FINAL REPORT

Project Title: Survey of Selected Bacteria in Irrigation Canal Water – Second Year

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Summary
Data of water quality in the irrigation canals was collected during 9 months (this an on-going study in its third-year stage) and correlated with environmental factors. The objective was to determine levels of bacteria indicators, including total coliform count, fecal coliform and generic E. coli, and determine a trend through the year. Collection included sampling of 4 sites in the Yuma Valley on a weekly basis. On the same days populations of insects captured during the week and count of birds during the 15 minutes prior to water sampling were recorded. Temperature and relative humidity data was also recorded. The results considered for these past 9 months showed what was observed in the previous 24 months. This meaning that bacteria indicators have higher levels before and after winter months when the winter vegetable are not harvested. However, several spikes have been observed during the winter which is a concern because some of those levels would not be acceptable using the metrics of the recent Arizona Marketing Agreement. During the last year we included the analysis of *E. coli* O157:H7 and the counts of specific insects such as various species of flies. No sample has been positive for *E. coli* O157:H7 as today. Moreover, this year we investigated the impact of different fly species that have been trapped in our collections. We have preliminarily correlated populations of the black scavenger fly with counts of E. coli in water, a finding the warrants further investigation.

Introduction

The reported illnesses cases associated with lettuce are now more publicized, and despite the fact that most cases have been due to cross commodity contamination (eg. meat to lettuce in kitchens), the cases of contaminated lettuce in the field are increasing. Eighteen outbreaks associated with consumption of lettuce were reported during the 1995-2005, due to contamination with pathogenic *E. coli.* Moreover, lettuce and leafy salad accounts for 25% of all the outbreaks traced to produce during the 1990-2003 period. After seafood, produce is the single food associated with the highest incidence of outbreaks. Consumers are becoming more aware of this information while retailers as well as Federal authorities are enforcing/stressing the importance of developing efficient food safety programs during the production and handling of lettuce. There is no doubt, that currently food safety is one of the most important concerns of the iceberg lettuce industry.

Despite the fact that no Arizona lettuce grower has been involved in any contaminated-lettuce outbreak, it is of paramount importance to determine the reasons why Arizona lettuce is regarded as safe. This can help lower possibilities of any emerging problem and prevent a catastrophic damage to the industry, as it has occurred in other regions when no control was taken to reduce risks of contaminated product.

This study was justified because more stringent food safety programs are currently requested by buyers, and the quality of surface water in Arizona is not well defined. Despite the fact that no Arizona grower has been implicated in any contaminated-lettuce outbreak, it is of paramount importance to determine the reasons why Arizona lettuce is regarded as safe, as to continue ensuring safety of the Arizona head lettuce. The objectives of this study (for all three years) are:

a) To develop a survey of bacteria indicator in irrigation canal water of the Yuma Valley;

b) To examine possible relationship between bacteria indicator population and environmental factors and/or biological agents;

c) To determine any implications of the survey on current and future food safety regulations.

Methodology

Samples of water were taken in four different sites across the Yuma Valley. Two were near the city of Yuma, one in the Sommerton area and one in the San Luis area. The samples were taken every week, and this has been the norm during the third year as well. We submitted the samples to AgriTrend Lab were analysis of bacteria was conducted following specifications of the Membrane Filtration Method m-Coliblue 24™ (USEPA Method No. 10029). Preparation of samples, inoculation, incubation and count of microbes were performed following manufacturer's recommendation. We analyzed water for total coliforms, and fecal coliforms. We also evaluated for generic *Escherichia coli*, as this a very important human fecal indicator used for reference of pathogenic growth in the environment. Since the second year (and currently) we also have been analyzed for *E. coli* O157:H7 during 6 months of the year (October-March) to determine any correlation with any of the bacteria indicator counts.

Data of waterborne bacteria were matched with data of wind speed, air temperature, solar radiation, relative humidity, sourced from the weather station. Insect traps were placed nearby the water sampling sites and the number of caught insects were counted weeks. Birds roaming in a 200 feet diameter around the water sampling site were counted for 15 minutes before taking the water samples. We identify flies in traps and conducted correlation analysis with the bacteria counts.

