasons in southern

Georgia we conducted a pilot study to assess the presence of *Salmonella* in irrigation water exiting four different irrigation systems used to irrigate tomatoes, squash, peppers, eggplant, cantaloupe, and two types of leafy greens on three farms. *Salmonella* and generic *E. coli* were found in samples collected from all three irrigation systems fed by pond water but not in irrigation systems fed by well water. These findings are intriguing and a more comprehensive study is underway to draw defensible conclusions. However, that project does not address potential contamination by irrigation-generated splash. In the southeastern Coastal Plain, overhead sprinkler irrigation is most frequently used with leafy greens, cabbage, cantaloupe, and watermelons, some of which are highly susceptible to contamination from splash. During our pilot irrigation water study, we observed that overhead sprinkler irrigation systems apply irrigation water at very high rates and this quickly overwhelms the infiltration capacity of the soils. As a result, irrigation water ponds on the soil surface and begins to run off. As more irrigation water is applied, the ponded water and saturated surface soils begin to splash.

With that in mind, the objective of this study was to better understand whether *Salmonella* contamination can stem from splash associated with farm soils, directly-applied irrigation water, or both. We measured *Salmonella,* generic *E. coli*, and total suspended solids concentrations in overhead sprinkler irrigation water reaching the crop as well as in splash generated by the irrigation water. We also measured *Salmonella* concentrations on produce and associated it with measured organic matter and soil residues deposited on the produce by splash.

Research Methods and Results

We spent the first four months of the project developing and assessing the methods we used for collecting samples and the methods we used for storing, resuscitating, and analyzing produce samples after they were collected.

Objective 1 – Quantifying the Levels of *Salmonella* in Splash

We evaluated a number of different potential samplers for capturing both the irrigation water from the overhead sprinklers and the splash resulting from the irrigation water droplets. Some were fabricated in-house while others were purchased. In the end, we found that the best and most cost-effective sampler was an industrial-style aluminum baking pan. The pans are square (12 × 12 inches), not tapered, and 4 inches deep (Figure 1). The 144 square inch surface area allows us to capture enough sample volume for our analyses even at small irrigation application depths. We removed a ½ inch lip around the top edges of the pans to ensure that it did not intercept any splash.

Based on field testing, we selected 2, 4, 8, 16, and 32 inches above the soil surface as the 5 heights at which to install the sampling pans during sampling rather than the 6, 12, 18, 24, and 48 inches suggested in the proposal. To sample at 2in above the soil surface requires the sampling pan to be recessed 2 inches into the soil. To sample at 4in above the soil surface simply requires that the sampling pan be placed on the soil surface. We then fabricated steel stands to support the sampling pans at 8, 16, and 32 inches above the soil surface (Figure 1). The vertical increments allow us to discriminate between the quality of the irrigation water alone (top sampling pan, 32 in), the quality of irrigation water + associated splash (ground-level sampling pans, 2 in) and the height to which splash has an effect (intermediate heights).

We selected a vegetable farm near Tifton, Georgia on which to conduct our study. The farm was a partner in other past and ongoing CPS-funded projects. We selected three fields, all of which are irrigated from a 1.4 ac pond which has been used for the other studies and is consequently well characterized and understood. Approximately 38% of samples collected from this pond over an 18 month sampling period in 2012 and 2013 were positive for *Salmonella*. The mean *Salmonella* concentration of those samples however was below 0.1 MPN/100mL. Two of the study fields are

irrigated by a center pivot irrigation system while the third is irrigated by a solid set irrigation system (Figure 2).

The selected fields were fallow during the late summer-early fall growing season. We began sampling on 02 December 2013 with the goal of conducting three sampling events per crop cycle. However extremely wet and cold conditions during the winter and early spring months greatly reduced the number of irrigation events scheduled by the farm. This required us to expand the project from two crop cycles to three crop cycles in order to collect the number of samples indicated in the proposal. In the end we sampled broccoli twice during December 2013, mustard greens once during April 2014 and cantaloupe three times during May and June 2014. Table 1 summarizes our sampling schedule and also presents the results for *Salmonella*, generic *E. coli*, and total suspended solids. We also collected samples during three rain events in January, February, and April. These results are summarized in Table 2.

