CENTER for PRODUCE SAFETY

CPS 2013 RFP
FINAL PROJECT REPORT

Project Title
Evaluation of risk-based water quality sampling strategies for the fresh produce industry

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Objectives

1) Determine the variability of E.coli/STEC/Salmonella occurrence in irrigation waters over time based on historic data at specific locations in Arizona/Southern California. This data will be used to initially assess the impact of rainfall events and water quality factors (e.g., temperature, turbidity), canal size, and watershed characteristics (e.g., bridges, drainage ways, urban development), on the occurrence of these organisms.

2) Assess the impact of occurrence, duration, and intensity of rainfall events on the presence of E.coli/Salmonella in irrigation waters and to determine the effect of sample volume on being able to detect E.coli and Salmonella in water in comparison to the traditional 100 ml IDEXX Colilert Quantitray® test.

3) Use an exposure scenario risk based model for E. coli/Salmonella in irrigation waters to quantify the risks of infection with different sampling frequencies of irrigation waters based on environmental factors (e.g., rainfall), irrigation methods, and the type of produce.

4) Develop a cell-phone/computer application (App) that can be used for guidance for frequency of sampling based on risk factors (e.g., after rainfall events).

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FINAL REPORT

Abstract
Irrigation water has been implicated in a number of outbreaks associated with fresh produce. General guidelines for water quality sampling for indicator bacteria (*Escherichia coli*) and sampling frequency have recently been proposed by the Food and Drug Administration (Federal Register, 2013), however, it is not apparent if they are based on site specific conditions with quantifiable benefits related to risk reduction. This research had four major goals. 1) Assess and quantify factors which determine variability of generic (indicator) *Escherichia coli, pathogenic E. coli (Shiga-toxin producing strains – STEC),* and *Salmonella* occurrence in irrigation water over time, based on historic data and data collected as part of this study, at specific locations in Arizona and Southern California. This data was used to assess the impact of risk events such as rainfall, water quality factors including temperature and turbidity, canal size, and watershed characteristics (potential sources of fecal contamination), on the occurrence of these organisms. 2) Assess the impact of occurrence, duration and intensity of rainfall events on *E. coli* and *Salmonella* in irrigation waters with the goal to determine how long after a specific rainfall event the irrigation water quality will be impacted. 3) Use an exposure scenario risk based model for *E. coli* and *Salmonella* in irrigation waters to quantify the risks of infection with different sampling frequencies of irrigation waters based on environmental factors (e.g. rainfall), irrigation methods, and type of produce. 4) Develop a cell-phone/computer application (app) that can be used for guidance for frequency of sampling after high-risk (rainfall) events. This study offers recommendations towards risk-based sampling strategies (frequency, timing, location, volume) for *E. coli* indicator bacteria in irrigation waters that provide the greatest risk reduction to produce.

Background
Irrigation water has been suspected in outbreaks of various types of produce (Gerba and Choi, 2009), and a recent review concluded that reducing the amount of microbial contamination of irrigation water and soil is the most effective way to prevent and control produce contamination during production (Park et al., 2012). The U.S. Food and Drug Administration (FDA) has recently finalized guidelines for the sampling of irrigation waters for generic *Escherichia coli* when used for produce production as an indicator of the potential presence of fecal contamination. These general guidelines are designed to reduce the risk of produce contamination and provide guidance on sampling frequency, location, and source of irrigation water. The guidelines also acknowledge that they may differ depending upon the types of produce, rainfall events, the methods of irrigation, and source water protection. We believe that proper documentation and quantification of the impact of these environmental factors can lead to a risk-based water quality monitoring program that provides the greatest risk reduction with available resources for sampling.

In Arizona and Southern California, irrigation waters originate from constructed canals, which are more protected than rivers or lakes from non-point sources of fecal contamination since runoff from rainfall events is largely controlled. Still, rainfall events have been shown to increase *E. coli* numbers in canals in Arizona (Kayed, 2004). The occurrence of rainfall is known

to increase the risk of produce outbreaks and is related to both the duration and the intensity of the event (Semenza et al., 2012). Kayed (2004) found that rainfall within seven days prior to sampling correlated strongly with elevated average *E. coli* concentrations in Yuma and Maricopa Counties in Arizona. Increases in *E. coli* may be related to runoff from canal banks, bridges, road ways, etc. and the re-suspension of sediments. Pathogens and *E. coli* survive longer and occur in greater concentrations in sediments than the overlaying water (Pachepsky and Shelton, 2011). Carpenter (2007) found that *E. coli* geometric average concentrations in sediments in Yuma canals were ten times greater than that in the overlaying water. Extreme rainfall events may also enhance the internalization of *Salmonella* in lettuce (Ge et al., 2011). Warm temperatures and humidity are other environmental factors that affect produce contamination (Park et al., 2012). Watershed characteristics, including landscape and design features, can also affect *E. coli* and pathogen occurrence (Strawn et al., 2013). Kayed (2004) found that main canals tended to have lower *E. coli* numbers than lateral canals. Other features such as bridges, roads, and urban development will also affect the prevalence of foodborne pathogens in the water (Strawn et al., 2013).

Using cultural and molecular methods, Kayed (2004) detected *Salmonella* (in one-liter water samples) in 24 of 83 samples (29%) in Maricopa. While there was no correlation with the detection of *E. coli,* in a 2013 study of Yuma canals, Bright (unpublished data) detected *Salmonella* in up to 17% of the samples collected from canals in Yuma by cultural methods.

Assessing the factors controlling the presence of generic indicator *E. coli* and *Salmonella/*Shiga-toxin producing *E. coli* (STEC) in irrigation waters will help determine both the frequency necessary and the location of sampling to minimize the risk of contamination in irrigation waters. Since risks are associated with the method of irrigation and the type of produce, these also need be considered (Choi et al., 2004).

Research Methods and Results

Over the course of this study three datasets were gathered from the field by the authors and their students from irrigation canals at the Yuma and Maricopa, AZ and Imperial Valley, CA starting in 2001. The datasets have measurements of *E. coli* and coliforms counts per 100 ml of irrigation water as well as the physical characteristics of the irrigation water. Supplemental datasets included pathogen presence information for some sampling locations and represented *Salmonella*, STEC, and enterococci data. Each region's dataset was analyzed separately to arrive at a regional model for prediction of *E. coli* and coliforms. For the purposes of this report, only Yuma datasets will be discussed below; additional analysis for the supplemental regions can be found in Appendix C. Additionally, the tables and figures section of Appendix C overviews effects of both physical and environmental parameters on *E. coli* and coliform numbers.

Yuma datasets

Counts of coliforms, *E. coli,* enterococci, *Clostridium*, *Salmonella* and *Campylobacter* per 100 ml of irrigation water were collected from Yuma irrigation canals for years 2001 through 2003,

2007, 2011, 2012, 2013 and 2014. These historical datasets were also supplemented with new data collected by the research team as part of this study. Physical characteristics of the irrigation water were also collected. Environmental factors were downloaded from the Arizona Meteorological Network (AZMET, 2015) for the Yuma Valley weather station that best represented the agricultural area being assessed for this work. The Yuma Valley weather station is located at the Yuma Agricultural Center (YAC), College of Agriculture and Life Sciences, University of Arizona. Additionally, in reviewing the historical data, the research team determined that there was not enough pathogen data collected to make accurate correlations and/or much of the data was not quantitative (for *Salmonella* was presence/absence data only) and thus could not be used.

Coliform and *E. coli* counts

Coliform and *E. coli* counts per 100 ml of irrigation water were tabulated with physical and environmental factors for the different dates and sites. The number of records totaled 693 for Yuma. Some of the physical characteristics for the coliforms dataset were missing and a multivariate imputation by chained equations (MICE) developed by van Buuren (2011) was used to regenerate the missing data through utilizing the MICE package in the R-Language (R Core Team, 2013).