Results

AILRC awarded us funds to develop a water survey study for three years. Up to date the pattern has been very consistent: with coliforms and *E. coli* declining significantly during the winter showing a similar trend to that of environmental and soil temperature. This can be seen in the data collected during this past year (Figures 1-4). It is relevant to mention that the low temperature during the winter accompanied with

<60% relative humidity prevalent in Yuma, may substantially decline *E. coli* as suggested by the literature.

The new metrics used for the California Marketing Agreement and adopted for the Arizona Marketing Agreement, states that acceptable levels should include <126 cfu E. Coli/100 ml). However, it is important to mention that we found that in certain weeks and locations "spikes" of the bacteria population are possible. This past year we found this occurring in two of the four locations. This is a concern because it would imply exhaustive subsequent sampling under the new Arizona Marketing Agreement. No clear relationship has been observed to this point between bacteria indicator counts and bird. Starting during the second year we initiated with the analysis of *E. coli* O157, and up to date no sample has been positive for this pathogen.

Final Remarks

Our results have shown a clear trend indicating decline of bacteria indicators during the season when lettuce is grown in Arizona, which correlates with a decline in average temperature. However, we have also observed "spikes" in the data that alert growers as this would involve more sampling (following the spike) according to the new Arizona Marketing Agreement. The benefit of these results is two fold. First, growers, national regulatory entities and buyers are starting to have information about the quality of the water in the Yuma valley, and how this fluctuates during the year. More importantly, the data is used to determine whether there is any connection with environmental or biological factors. In the latter case, we believe is important to study more profoundly the potential connection of flies as vectors of *E. coli* to water as our results have preliminarily suggesting this. Despite the fact that Arizona leafy greens has never been associated with foodborne outbreaks, this study is contributing with relevant information that can be used for future regulatory guidelines.