Water Sample Collection and Analysis

During sampling, we installed five sampling pans at each height so a total of 25 sampling pans were deployed during each sampling event. The sampling pans were installed in groups or nodes of five. Each node contained a sampling pan at 2, 4, 8, 16, and 32 inches but their positions within the node were randomized. The nodes were deployed to represent a large area of the field. Under the pivot they were deployed to capture samples from 5 different spans of the pivot. Under the solid set system, we selected two blocks of the field with the most uniform coverage by the sprinklers, as indicated from aerial photographs. Within these two blocks, the location of the five nodes was randomly located (Figure 3). The location of each sampling pan was marked with a flag and sampling pans were reinstalled in the same location at subsequent sampling events. However the height of the sampling pan at that location was randomly selected for each event.

The sampling pans were installed in the study fields prior to an irrigation event and used to capture irrigation water and water splashing from the soil surface during the irrigation event. After the irrigation event was completed (or the pivot had moved past the sampling pans), water and any solids collected in the sampling pans was poured into a sterile sample bottle and returned to the lab for analysis (Figure 4). At the laboratory, the replicates from each height were composited. Only one composite sample was analyzed per height.

During the irrigation event, we collected a water sample from the pond near the intake of the irrigation system (Figure 5). We also used a multiparameter water quality sonde to measure *in situ* temperature, pH, dissolved oxygen concentration, turbidity, and specific conductivity at the same time.

All samples were analyzed for *Salmonella*, *E. coli*, Total Coliform, and total suspended solids under the supervision of Ms. Debbie Coker – our Water Quality Laboratory's manager. A novel cross-streaking method developed by Dr. Anita Wright (Luo et al., 2014) was used to isolate, confirm, and enumerate *Salmonella*. The method uses a MPN protocol with three dilutions cultured in enrichment broth, selective broth, and plated on two types of selective media. With the dilutions we were using, our lower detection limit was 0.0548 MPN/100mL and our upper detection limit was 11 MPN/100mL. All presumptive positive *Salmonella* colonies were confirmed by PCR targeting the InvA gene in Dr. Sree Rajeev's laboratory at the University of Georgia's Veterinary Diagnostic Laboratory in Tifton. The IDEXX Colilert reagent and the Quanti-tray system were used to analyze water samples for *E. coli* (IDEXX Laboratories, 2013).

Water Sample Results – *Salmonella* and Generic *E. coli* from Irrigation and Irrigation Splash

As Table 1 indicates, four of 30 (13%) of the composited samples resulting from irrigation and irrigation splash were positive for *Salmonella* during the study. None of the six pond samples collected during irrigation were positive. There were no positive irrigation or irrigation splash samples during the December sampling, two during the April sampling, and two more during the June sampling events. One

positive sample was collected at the 2in height, two at the 4in height, and one at the 32in height. Past experience with pond and irrigation water sampling indicates that the lowest concentrations and percent positives are found in winter so the trend found here matches previous studies. The number of positives measured during the spring and summer sampling events (four of 20 samples positive or 20%) is in the same range but lower than that measured during the pilot irrigation water study conducted during the summer months of 2012 during which 8 of 27 (30%) samples were positive.

Four of 30 (13%) samples resulting from irrigation and irrigation splash were positive for generic *E. coli* during the study (Table 1). This is the same number of positives as for *Salmonella* but the samples in which the *E. coli* positives were found were not the same as those for *Salmonella*. Three of the four samples positive for generic *E. coli* were collected during the final sampling event on 30 June 2014 and they came from the three highest sampling locations. Because of canopy cover, not enough sample was collected at the 2in and 4in heights for this analysis (priority was given to *Salmonella* analyses.) Pond samples were positive for generic *E. coli* during the final three sampling events, which coincided with warmer temperatures. In all cases, the concentration of the positive samples was low and much lower than the limit proposed by FDA.