Indicator counts and physical characteristics

Figure 1 shows scatter plots for irrigation water coliforms and *E. coli* counts versus irrigation water physical characteristics for the Yuma records. Scatter plots are used to give a quick "snap shot" of the different variables to determine if there are correlations amongst the variables. The following parameters are plotted in Figure 1; coliforms, *E. coli*, tw (water temperature), pH, turbidity, and ec (electrical conductivity).

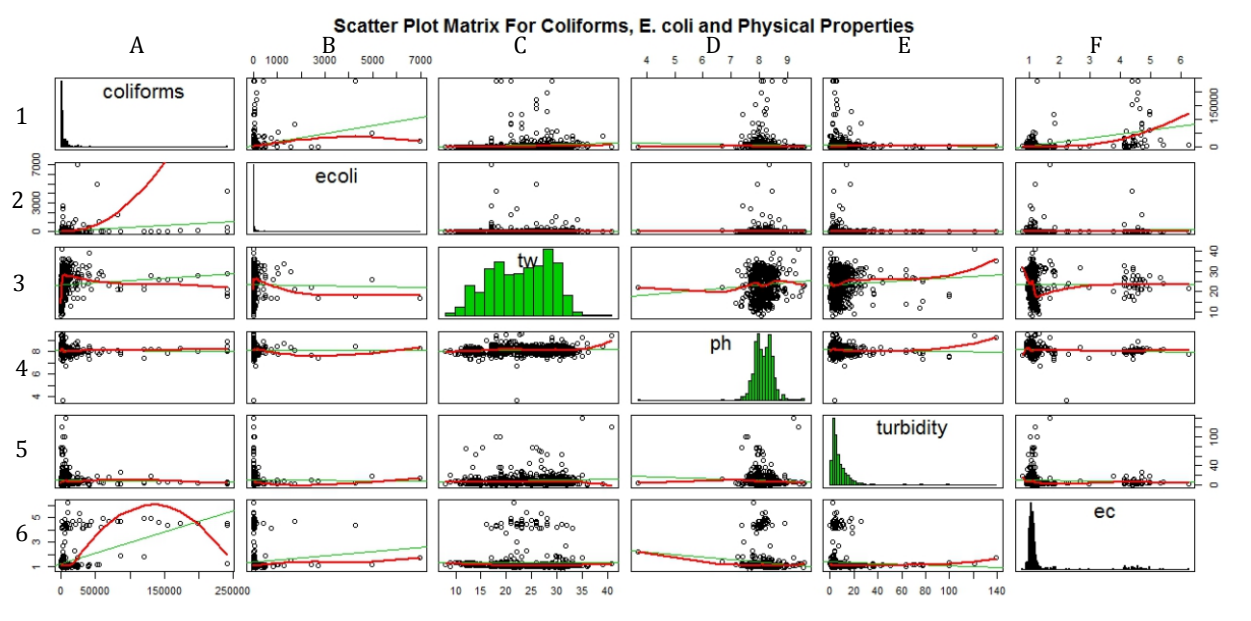


Figure 1: Scatter Plot for Yuma Coliforms, *E. coli* and Physical Characteristics Records

Figure 1 shows the bivariate relationships among all the variables specified (coliforms, ecoli: *E. coli*, tw: irrigation water temperature (°C), ph: pH of the irrigation water being the hydrogen ion concentration, turbidity: irrigation water turbidity (NTU), and ec: irrigation water electrical conductivity (dS/m)). Linear and smoothed curves to fit the data points are shown for each plot. A histogram is shown for each variable in the matrix diagonal. For example, the scatter plot of coliforms counts vs. water temperature (tw) is found at the row and column intersection of those two variables (box A3: A on the horizontal axis and 3 on the vertical axis). Initial possible correlations between the variables can be observed in the coliforms vs. electrical conductivity and between coliforms and *E. coli* (box A6).

To determine the strength of a correlation between the different variables, the research team utilized the "cor" function in the R-Language. Table 1 shows the generated correlation factors amongst the different variables assessed. Results shown in Figure 1 and Table 1 assume that the dependent variable is normally distributed and outliers do not have much effect on the correlation. Numbers in bold indicate significant correlations between variables.

Inspecting Table 1, there is a weak positive correlation between coliform and *E. coli* counts with a factor of 0.242. In addition, there exist a stronger positive correlation between coliforms counts and water electrical conductivity with a factor of 0.507. Electrical conductivity relates to the salinity of water and is often measured in mg/l.

Table 1: Correlation between Coliforms and *E. coli* Counts and Irrigation Water Physical Characteristics

	Coliforms	***E. coli***	**Water Temperature**	**pH**	**Turbidity**	**EC**
Coliforms	1	**0.242**	0.091	-0.022	-0.028	**0.507**
E. coli	**0.242**	1	-0.011	-0.001	-0.004	0.086
Water Temperature	0.091	-0.011	1	0.076	0.076	-0.007
pH	-0.022	-0.001	0.076	1	-0.055	-0.085
Turbidity	-0.028	-0.004	0.076	-0.055	1	-0.049
EC	**0.507**	0.086	-0.007	-0.085	-0.049	1

The same analysis was then repeated to compare correlations between indicator bacterial counts and environmental characteristics.

Indicator counts and environmental characteristics

Figure 2 shows a scatter plot of irrigation water coliform and *E. coli* counts vs. various environmental characteristics obtained from Yuma Valley weather station. In Figure 2, coliforms and *E. coli* are defined as before, tair: mean daily air temperature (°C), rh: mean daily relative humidity (%), sr: total daily solar radiation (Watt/m^2), u: total wind speed (m/s) and hu: heat units. Heat Units is a calculated factor obtained from AZMET; they are calculated based on 86 °F and 55 °F. Daily heat units represent the range of temperature during which plants and insects grow. Daily heat units are calculated as the difference between the average of the daily maximum and daily minimum temperatures and a certain critical basal and maximum "threshold" temperatures.

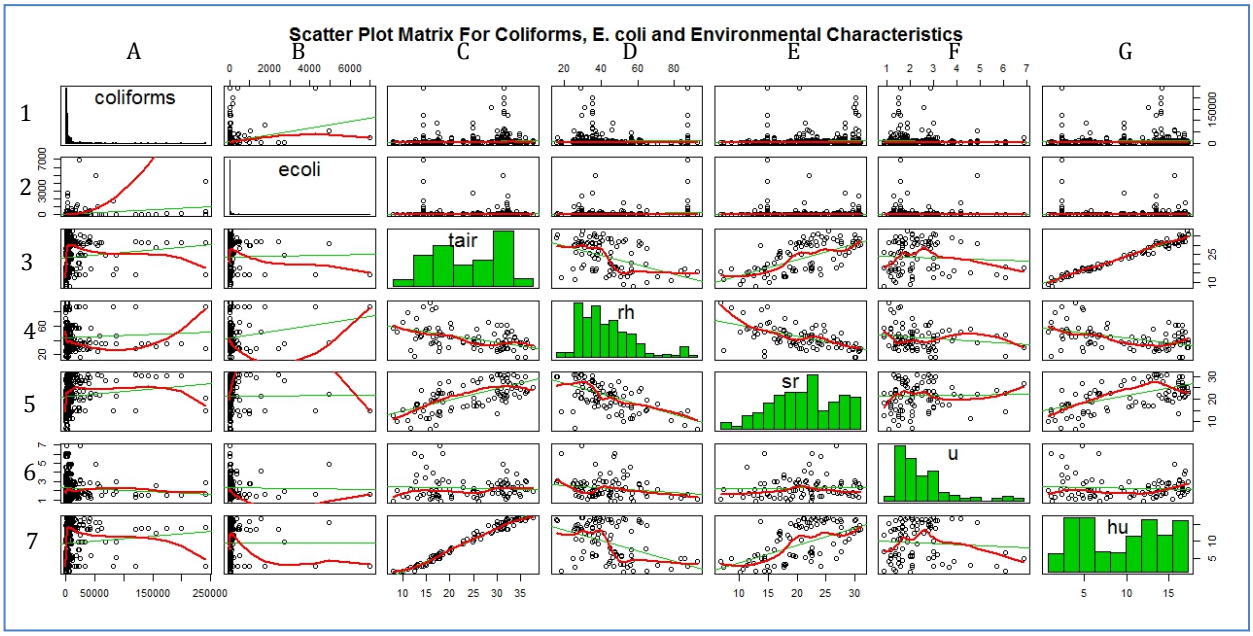


Figure 2: Scatter Plot for Yuma Coliforms, *E. coli* and Physical Characteristics Records

Figure 2 shows the bivariate relationships among all the variables specified (coliforms, *E. coli*, and environmental characteristics). Linear and smoothed curves to fit the data points are shown for each plot. The "cor" function in the R-Language was again used to determine the strength of a correlation between the different variables.