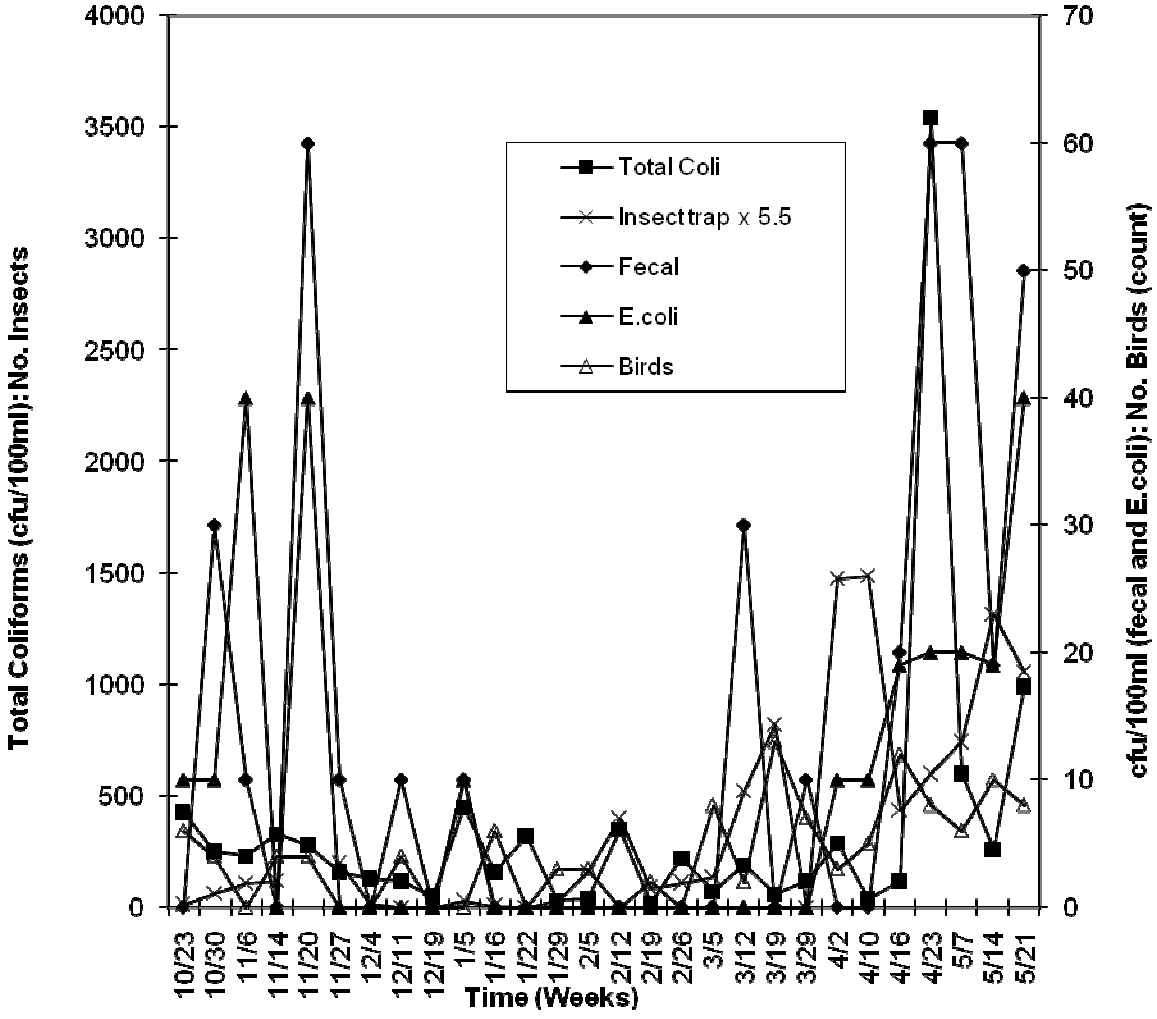

Figure 1. Bacteria, birds and insect counts in a sampling site in a secondary canal in the west side of the city of Yuma during the period October 2007-May 2008.