We analyzed the data using a variance generalized linear model (GLM) procedure followed by a means separation least significant difference (LSD) test. There were no statistical differences between the results from irrigation and irrigation splash for *Salmonella*, as indicated in Table 3, nor for generic *E. coli*, as indicated in Table 5, but our sample sizes were low so we probably did not have the power to detect a difference. Although not conclusive, because three of the four positive *Salmonella* samples were from the sampling pans at 2 and 4 inches, the results may indicate that irrigation water splash may be more frequently positive than irrigation water itself. A more comprehensive study that includes intensive sampling during the entire summer growing season is needed to answer this question conclusively.

Water Sample Results – *Salmonella* and *E. coli* from Rain and Rain Splash

Two of the three pond samples collected during the rain event sampling were positive for *Salmonella*. No other samples were positive (Table 2). The concentration of the pond water collected during rainfall events was significantly different from the rain and rain splash samples (Table 4). This is consistent with findings from an earlier CPS-funded runoff study in which pond *Salmonella* and *E. coli* concentrations were consistently higher after runoff events. All three rain and rain splash samples from the 2in and 4in sampling pans were positive for generic *E. coli* (Table 2), while none of the higher sampling pans were positive, indicating that perhaps splash may indeed be a vector for contamination.

Water Sample Results – Total Suspended Solids

Total suspended solids mean concentrations were much larger at 2in and 4in (Tables 7 and 8) than at the other three heights. There was a clear trend of decreasing concentration with height. The pond samples had the lowest mean concentrations. These data indicate that soil particles and other solids are detached from the soil by irrigation splash and can contaminate produce at least 16in and perhaps as high as 32in above the soil surface. Because the very high variability in concentrations from one sampling event to the next and the relatively small number of samples resulted in a high standard deviation, only the samples collected from 2in were statistically significantly different. It was somewhat surprising to find that mean concentrations from the irrigation events (Table 7) were considerably higher than mean concentrations from rain events (Table 8).

Objective 2 – Produce Sampling

At the end of each growing cycle, we collected produce samples for analyses. The produce samples were harvested from the same areas in which the sampling pan nodes had been installed, at randomly selected distances and directions from the sampling pan nodes . A duplicate sample was also collected

from one of the node areas. This resulted in six produce samples being collected during each produce sampling event. The samples were harvested in Whirl-pak bags (Figure 6). The produce was sampled either immediately before or during commercial harvest operations. Broccoli samples were harvested on 13 February 2014, mustard greens were harvested on 08 April 2014, and cantaloupes were harvested on 02 July 2014.

Once collected the produce samples were put on ice (Figure 6) and transported to Dr. Karen Levy's laboratory at Emory University within 24 hours of harvest for analysis. Because we were sampling commercial fields, to avoid regulatory compliance issues, pathogen testing of the produce samples was delayed for 90 days by using a storage and resuscitation procedure developed from Gorski et al. (2011). When the samples arrived at the laboratory 2L of peptone were added to the bag to wash the produce (30s shaking, 60s massaging, 30s shaking). The rinseate was then poured into 9 replicate bottles that contain lactose broth. 3 bottles with 500mL of lactose and 500mL of rinsate, 3 bottles with 100mL of lactose and 100mL of rinsate, and 3 bottles with 10mL of lactose and 10mL of rinsate. Samples were incubated for 24 hours at 37C. These methods mirror those used for water samples. After 24 hours, 8.5mL of each incubated sample was mixed with 1.5 mL of glycerol and then frozen at -80C for 90 days. After the 90 day waiting period, samples were thawed to room temperature for 2 hours and incubated at 37C for 2 hours. Then, 1mL of sample was added to 10mL of tetrathionate. The sample was incubated at 37C for 24 hours and then streaked onto XLT4 plates and analyzed for *Salmonella*, as described earlier (Figure 7). Optimization of these produce sampling and resuscitation protocols was the subject of Emory Master in Public Health student Whitney Pennington's thesis.