Table 2 shows the generated correlation factors amongst the different variables. Results shown in Figure 2 and Table 2 were completed assuming that the dependent variables (coliforms and *E. coli* counts) are normally distributed and outliers do not have much effect on the correlation. Numbers in bold indicate significant correlations between variables. Results in Table 2 show that coliforms and *E. coli* counts have no correlation with environmental characteristics. As the case in Table 1, there is a weak relationship (0.242) between the occurrence of *E. coli* and the occurrence of coliforms. There are strong relationships between weather factors since they are dependent on each other.

Table 2: Correlation between Coliforms and *E. coli* Counts and Environmental Characteristics

	Coliforms	***E. coli***	**Air Temperature**	**Relative Humidity**	**Solar Radiation**	**Wind Speed**	**Heat Units**
Coliforms	1	**0.242**	0.092	0.050	0.096	-0.056	0.066
E. coli	**0.242**	1	0.012	0.113	0.005	-0.009	-0.001
Air Temp.	0.092	0.012	1	-0.497	0.651	-0.072	0.986
Rel. Humidity	0.050	0.113	-0.497	1	-0.590	-0.158	-0.458
Solar Rad.	0.096	0.005	0.651	-0.590	1	0.030	0.591
Wind Speed	-0.056	-0.009	-0.072	-0.158	0.030	1	-0.080
Heat Units	0.066	-0.001	0.986	-0.458	0.591	-0.080	1

Transformation of datasets

After the initial data analysis, it became apparent that the historical data was not normally distributed. In order for the research team to make accurate correlations for risk modeling, the data would need to be transformed. It is possible to transform the dependent variables to improve statistical analysis. The most common transformation used for microbiological datasets is a log transformation ($\log_{10}(Y)$) of the counts, i.e. the arithmetic values are to $\log_{10}$ values. The R-Language has a *powerTransform* function that can take a dataset that does not satisfy the normality assumption and provides a λ value that makes the transformed dataset fulfill the normality assumption. These same conditions and techniques can also be used when conducting regression analysis to determine correlations between two or more variables.

The following tables outline the relationship between indicator counts, *E. coli*, physical and environmental characteristics on transformed datasets (Tables 5–8). Additionally, these transformed data were then used to assess correlation associated with rainfall events occurring 5 days prior to sampling. Historical rainfall data was collected from the AZMET system and was limited to the weather station in Yuma (Tables 9–10). Numbers in bold indicate significant correlations between variables.

Table 5: Correlation between Coliforms Counts and Irrigation Water Physical Characteristics after Transformation of Coliform Counts

	Coliform Count	Water Temperature	Water pH	Water Turbidity	Electrical Conductivity
Coliform Count	1.000	**0.573**	-0.082	0.081	**0.385**
Water Temperature	**0.573**	1.000	0.076	0.076	-0.007
Water pH	-0.082	0.076	1.000	-0.055	-0.085
Water Turbidity	0.081	0.076	-0.055	1.000	-0.049
Electrical Conductivity	**0.385**	-0.007	-0.085	-0.049	1.000

Table 6: Correlation between *E. coli* Counts and Irrigation Water Physical Characteristics after Transformation of *E. coli* Counts

	E. coli Counts	Water Temperature	Water pH	Water Turbidity	Electrical Conductivity
***E. coli* Counts**	1.000	**-0.227**	-0.014	0.031	-0.066
Water Temperature	**-0.227**	1.000	0.076	0.076	-0.007
Water pH	-0.014	0.076	1.000	-0.055	-0.085
Water Turbidity	0.031	0.076	-0.055	1.000	-0.049
Electrical Conductivity	-0.066	-0.007	-0.085	-0.049	1.000

Table 7: Correlation between Transformed Coliform Counts and Environmental Characteristics

	Coliforms Counts	Air Temperature	Relative Humidity	Solar Radiation	Wind Speed	Heat Units
Coliform Counts	1.000	**0.560**	**-0.213**	**0.396**	-0.021	**0.538**
Air Temperature	**0.560**	1.000	-0.497	0.651	-0.072	0.986
Relative Humidity	**-0.213**	-0.497	1.000	-0.590	-0.158	-0.458
Solar Radiation	**0.396**	0.651	-0.590	1.000	0.030	0.591
Wind Speed	-0.021	-0.072	-0.158	0.030	1.000	-0.080
Heat Units	**0.538**	0.986	-0.458	0.591	-0.080	1.000

Table 8: Correlation between Transformed *E. coli* Counts and Environmental Characteristics

	E. coli Counts	Air Temperature	Relative Humidity	Solar Radiation	Wind Speed	Heat Units
***E. coli* Counts**	1.000	**-0.269**	0.055	**-0.197**	-0.006	**-0.249**
Air Temperature	**-0.269**	1.000	-0.497	0.651	-0.072	0.986
Relative Humidity	0.055	-0.497	1.000	-0.590	-0.158	-0.458
Solar Radiation	**-0.197**	0.651	-0.590	1.000	0.030	0.591
Wind Speed	-0.006	-0.072	-0.158	0.030	1.000	-0.080
Heat Units	**-0.249**	0.986	-0.458	0.591	-0.080	1.000

Table 9: Correlation between Transformed Coliform Counts and Rainfall

		Coliform Counts	Rainfall Occurring On					
			Sampling Day	1 Day Before Sampling	2 Days Before Sampling	3 Days Before Sampling	4 Days Before Sampling	5 Days Before Sampling
Coliform Counts		1.000	**0.123**	0.093	-0.036	-0.040	-0.097	0.065
Rainfall Occurring On	**Sampling Day**	**0.123**	1.000	-0.026	-0.018	-0.027	-0.020	-0.022
	1 Day Before	0.093	-0.026	1.000	-0.013	-0.019	-0.024	-0.015
	2 Days Before	-0.036	-0.018	-0.013	1.000	-0.013	-0.017	-0.011
	3 Days Before	-0.040	-0.027	-0.019	-0.013	1.000	**0.383**	-0.016
	4 Days Before	-0.097	-0.020	-0.024	-0.017	**0.383**	1.000	0.001
	5 Days Before	0.065	-0.022	-0.015	-0.011	-0.016	0.001	1.000

Table 10: Correlation between Transformed *E. coli* Counts and Rainfall Amounts

		E. coli Counts	Rainfall Occurring On					
			Sampling Day	1 Day Before Sampling	2 Days Before Sampling	3 Days Before Sampling	4 Days Before Sampling	5 Days Before Sampling
E. coli Counts		1	**-0.174**	0.019	-0.016	-0.033	-0.024	0.004
Rainfall Occurring On	**Sampling Day**	**-0.174**	1	-0.026	-0.018	-0.027	-0.020	-0.022
	1 Day Before	0.019	-0.026	1	-0.013	-0.019	-0.024	-0.015
	2 Days Before	-0.016	-0.018	-0.013	1	-0.013	-0.017	-0.011
	3 Days Before	-0.033	-0.027	-0.019	-0.013	1	**0.383**	-0.016
	4 Days Before	-0.024	-0.020	-0.024	-0.017	**0.383**	1	0.001
	5 Days Before	0.004	-0.022	-0.015	-0.011	-0.016	0.001	1

After data transformation, the results allowed the research team to have significantly better ability to apply correlation statistics to the datasets collected and for use in risk model development. Results of note include the following:

1) Correlation between *E. coli* counts and rainfall amounts occurring on sampling day.
2) A positive correlation between rainfall amounts occurring on day 3 and day 4 prior to sampling day, possibly due to the travel time for water sources as well as the disturbance of canal sediments that could provide adequate environment for bacterial re-suspension and growth.
3) A significant positive correlation between coliform counts, air temperature, solar radiation and heat units.
4) A positive correlation between coliform counts and electrical conductivity and positive correlation between coliform counts and irrigation water temperature.