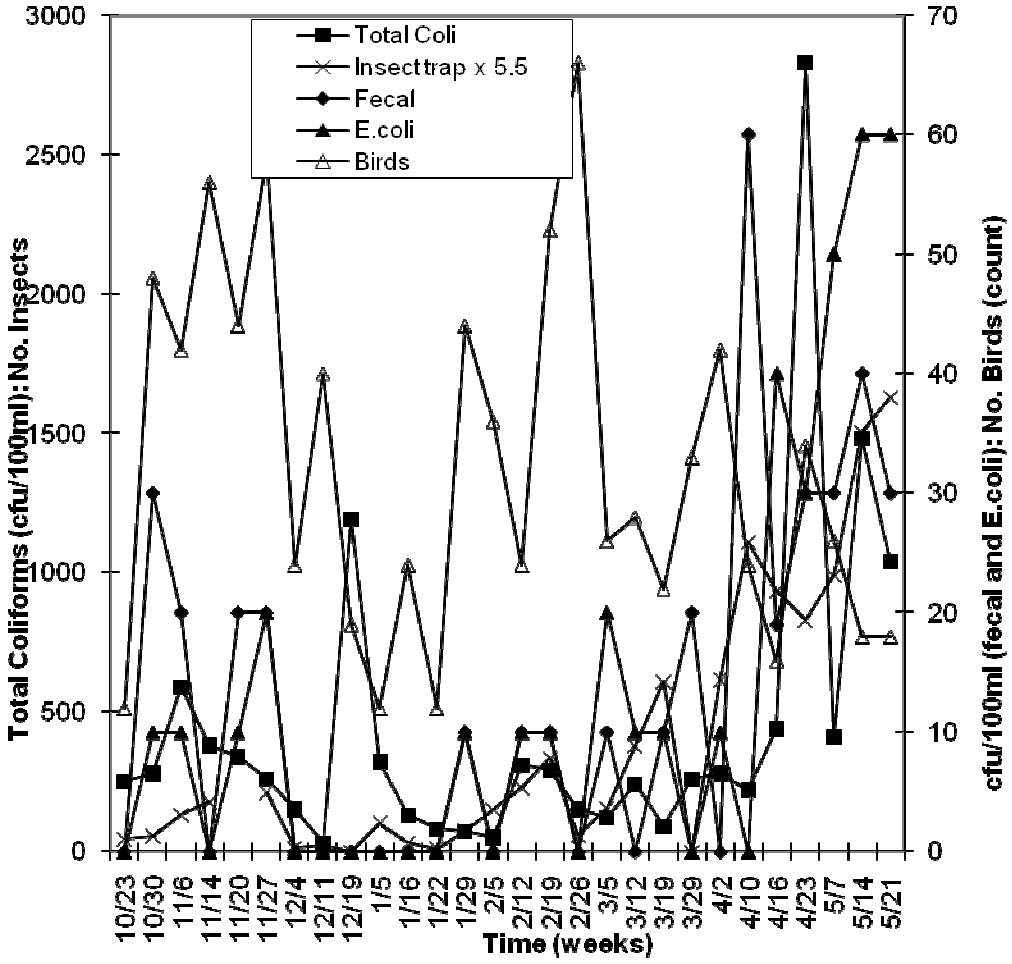

Figure 2. Bacteria, birds and insect counts in a sampling site near the main west canal in the Yuma Valley during the period October 2007-May 2008.

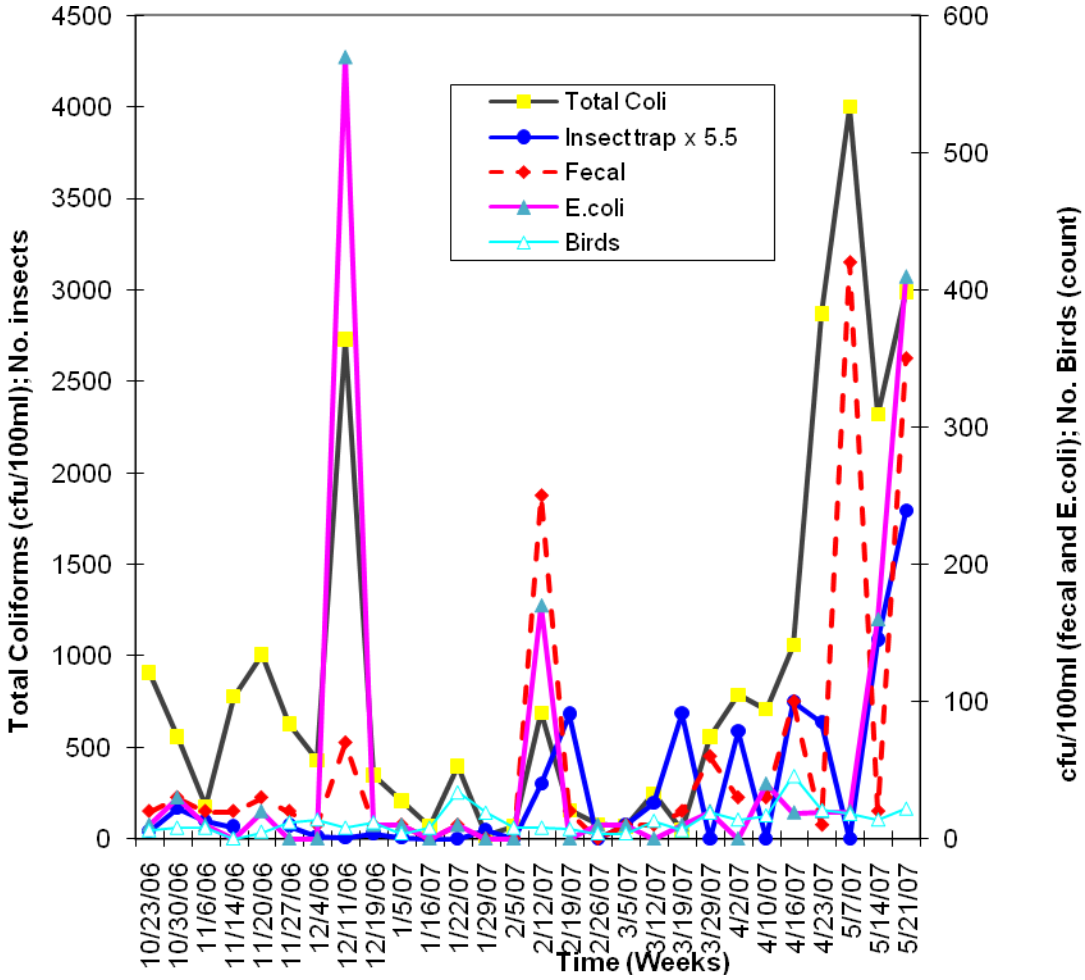

Figure 3. Bacteria, birds and insect counts in a sampling site near the city of Sommerton, AZ during the period October 2007-May 2008.