Produce Sampling Results

To date only the results from the broccoli and mustard greens sampling are available as the samples from the cantaloupe are still frozen. All six broccoli produce samples were negative for *Salmonella*. Likewise, all six mustard green samples were negative for *Salmonella*. These results were not surprising as only two of 15 sampling pan water samples were positive for *Salmonella* and the concentration of those positive samples was very small (Table 1). *We will send CPS a supplement to this report when we have received all the produce sample results.*

Outcomes and Accomplishments

This project provided many challenges, ranging from logistical to scientific, but resulted in a series of positive outcomes and accomplishments. With support from the Center for Produce Safety, we developed a strong and dynamic multi-state, multi-institutional team dedicated to developing knowledge, which will allow vegetable producers who rely on untreated surface sources of irrigation water to effectively address recently proposed FDA rules. The partner institutions include the University of Georgia, Emory University, and the Western Center for Food Safety. The team consisted of microbiologists, water quality experts, hydrologists and a vegetable producer in southern Georgia. The partnerships and trust we developed with the vegetable producers will be long-lasting and will allow us to conduct important on-farm projects in the future. In addition, we trained several young scientists during the project. Two graduate students (two at Emory and one at the University of Georgia) were involved with several components of the project and were critical in developing the methods used. We also employed a post-doctoral researcher, two undergraduate student workers, a field technician and a lab analyst.

We developed innovative analytical techniques for the laboratory and innovative sampling techniques for the field which will make future projects easier, more cost-effective, and more productive. We were excellent stewards of the Center for Produce Safety funds provided to us for this project. We successfully completed the goals of our proposal even though we were faced with a series of operational obstacles such as fields being left fallow, severe cold, and extremely rainy conditions.

Summary of Findings and Recommendations

- The sampling methodology we developed successfully provided us with a vertical gradient of splash contamination. This is evidenced by the clear trend of decreasing total suspended solids concentrations with height.
- It is not clear if our tallest sampling height (32in) was high enough to eliminate the possibility of splash contamination because the mean total suspended solids concentration at this height is slightly larger than that of pond water. If a follow-up study is conducted, an additional sampler should be installed at 64in.
- The *Salmonella* data are inconclusive. However, because three of the four positive samples were from the sampling pans at 2 and 4 inches, the results may indicate that irrigation water splash may be more frequently positive than irrigation water itself. The *E. coli* data also support this conclusion.
- Data from rain events more clearly indicate the effect of splash because they avoid the noise caused by irrigation water which is occasionally positive for pathogens and indicator organisms.
- A more comprehensive study that includes the entire summer growing season is needed to answer this question conclusively. If a more comprehensive study is conducted, the sampler design should be modified to capture splash only and not irrigation water and splash. In addition, future studies should collect concurrent soil samples and analyze them for *Salmonella*.

References

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APPENDICES

Publications and Presentations

Presentations

To date, we have made two presentations on this project:

- Oral presentation at the 2014 Center for Produce Safety Symposium
- Oral presentation at the workshop on Survival of Foodborne Pathogens in Pre-harvest and Postharvest Produce Environments: Research Funded by the Western Center for Food Safety

Planned presentations include:

- January 2015 Southeast Regional Fruit and Vegetable Conference. This conference is the largest educational conference and trade show in the southeastern United States that unites growers, vendors and suppliers.

Publications

None to date. We are assessing whether this data set is publishable in a scientific journal.

Budget Summary

The funds provided by the Center for Produce Safety were appropriate for the effort required to accomplish the project's objectives. Funds were expended as indicated below.

Expenditures 01 July 2013 – 30 June 2014		
Total Salaries	$	19,121.78
Total Benefits	$	6,703.76
Travel	$	0
Operating*	$	32,646.46
Indirect Costs	$	23,389.00
Total Expenditures	$	81,861.00

*Operating includes Supplies and Materials, Subaward to Emory University, and Other Costs.

Tables and Figures

Table 1. *Salmonella, E. coli* and total suspended solids concentrations from samples resulting from irrigation and irrigation splash. NS indicates no sample.