Development of water quality/risk assessment models

Using the transformed data (above) and associated correlations, the research team was then able to build a set of models that could be used to predict water quality conditions related to coliform counts or the presence of *E. coli* bacteria. At the onset of this project the research team anticipated the development of only one model to be available to industry, however, it was determined that multiple models could be produced for users based on data input available as well as confidence level needed.

In modeling, the research team used ordinary least square (OLS) regression, to try to predict the response (dependent) variable from a set of predictor (independent) variables. The OLS regression fits equation 1 (Ott and Longnecker, 2001):

$$Y_i = \beta_0 + \beta_1 X_{1,i} + \cdots \ldots \ldots \ldots + \beta_k X_{ki} \qquad for\ i = 1\ to\ n \tag{1}$$

In which, n is the number of observations; k is the number of predictor variables; Y_i is the predictor dependent variable; β_0 is the intercept: predicted value of Y when all the predictor values are equal to zero; $X_{j,i,}$ is the j^{th} predictor value for the i^{th} observation; and β_j is the regression coefficient for the j^{th} predictor. The objective in performing the OLS regression model was to find model parameters (intercept and predictor variables) that minimize the difference between actual response values (measured values) and those predicted by the OLS regression model. Linear regression calculates an equation that minimizes the distance between the fitted line and all of the data points. Technically, OLS regression minimizes the sum of the squared residuals. In general, a model fits the data well if the differences between the observed values and the model's predicted values are small and unbiased.

Overall the team developed a total of 13 models that can be used to predict water quality based on the user data available to be input for calculation. A complete list of all developed models can be found in Tamimi et al. (2016). In this report we present the three main models that provided the most straightforward interpretation and with the most appropriate

confidence level for the user. These models have been named as follows: 1) complete model (includes both physical and environmental variables for risk calculation), 2) physical model (includes only physical parameters in risk calculation), and 3) environmental model (that includes only environmental parameters collected from AZMET in risk calculation). The overall complete model (equation) is presented below along with definitions of each of the input variables.

$$coliforms = \lceil 1.021 + 2.52 \times 10^{-4} tw - 1.38 \times 10^{-3} ph + 2.76 \times 10^{-5} turbidity + 2.53 \times 10^{-3} ec + 8.56 \times 10^{-5} sr + 4.25 \times 10^{-4} u + 3.60 \times 10^{-4} hu + 1.21 \times 10^{-3} prec0 + 3.62 \times 10^{-3} prec1 - 2.53 \times 10^{-3} prec2 \rceil^{\left(\frac{1}{0.0034}\right)} \quad (2)$$

coliforms: Predicted count of coliforms in irrigation water (CFU/100 ml);
tw: Temperature of water (°C);
ph: Hydrogen ion concentration of irrigation water (pH);
turbidity: Turbidity of irrigation water (NTU);
ec: Electrical conductivity of irrigation water (dS/m);
sr: Total solar radiation for the sampling day (Watt/m^2);
u: Average wind speed for sampling day (m/s);
hu: Heat units based on 86 °F and 55 °F (30 °C and 12.7 °C)
prec0: Total rainfall depth on sampling day (mm)
prec1: Total rainfall depth one day before sampling day (mm)
prec2: Total rainfall depth two days before sampling day (mm)

Table 11: Summary of the Three Main Models

Regression Model	Variables Used in Model	Ranking Significance of Variables	Regression Coefficients	R^2
Complete Model	**prec1**	1	3.467E-03	0.5696
	ec	2	2.543E-03	
	prec2	3	-2.475E-03	
	ph	4	-1.355E-03	
	prec0	5	1.327E-03	
	hu	6	4.594E-04	
	u	7	4.047E-04	
	tw	8	2.657E-04	
	sr	9	7.860E-05	
	turbidity	10	2.642E-05	
Physical Model	**ec**	1	2.56E-03	0.4913
	ph	2	-1.40E-03	
	tw	3	5.38E-04	
Environmental Model	**prec1**	1	3.25E-03	0.4006
	prec2	2	-1.69E-03	
	prec0	3	1.50E-03	
	u	4	2.97E-04	
	sr	5	1.14E-04	

Figure 3 (below) depicts the complete model of predicted coliform bacteria (dark blue line) when compared with measured coliform counts (from low to high concentrations of *E. coli*) within 95% confidence intervals (red and light blue lines). The model predicts accurate bacterial numbers within the assigned confidence interval with very few outliers present.

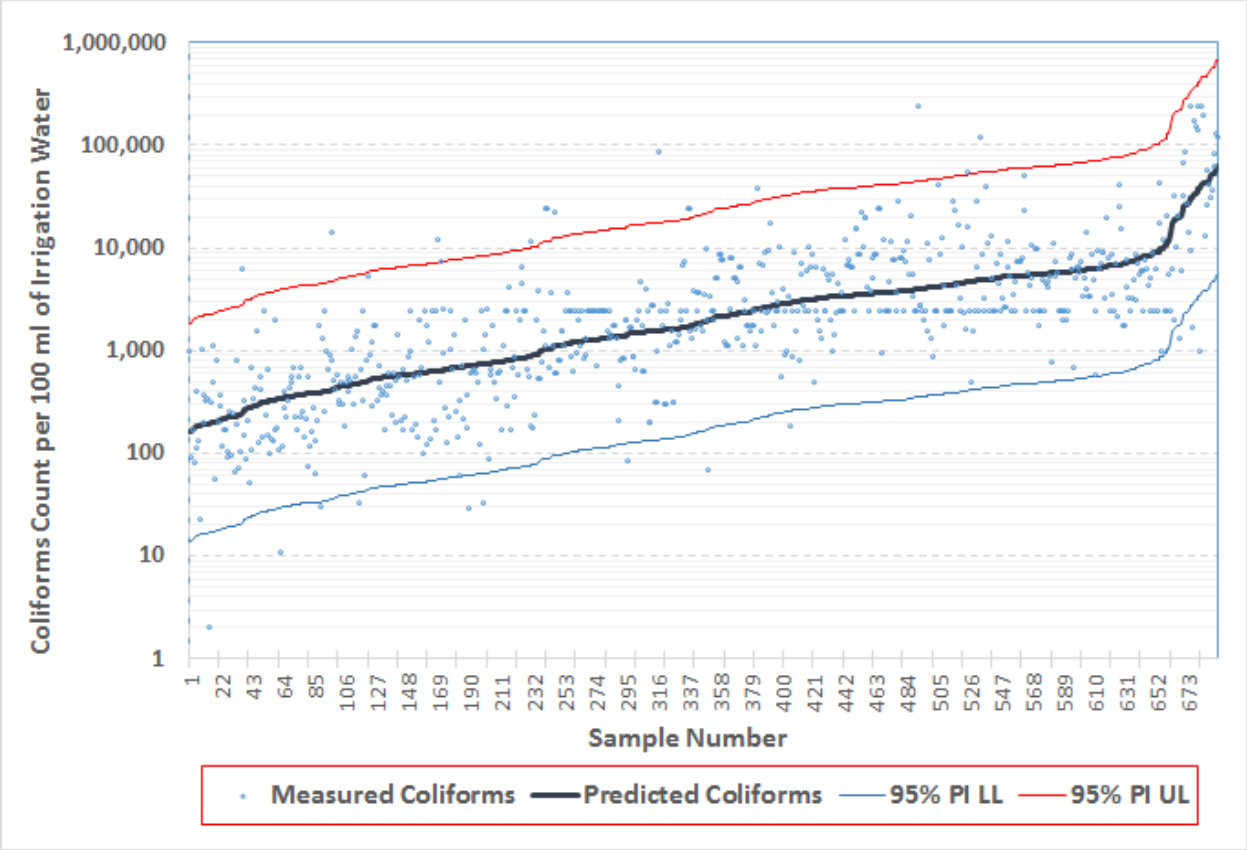


Figure 3: Complete Model: Predicted Coliforms Counts vs. Measured Coliforms for the Yuma Area with 95% Upper and Lower Prediction Limits