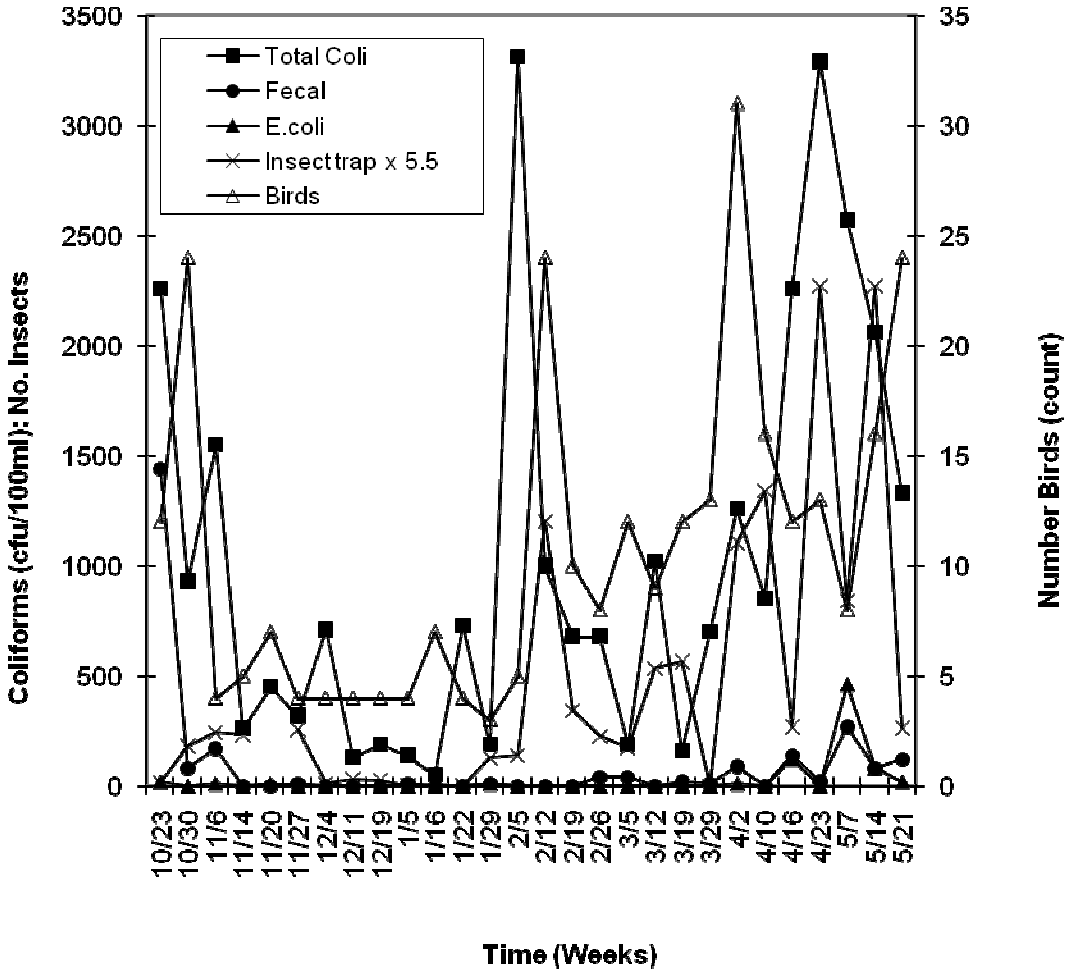

Figure 4. Bacteria, birds and insect counts in a sampling site near the main west canal in the Yuma Valley during the period October 2007-May 2008.