Date	Crop	Height (inch)	*Salmonella* (MPN/100mL)	*E. coli* (MPN/100mL)	Suspended Solids (mg/L)	Temp (°C)
2-Dec-2013	Broccoli	Pond	0	0	0.8	11.9
		2in	0	0	220.6	
		4in	0	2	85.8	
		8in	0	0	19.4	
		16in	0	0	2.9	
		32in	0	0	0.9	
18-Dec-2013	Broccoli	Pond	0	0	1.4	11.7
		2in	0	0	112.5	
		4in	0	0	33.7	
		8in	0	0	5.9	
		16in	0	0	2.6	
		32in	0	0	2.1	
1-Apr-2014	Mustard Greens	Pond	0	0	1.9	21.66
		2in	0	0	74.5	
		4in	0.056	0	12.4	
		8in	0	0	4.4	
		16in	0	0	2.9	
		32in	0.064	0	5.1	
20-May-2014	Cantaloupe	Pond	0	2.5	1.3	25.2
		2in	0	0	707.5	
		4in	0	0	556.3	
		8in	0	0	NS	
		16in	0	0	24.0	
		32in	0	0	9.4	
5-Jun-2014	Cantaloupe	Pond	0	9.1	1.5	22.3
		2in	0	0	881.8	
		4in	0.064	0	473.6	
		8in	0	0	38.4	
		16in	0	0	8.5	
		32in	0	0	3.1	
30-Jun-2014	Cantaloupe	Pond	0	6.9	1.3	29.8
		2in	0.17	NS	NS	
		4in	0	NS	NS	
		8in	0	14.8	5.2	
		16in	0	57.3	NS	
		32in	0	5.2	NS	

Table 2. *Salmonella, E. coli* and total suspended solids concentrations from samples resulting from rain and rain splash.

Date	Crop	Height (inch)	*Salmonella* (MPN/100mL)	E Coli (MPN/100mL)	Suspended Solids (mg/L)	Temp (°C)
14-Jan-2014	Broccoli	Pond	0	1	1.5	11.9
		2in	0	2	31.2	
		4in	0	1	9.7	
		8in	0	0	2.7	
		16in	0	0	0.6	
		32in	0	0	0.6	
12-Feb-2014	Broccoli	Pond	0.16	7.4	1.9	11.7
		2in	0	1	94.0	
		4in	0	1.5	11.3	
		8in	0	0	2.7	
		16in	0	0	0.9	
		32in	0	0	9.8	
8-Apr-2014	Mustard Greens	Pond	0.056	11	1.2	29.8
		2in	0	67.2	85.5	
		4in	0	5.2	17.2	
		8in	0	0	5.1	
		16in	0	0	3.9	
		32in	0	0	1.9	

Table 3. Mean *Salmonella* concentrations from samples resulting from irrigation and irrigation splash.

Means with the same letter are not significantly different.			
T Grouping	Mean (MPN/100mL)	N	Height (inches)
A	0.028	6	2
A			
A	0.020	6	4
A			
A	0.011	6	32
A			
A	0.000	6	16
A			
A	0.000	6	8
A			
A	0.000	6	Pond

Table 4. Mean *Salmonella* concentrations from samples resulting from rain and rain splash.

Means with the same letter are not significantly different.			
T Grouping	Mean (MPN/100mL)	N	Height (inches)
A	0.072	3	Pond
B	0.000	3	2
B			
B	0.000	3	4
B			
B	0.000	3	8
B			
B	0.000	3	16
B			
B	0.000	3	32

Table 5. Mean Generic *E. coli* concentrations from samples resulting from irrigation and irrigation splash.

Means with the same letter are not significantly different.			
T Grouping	Mean (MPN/100mL)	N	Height (inches)
A	9.551	6	16
A			
A	3.076	6	Pond
A			
A	2.467	6	8
A			
A	0.867	6	32
A			
A	0.400	5	4
A			
A	0.000	5	2

Table 6. Mean Generic *E. coli* concentrations from samples resulting from rain and rain splash.

Means with the same letter are not significantly different.			
T Grouping	Mean (MPN/100mL)	N	Height (inches)
A	23.41	3	2
A			
A	6.467	3	Pond
A			
A	2.567	3	4
A			
A	0.000	3	8
A			
A	0.000	3	16
A			
A	0.000	3	32

Table 7. Mean suspended solids concentrations from samples resulting from irrigation and irrigation splash.