Outcomes and Accomplishments

The overall objective of the project was to integrate the water quality predictor models presented above into a user-friendly application. Working with an external computer modeler and App developer we developed an online or "web App" as well as an App that is available for download for both iOS and Android devices.

Development of water quality risk App and online calculator

This App, called the *AgWater App,* integrates user information related to location of the water source, any available physical water quality data, locally available environmental data in real-time, as well as historical knowledge to predict the likelihood of a water quality exceeding current LGMA or FSMA standards. Based on the information available, the App automatically selects the most appropriate model (of the 13 created) to determine the likelihood of coliform or *E. coli* bacteria in the water source.

Additionally, during the development of this App the research team was approached by members of the Western Center for Food Safety and FDA to incorporate App user functions to help industry calculate their Microbial Water Quality Profile (MWQP) and Statistical Threshold Value (STV). Due to the finalization of the FDA Food Safety Modernization Act (FSMA) in November of 2015, we felt that this was an important component to include in the final developed App.

The following text provides an overview of the online calculator, native web App, and downloadable App for iOS and android devices. It should be noted that we have provided user workshops in order to test the functions of the App, user friendliness, and calibration of the model. We anticipate that future revisions of the App will be necessary to maintain usability as well as to respond to industry feedback.

Online calculator http://agwater.arizona.edu/onlinecalc/

Overview

The Produce Safety Rule (PSR) requires growers to initially establish a Microbial Water Quality Profile (MWQP) for each untreated surface agricultural water source and conduct annual surveys for that water source in subsequent years. The water quality profile is based on the levels of generic *E. coli* in your agricultural water. *Note: Agricultural water is defined in part "as water that is intended to, or likely to, contact the harvestable portion of covered produce or food-contact surfaces.*

The MWQP must initially be established with a minimum of 20 water samples collected as close to harvest as possible over a period of at least 2 to a maximum of 4 years. Geometric mean (GM) and Statistical Threshold Value (STV) are calculated from these 20 samples (minimum). These GM and STV values are your MWQP and must be compared to the microbial quality criteria provided in the Produce Safety Rule.

The GM and STV values must be updated annually with a minimum of 5 new samples. "Rolling" GM and STV values are thus calculated using a combination of the 5 annual sample values plus 15 sample values from previous years. The updated MWQP will confirm that the water is still being used appropriately.

More information on the Produce Safety Rule and agricultural water can be found here: http://www.fda.gov/Food/GuidanceRegulation/FSMA/ucm334114.htm

This multi-table tool was developed to make it easy to calculate the GM and STV and to determine if your water meets the standards for unrestricted application to produce before harvest. The tool is also designed to assist you with making food safety management decisions if your water does not meet the standards in the rule.

Figure 4. Screen shot of online calculator

Native/Web App http://agwater.arizona.edu/

The Ag Water App was designed to aid in determining compliance with national FSMA regulations and Leafy Green Marketing Agreement (LGMA) recommendations as well as predict the likelihood of microbial contamination based on local environmental conditions.

Overview

The Produce Safety Rule requires growers to initially establish a Microbial Water Quality Profile (MWQP) for each untreated surface agricultural water source and conduct annual surveys for that water source in subsequent years. The water quality profile is based on the levels of generic *E. coli* in your agricultural water, which must fall below a geometric mean (GM) of 126 CFU/100ml and a Statistical Threshold Value (STV) of 410 CFU/100ml. Ag Water app was designed to:

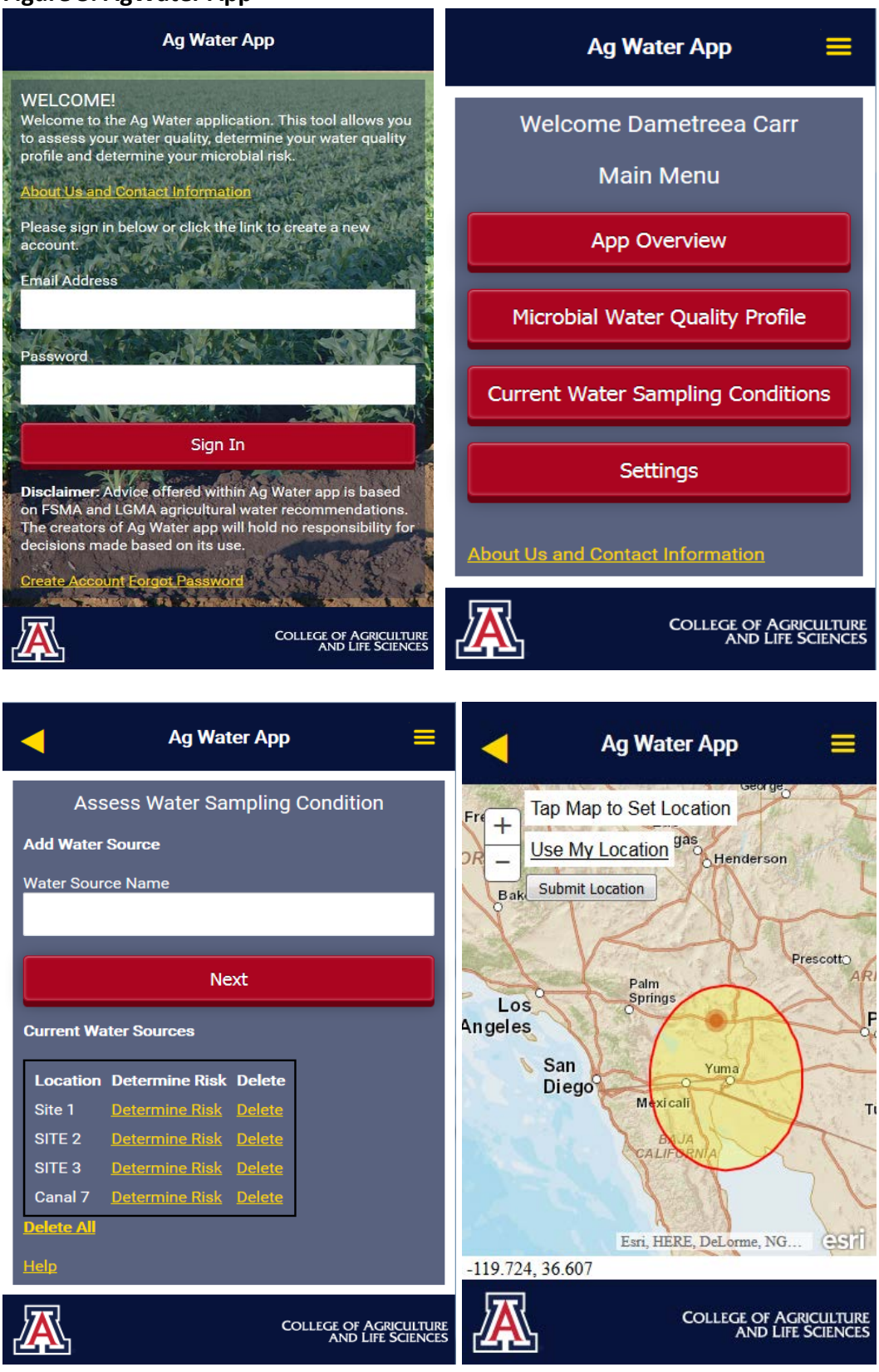
- Assist growers with GM and STV calculations to determine if their water meets the standards for unrestricted application to produce before harvest
- Assist growers with making food safety management decisions if their water does not meet the standards in the rule, and
- Evaluate current sampling conditions for irrigation water quality to determine the probability of microbial contamination

This app will help you calculate the GM and STV and to determine if your water meets the standards for unrestricted application to produce before harvest. The app will also assist you with making food safety management decisions if your water does not meet the standards in the rule.