Means with the same letter are not significantly different.				
T Grouping		Mean (mg/L)	N	Height (inches)
	A	417.1	5	2
	A			
B	A	231.4	5	4
B				
B		14.7	5	8
B				
B		8.2	5	16
B				
B		4.1	5	32
B				
B		1.3	6	Pond

Table 8. Mean suspended solids concentrations from samples resulting from rain and rain splash.

Means with the same letter are not significantly different.			
T Grouping	Mean (mg/L)	N	Height (inches)
A	67.2	3	2
B	12.4	3	4
B			
B	4.0	3	32
B			
B	3.5	3	8
B			
B	1.8	3	16
B			
B	1.5	3	Pond

Figure 1. Square aluminum pans were used as samplers for the project. The rim of the pans was installed at five different heights above the soil surface. For each sampling event, there were five replicates of each height.

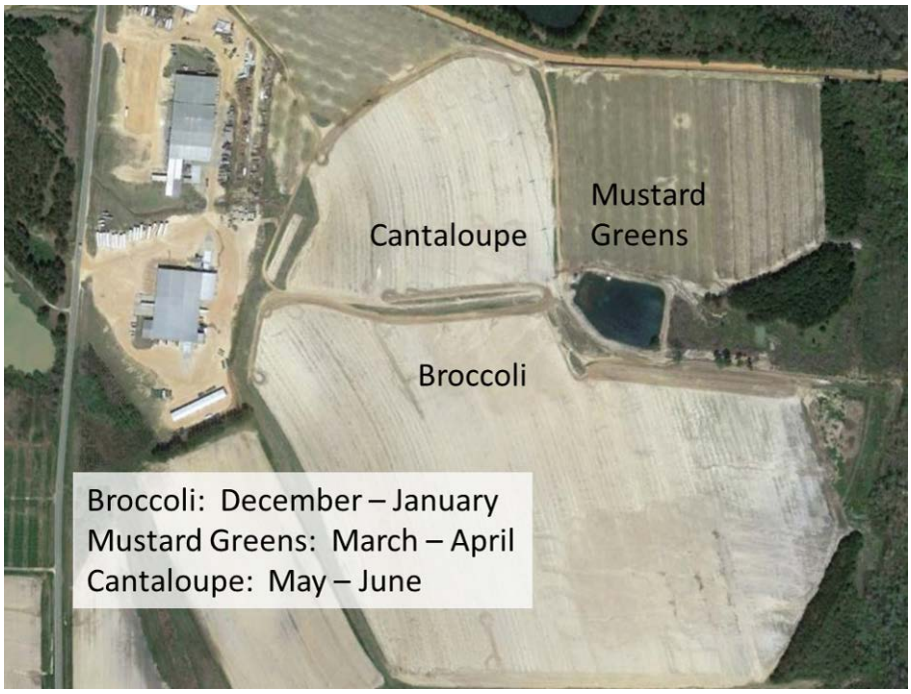


Figure 2. The project was conducted around one irrigation pond on a large vegetable farm in southern Georgia. Samples were collected during the growth cycle of three different crops in the fields surrounding the pond. Broccoli and Cantaloupe were grown under a center pivot irrigation system and the Mustard Greens under a solid set irrigation system.

Figure 3. The sampling pans were installed in the study fields prior to an irrigation event and used to capture irrigation water and water splashing from the soil surface. Pans were installed so that pan lips were 2, 4, 8, 16, and 32 inches above the soil surface. Five replicates of each height were installed during each sampling event.

Figure 4. Water and any solids collected in the sampling pans was poured into a sterile sample bottle and returned to the lab for analysis. At the laboratory, the replicates from each height were composited. Only one composite sample was analyzed per height.