Microbial water quality profile (MWQP):
This feature allows you to determine your MWQP. The MWQP must initially be established with a minimum of 20 water samples collected as close to harvest as possible over a period of at least 2 to a maximum of 4 years. Geometric mean (GM) and Statistical Threshold Value (STV) are calculated from these 20 samples (minimum). These GM and STV values are your MWQP and must be compared to the microbial quality criteria provided in the Produce Safety Rule.

Current water sampling conditions:
This feature will evaluate current environmental conditions to determine the potential for microbial contamination in a water source. The probability of contamination is determined using environmental data, current weather conditions, and a user survey that includes water quality parameters specific to the water source in question.

Figure 5. AgWater App



Summary of Findings and Recommendations

Overall, the findings from this project support previous work by the project PIs to determine risk related factors that are likely to influence water quality. More specifically, this project has the following recommendations:

- Data assessment indicates that water quality is highly dependent on localized environmental conditions, and every effort should be made by industry to better understand their water sources through collection of water quality data and historical analysis.

- Scientific data collected and analyzed by our research team indicate that the main influential factors in the region evaluated were air temperature, solar radiation, rainfall and electrical conductivity (Appendix C). Surprisingly, the ability of a user to input electrical conductivity into developed models greatly increased risk assessment confidence. This lends itself towards the recommendation to industry to include EC in routine water quality monitoring plans to increase the likelihood of predicting coliform bacteria and *E. coli* in water sources.

- There is no "one-size fits all" model to predict water quality, however, the development of multiple models allows for a wider range of users based on available data and location. The complete model developed by our research team provides excellent predictions of water quality based on the data available and the region evaluated.

- Grower Apps can be useful tools that allow industry to make more informed decisions about their water sources from both a water use and sampling perspective.

- Future work should include testing of additional regional water sources and comparison of water quality data against development models (n=13) in order to validate their use in regions beyond the desert Southwest.

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APPENDICES

Appendix A. Publications and Presentations

Appendix B. Budget Summary

Appendix C. Tables and Figures

Appendix D. Suggestions to CPS

Appendix A.

Publications and Presentations

- The project PI, Dr. Channah Rock, presented the project status in an oral presentation at the 2015 UA Desert Collaborative Field Conference. This conference was held on March 31st in Yuma, AZ and is a collaborative effort of UA Specialists, scientists and local industry.

- Dr. Channah Rock presented the project status in a poster presentation at the annual Hartnell College Western Food Safety Summit on May 7-8, 2015, in Salinas, CA.

- Dr. Channah Rock presented a research update related to this work at the local Yuma Safe Produce Council monthly meeting and Water Sampling 101 training, May 20, 2015.

- Dr. Channah Rock presented the project status in an oral presentation at the 2015 CPS Produce Research Symposium, June 23-24 in Atlanta, Georgia. This is an important event due to the interaction between research scientists, produce industry members and government regulatory personnel.

- Dr. Channah Rock and Assistant Health Educator, Dametreea Carr, presented the project status as well as held an industry-testing event with the Yuma Safe Produce Council in Yuma, AZ on November 12, 2015.

- Dr. Channah Rock was an invited speaker at the Washington State Tree Fruit Association Annual meeting held in Yakima, WA on December 9, 2015.

- Dr. Channah Rock was an invited speaker at the Desert Produce Safety Collaborative Field Conference held in Yuma, AZ on January 12, 2016.

- Dr. Channah Rock and Assistant Health Educator, Dametreea Carr, will be providing a hands-on workshop and industry-testing event with the Yuma Safe Produce Council in Yuma, AZ on February 3, 2016.

- Dr. Channah Rock will be presenting the final overview of this project at the Southwest Ag Summit in Yuma, AZ on February 26, 2016.

Appendix B.

Budget Summary

Over the course of the project, grant funds were used to cover costs associated with the following:

- Salaries for research specialists and students. Responsibilities included collection of irrigation water samples from Maricopa and Yuma, AZ as well as Imperial Valley, CA. Additionally, these specialists and students were responsible for support with data assessment, data cleaning, and data entry. A substantial amount of time was spent by our health educator, Dametreea Carr, in working with the App developer, communicating edits to the App, and tracking progress of the AgWater App development. She should also be commended by the level of effort that she provided to this project well beyond the scope and budget.

- Salary for research scientist. Responsibilities included assisting Dr. Gerba and Dr. Tamimi with risk assessment and model development and data reporting where appropriate. Also, advising the project members on data interpretation.

- Travel. Costs were allocated in the budget for travel from Tucson and Maricopa, AZ to Yuma, AZ and Imperial Valley, CA. Costs included mileage, per diem for lodging and meals, as well as any incidentals incurred.

- Consumables. A portion of the budget was dedicated to expendable laboratory supplies, water sample testing, consumables for molecular confirmatory work and sample shipping costs.

- Subcontractor. A sufficient amount of funds were allocated to hire an App developer to complete development of the online calculator, native App, and Ag Water App for iOS and android devices.

- It should be noted that originally the App development funds were allocated to University personnel. While all work was completed within the budgetary limitations of the grant, the team was forced to use an external subcontractor due to time constraints of the University IT and MobileMatters support staff.

Appendix C.

Tables and Figures

The following text provides an overview of both *E. coli* and coliform correlations. It is important to note that in the ultimate model development the research team selected to model coliforms as there was a more robust dataset to include in modeling as well as the statistical significance to pathogen loading and other physical and environmental factors observed.

E.coli Correlations

Both the air and water temperature have significant effects on the numbers of *E. coli* found in irrigation water, with greater numbers found with increasing air and water temperatures (see Tables 1 and 2 below).

Table 1. Comparisons between the numbers of *E. coli* found in irrigation waters at different air temperature levels. A *P*-value of ≤ 0.05 is considered statistically significant.

Air Temperature (°C)	*E. coli* (geometric mean)/100 ml	*P*-value
< 24.0 versus > 24.0	6.3 versus 17.2	*< 0.0005*
< 14.3 versus > 14.3	5.3 versus 12.5	*< 0.0005*
< 8.8 versus > 8.8	3.6 versus 11.2	*0.032*

Table 2. Comparisons between the numbers of *E. coli* found in irrigation waters at different water temperature levels. A *P*-value of ≤ 0.05 is considered statistically significant.

Water Temperature (°C)	*E. coli* (geometric mean)/100 ml	*P*-value
< 24.0 versus > 24.0	7.3 versus 17.6	*< 0.0005*
< 15.0 versus > 15.0	4.1 versus 11.6	*< 0.0005*
< 10.0 versus > 10.0	1.3 versus 11.2	*0.016*

The turbidity of the water has a significant effect on the numbers of *E. coli* in irrigation waters, with greater numbers found with increasing water turbidity (see Table 3).

Table 3. Comparisons between the numbers of *E. coli* found in irrigation waters with different turbidity levels. A *P*-value of ≤ 0.05 is considered statistically significant.

Turbidity (NTU)	*E. coli* (geometric mean)/100 ml	*P*-value
< 10.6 versus > 10.6	10.1 versus 15.1	*< 0.0005*
< 6.0 versus > 6.0	9.3 versus 13.3	*< 0.0005*
< 3.0 versus > 3.0	8.8 versus 11.8	*0.02*

The pH of the water has a significant effect on the numbers of *E. coli* in irrigation waters, with greater numbers found with increasing water pH (see Table 4).

Table 4. Comparisons between the numbers of *E. coli* found in irrigation waters at different pH levels. A *P*-value of ≤ 0.05 is considered statistically significant.

pH	*E. coli* (geometric mean)/100 ml	*P*-value
< 8.5 versus > 8.5	10.6 versus 15.7	*0.009*
< 8.1 versus > 8.1	9.6 versus 13.4	*0.001*

With increasing electro-conductivity of irrigation waters, higher *E. coli* numbers were observed; however, this difference was not usually statistically significant (see Table 5).

Table 5. Comparisons between the numbers of *E. coli* found in irrigation waters with different electro-conductivity levels. A *P*-value of ≤ 0.05 is considered statistically significant.

Electro-conductivity (dS/m)	*E. coli* (geometric mean)/100 ml	*P* value
< 2.5 versus > 2.5	11.0 versus 13.2	0.461
< 1.2 versus > 1.2	10.4 versus 13.6	*0.021*
< 1.1 versus > 1.1	10.9 versus 11.2	0.736

Solar radiation appears to have a significant effect on the numbers of *E. coli* in irrigation waters, with higher numbers found with increasing radiation (see Table 6). Nevertheless, since solar radiation is likely closely tied to temperature, the increasing numbers are probably due to the effects of higher temperatures rather than the greater solar radiation (which would presumably be somewhat antimicrobial).

Table 6. Comparisons between the numbers of *E. coli* found in irrigation waters at different solar radiation levels. A *P*-value of ≤ 0.05 is considered statistically significant.

Solar Radiation (Watt/m^2)	*E. coli* (geometric mean)/100 ml	*P*-value
< 30.0 versus > 30.0	10.6 versus 20.9	*< 0.0005*
< 22.0 versus > 22.0	7.9 versus 15.0	*< 0.0005*
< 13.0 versus > 13.0	7.4 versus 11.6	*0.007*

The heat index has a significant effect on the numbers of *E. coli* in irrigation waters, with higher numbers found with an increasing heat index (see Table 7). Since the heat index is tied to temperature, the trend for heat index resembles those for *E. coli* at various air and water temperatures.

Table 7. Comparisons between the numbers of *E. coli* found in irrigation waters at different heat index levels. A *P*-value of ≤ 0.05 is considered statistically significant.

Heat Index	*E. coli* (geometric mean)/100 ml	*P*-value
< 15.0 versus > 15.0	10.2 versus 16.4	*< 0.0005*
< 10.0 versus > 10.0	6.4 versus 17.7	*< 0.0005*
< 5.0 versus > 5.0	5.6 versus 12.9	*< 0.0005*

Some significant differences were observed between *E. coli* numbers in irrigation waters with varying wind speeds; however, no consistent trend was observed (see Table 8). No significant difference was observed between the extremes (low and high wind speeds), suggesting that wind speed is likely not a relevant factor for *E. coli* levels in irrigation waters.

Table 8. Comparisons between the numbers of *E. coli* found in irrigation waters at different wind speeds. A *P*-value of ≤ 0.05 is considered statistically significant.

Wind Speed (m/s)	*E. coli* (geometric mean)/100 ml	*P*-value
< 5.0 versus > 5.0	11.3 versus 8.0	*0.028*
< 2.2 versus > 2.2	10.5 versus 11.9	0.201
< 1.3 versus > 1.3	6.8 versus 12.5	*< 0.0005*
Low (< 1.3) versus High (> 5.0)	6.8 versus 8.0	0.458

The air relative humidity has a significant effect on the numbers of *E. coli* in irrigation waters, with fewer numbers found with increasing relative humidity (see Table 9).

Table 9. Comparisons between the numbers of *E. coli* found in irrigation waters at different air relative humidity percentages. A *P*-value of ≤ 0.05 is considered statistically significant.

Relative Humidity (%)	*E. coli* (geometric mean)/100 ml	*P-value*
< 65.0 versus > 65.0	11.6 versus 6.8	*0.004*
< 44.0 versus > 44.0	13.2 versus 9.0	*< 0.0005*
< 20.0 versus > 20.0	26.1 versus 10.6	*< 0.0005*
Low (< 20.0) versus High (> 65.0)	26.1 versus 6.8	*< 0.0005*

Rainfall within 2 days prior to the sampling date results in higher numbers of *E. coli* in irrigation waters (see Table 10). Nevertheless, this difference was only statistically significant when it rained on the sampling date and not on any days prior. This suggests that higher *E. coli* numbers are only transiently present in the irrigation water following a rainfall event.

Table 10. The effect of rainfall within 5 days prior to a sampling event on the numbers of *E. coli* found in irrigation waters. A *P*-value of ≤ 0.05 is considered statistically significant.

Rainfall versus No Rain (within 5 Days Prior to Sampling)	*E. coli* (geometric mean)/100 ml	*P*-value
Rainfall on Sampling Date vs. No Rain	25.3 versus 11.5	*0.005*
Rainfall within 1 Day Prior to Sampling vs. No Rain	15.5 versus 11.5	0.113
Rainfall within 2 Days Prior to Sampling vs. No Rain	12.6 versus 11.5	0.517
Rainfall within 3 Days Prior to Sampling vs. No Rain	8.7 versus 11.5	*0.022*
Rainfall within 4 Days Prior to Sampling vs. No Rain	9.6 versus 11.5	0.132
Rainfall within 5 Days Prior to Sampling vs. No Rain	10.2 versus 11.5	0.293

Total Coliform Correlations

Both the air and water temperature have significant effects on the numbers of total coliforms found in irrigation water, with greater numbers found with increasing air and water temperatures (see Tables 1b and 2b below).

Table 1b. Comparisons between the numbers of total coliforms found in irrigation waters at different air temperature levels. A *P*-value of ≤ 0.05 is considered statistically significant.

Air Temperature (°C)	Coliforms (geometric mean) per 100 ml	*P*-value
< 30.0 versus > 30.0	1,112 versus 5,080	*< 0.00001*
< 24.0 versus > 24.0	754 versus 4,015	*< 0.00001*
< 14.3 versus > 14.3	288 versus 2,594	*< 0.00001*
< 10.0 versus > 10.0	332 versus 1,953	*< 0.00001*

Table 2b. Comparisons between the numbers of total coliforms found in irrigation waters at different water temperature levels. A *P*-value of ≤ 0.05 is considered statistically significant.

Water Temperature (°C)	Coliforms (geometric mean) per 100 ml	*P*-value
< 30.0 versus > 30.0	1,538 versus 4,014	*< 0.00005*
< 24.0 versus > 24.0	861 versus 4,000	*< 0.00005*
< 15.0 versus > 15.0	231 versus 2,351	*< 0.00005*
< 10.0 versus > 10.0	298 versus 1,871	*0.0065*

The turbidity of the water has a significant effect on the numbers of total coliforms in irrigation waters, with greater numbers found with increasing water turbidity (see Table 3b).

Table 3b. Comparisons between the numbers of total coliforms found in irrigation waters with different turbidity levels. A *P*-value of ≤ 0.05 is considered statistically significant.