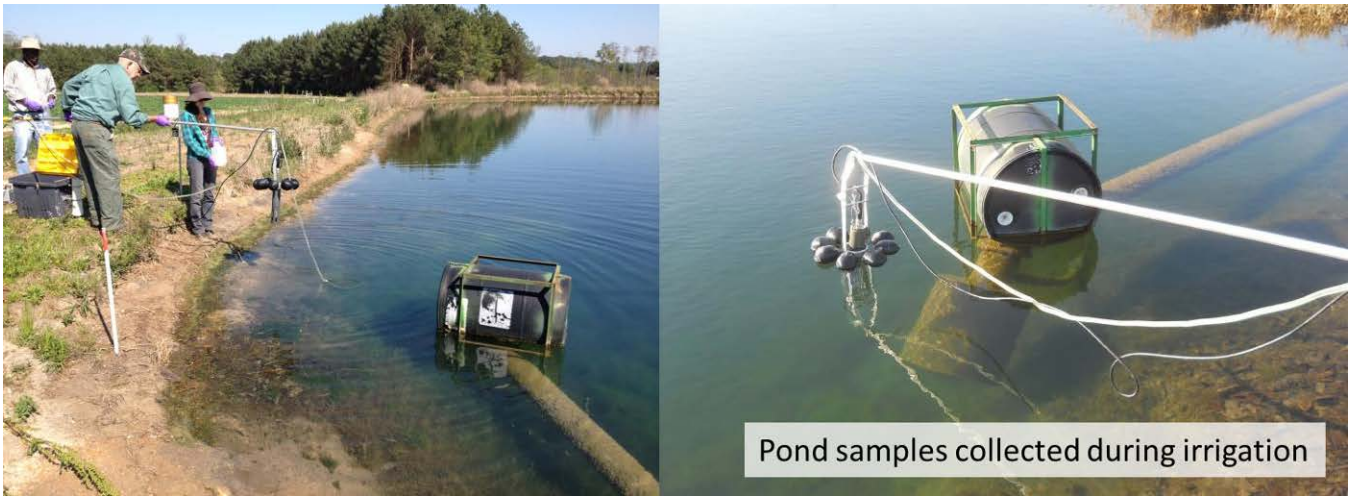
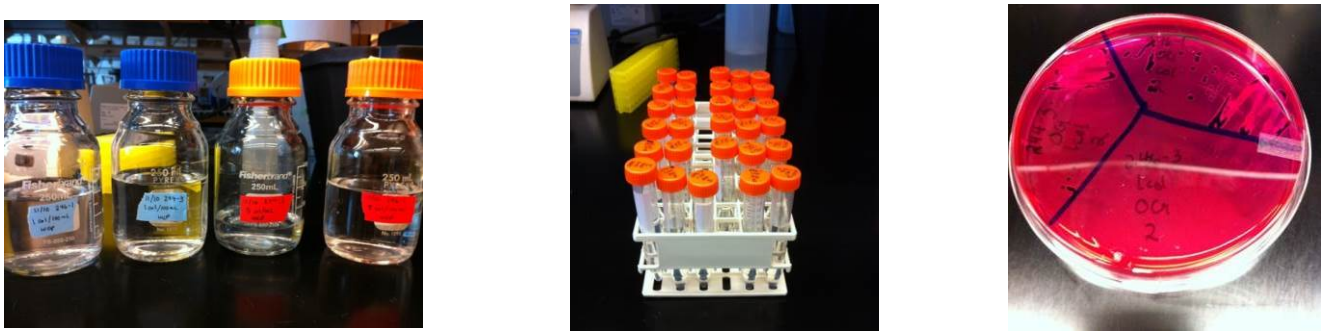


Figure 5. A sample was collected from the pond at the pump intake point during the irrigation event. The sample hose was approximately 80cm (31.5in) below the pond surface. At the same time, water quality parameters such as temperature, pH, dissolved oxygen concentration were measured with a YSI Water Quality Sonde.

Figure 6. Produce samples were collected during the regular harvest. The produce samples were placed in sterile Whirlpak bags, placed on ice, and transported to the laboratory.



(a) **(b)** **(c)**

Figure 7. (a) Water samples inoculated in the laboratory; (b) one set of thawed Lactose Broth/Glycerol samples; (c) XLT-4 plate with differential re-growth.