Turbidity (NTU)	Coliforms (geometric mean) per 100 ml	*P*-value
< 10.6 versus > 10.6	1,624 versus 2,763	*< 0.0003*
< 6.0 versus > 6.0	1,573 versus 2,218	*0.0063*
< 3.0 versus > 3.0	1,552 versus 1,936	0.14

The pH of the water has an effect on the numbers of total coliforms in irrigation waters, with greater numbers found with decreasing water pH; however, this difference was not usually statistically significant (see Table 4b). This is the opposite of the effect observed for *E. coli*.

Table 4b. Comparisons between the numbers of total coliforms found in irrigation waters at different pH levels. A *P*-value of ≤ 0.05 is considered statistically significant.

pH	Coliforms (geometric mean) per 100 ml	*P*-value
< 8.5 versus > 8.5	1,866 versus 1,631	0.51
< 8.1 versus > 8.1	2,094 versus 1,634	*0.046*
< 7.5 versus > 7.5	3,620 versus 1,821	0.17

At high levels of electro-conductivity of irrigation waters, higher total coliform numbers were observed; however, no statistically significant differences were found between total coliform numbers in waters with lower levels (see Table 5b).

Table 5b. Comparisons between the numbers of total coliforms found in irrigation waters with different electro-conductivity levels. A *P*-value of ≤ 0.05 is considered statistically significant.

Electro-conductivity (dS/m)	Coliforms (geometric mean) per 100 ml	*P* value
< 2.5 versus > 2.5	328 versus 5,602	*< 0.00001*
< 1.2 versus > 1.2	1,837 versus 1,845	0.95
< 1.1 versus > 1.1	1,756 versus 2,227	0.12
< 1.0 versus > 1.0	2,443 versus 1,781	0.12

Solar radiation appears to have a significant effect on the numbers of total coliforms in irrigation waters, with higher numbers found with increasing radiation (see Table 6b). Nevertheless, since solar radiation is likely closely tied to temperature, the increasing numbers are probably due to the effects of higher temperatures rather than the greater solar radiation (which would presumably be somewhat antimicrobial).

Table 6b. Comparisons between the numbers of total coliforms found in irrigation waters at different solar radiation levels. A *P*-value of ≤ 0.05 is considered statistically significant.

Solar Radiation (Watt/m^2)	Coliforms (geometric mean) per 100 ml	*P*-value
< 30.0 versus > 30.0	1,647 versus 5,315	*< 0.00001*
< 22.0 versus > 22.0	1,205 versus 2,861	*< 0.00001*
< 13.0 versus > 13.0	338 versus 2,181	*< 0.00001*

The heat index has a significant effect on the numbers of total coliforms in irrigation waters, with higher numbers found with an increasing heat index (see Table 7b). Since the heat index is tied to temperature, the trend for heat index resembles those for total coliforms at various air and water temperatures.

Table 7b. Comparisons between the numbers of total coliforms found in irrigation waters at different heat index levels. A *P*-value of ≤ 0.05 is considered statistically significant.

Heat Index	Coliforms (geometric mean) per 100 ml	*P*-value
< 15.0 versus > 15.0	1,450 versus 4,820	*< 0.00001*
< 10.0 versus > 10.0	803 versus 4,022	*< 0.00001*
< 5.0 versus > 5.0	592 versus 2,742	*< 0.00001*

Although some significant differences were observed between total coliforms numbers in irrigation waters with varying wind speeds, no consistent trend was observed (see Table 8b). No significant difference was observed between extremes (low and high wind speeds), suggesting that wind speed is likely not a relevant factor for total coliform levels in irrigation waters.

Table 8b. Comparisons between the numbers of total coliform found in irrigation waters at different wind speeds. A *P*-value of ≤ 0.05 is considered statistically significant.

Wind Speed (m/s)	Coliforms (geometric mean) per 100 ml	*P*-value
< 5.0 versus > 5.0	1,909 versus 972	*0.015*
< 2.2 versus > 2.2	1,673 versus 2,146	0.054
< 1.3 versus > 1.3	1,577 versus 1,901	0.264
Low (< 1.3) versus High (> 5.0)	1,577 versus 972	0.130

Although some significant differences were observed between total coliforms numbers in irrigation waters collected under varying air relative humidity levels, no consistent trend was observed (see Table 9b). No significant difference was observed between the extremes (low and high air relative humidity), suggesting that air relative humidity is likely not a consistently relevant factor for total coliform levels in irrigation waters.

Table 9b. Comparisons between the numbers of total coliforms found in irrigation waters at different air relative humidity percentages. A *P*-value of ≤ 0.05 is considered statistically significant.

Relative Humidity (%)	Coliforms (geometric mean) per 100 ml	*P-value*
< 65.0 versus > 65.0	2,002 versus 764	*< 0.00001*
< 44.0 versus > 44.0	2,912 versus 1,028	*< 0.00002*
< 20.0 versus > 20.0	1,184 versus 1,856	0.350
Low (< 20.0) versus High (> 65.0)	1,184 versus 764	0.563

Rainfall at any point within 5 days prior to and/or on the sampling date results in higher numbers of total coliforms in irrigation waters (see Table 10b). This difference was statistically significant when it rained on the sampling day and within 5 days prior with one exception (rainfall within 3 days prior). Nevertheless, the total coliform numbers were the highest if rainfall had occurred at any time within two days prior to sampling. This suggests that higher total coliform numbers persist for several days in irrigation water following a rainfall event, but are at their highest levels for ≤2 days.

Table 10b. The effect of rainfall within 5 days prior to a sampling event on the numbers of total coliforms found in irrigation waters. A *P*-value of ≤ 0.05 is considered statistically significant.

Rainfall versus No Rain (within 5 Days Prior to Sampling)		Coliforms (geometric mean) per 100 ml	*P*-value
Rainfall on Sampling Date	vs. No Rain	4,350 versus 1,823	*0.0053*
Rainfall within 1 Day Prior to Sampling	vs. No Rain	4,724 versus 1,823	*0.00032*
Rainfall within 2 Days Prior to Sampling	vs. No Rain	3,746 versus 1,823	*0.0034*
Rainfall within 3 Days Prior to Sampling	vs. No Rain	2,446 versus 1,823	0.145
Rainfall within 4 Days Prior to Sampling	vs. No Rain	2,524 versus 1,823	*0.0335*
Rainfall within 5 Days Prior to Sampling	vs. No Rain	2,506 versus 1,823	*0.0287*

Appendix D.

Suggestions to CPS

a) The research team found the CPS reporting requirements and communication with the grants manager to be pleasant and not burdensome as with some granting agencies. We suggest that CPS strive to maintain this level of commitment to their project PIs in the future.

b) The research team would like to work with CPS personnel to increase the distribution and dissemination of the research results in the future beyond the term of the grant. This could be in the form of sponsored workshops, conferences, round-table discussions, etc. While the research symposium is a wonderful opportunity to disseminate research results, we would like to build upon this and look towards working with CPS to identify these opportunities in the future and to collaboratively expand upon them.

c) One suggestion in relation to the comment above is for CPS to host monthly or quarterly "*webcasts*" or seminars where PIs are required to present their final report findings and answer questions to online participants at some point in time during the year following the end of their project. These webcasts can be as short as one hour and have as many participants as needed. This mechanism may be a successful way to reach/engage a broad number of stakeholders in the industry as well as other research scientists over an extended period of time.

d) At the onset of this project, the project team understood that the research goals and objectives were extremely ambitious to accomplish within a two-year time frame. While the work has been completed and the research team is very confident and proud of the resulting research and extension products, it would have been appropriate to build in some additional support from CPS to test the developed industry products at a larger scale after the completion of the project. As the *AgWater App* is used in the future, it is highly likely that revisions will be needed to strengthen its use and to broaden its application beyond the initial test case of the Southwest. Currently, there is no mechanism to update this application as the grant has now closed. It is suggested to CPS that for projects such as this, in the future, a separate fund may be applied for to meet the needs of the project, a fund that is separate from the main research call for proposals.