Microbiological risk assessment using QMRA in preharvest agriculture water treatment systems for leafy greens

Contact

Channah Rock, PhD
The University of Arizona
channah@cals.arizona.edu

Authors

Charles Gerba (Co-PI), Kerry Cooper (Co-PI), Natalie Brassill, Victoria Obergh – University of Arizona; and Kerry Hamilton (Co-PI) – Arizona State University

Project funding dates

January 1, 2023 – December 31, 2024

Acknowledgements

The University of Arizona and Arizona State University research and extension teams would like to acknowledge the support of BioSafe Systems and BlueTech Water for their generous contributions to this research project. Additionally, we would like to thank members of the leafy greens industry in the Southwest that allow our team to conduct this research.

Summary

While much research has been done on the efficacy of commonly used agricultural water treatment sanitizers including peroxyacetic acid (PAA), calcium/sodium hypochlorite, and chlorine dioxide to reduce pathogens/indicators in water, little research has focused on the potential added benefit of these sanitizers on pathogens that are already established on crop surfaces, plant tissues, or in soil. Also, variability in water treatment or "breakthrough" has not been properly characterized in an agricultural setting. This project aims to directly address these knowledge gaps by using laboratory and field evaluation coupled with quantitative microbial risk assessment (QMRA). Results of this study will provide growers and regulators with an improved understanding of the impact of water treatment on risk reduction for consumers.

Objectives

1. Determine the die-off or log-reduction of Shiga-toxigenic *Escherichia coli* (STEC) and generic *E. coli* surrogates pre-established on leaf surfaces and in soil following agricultural water treatment with commonly used water treatment sanitizers (PAA and calcium hypochlorite).
2. Conduct in-field evaluations of water treatment variability or "breakthrough" using traditional grab sampling techniques for microbiological indicators (generic *E. coli* and Total Coliform bacteria) coupled with real-time in-line monitoring for physical/chemical parameters (PAA, free chlorine, pH, temperature, ORP, flow rate in gpm).
3. Use real-world collected data from research Objectives 1 and 2 to conduct a QMRA for STEC in leafy greens (romaine and spinach).

Methods

Non-pathogenic E. coli TVS353 at ~1×10^4 CFU/100 mL was applied in field experiments (**Figure 1**). Following agricultural water treatment, plant tissue and soil samples were collected at 7-, 14-, and 21-days post irrigation. Ten heads of lettuce and ten 100-g soil samples at randomized locations within each plot were collected each day. For each head of lettuce or soil sample, duplicate 25-g samples were weighed and placed into individual Whirl-Pak bags containing 90 mL of 1X Phosphate Buffered Solution. Samples were stomached for 1 minute and two 100-ul aliquots were spread plated on ECC + Rifampicin (80 ug/mL). Plates were incubated at 37°C for 18 to 24 hours. Positive samples were confirmed as blue colonies and counted to determine bacterial concentrations.

Results to Date

To date the research team has evaluated both PAA and calcium hypochlorite agricultural water treatment(s) and the ability of residual chemical(s) to inactivate previously inoculated surrogate TVS353 on plant tissue (romaine lettuce). An in-line sensor monitoring unit is being used for in-field evaluations (**Figure 2**). Initial findings indicate between 12 and 20% reduction in the ability to detect the presence of generic *E. coli* TVS353 on plant tissue post treatment for PAA and calcium hypochlorite (chlorine), respectively (**Figures 3** and **4**). Results of agricultural water treatment demonstrate time periods where residual (ppm) dropped below critical limit threshold(s) for up to 56 minutes during a 6-hour irrigation event, with the majority of "breakthrough" occurring at system start up and stabilization. Additional field trials will be conducted to capture the full range in variability of breakthrough events to better inform the fresh produce industry.

Benefits to the Industry

This project will result in documented scientific data which growers can use as justification for validity of enhanced benefits of their antimicrobial water treatment beyond die-off of organisms found in agricultural water alone. With the use of our "in-line sensor," this will effectively allow the research team to study treatment systems at commercial-scale production and document critical time periods and durations of treatment variability that may result in a loss of effectiveness due to decreased residual. Ultimately, results of this project will allow the research team to better understand and describe the cumulative impacts of water treatment variability on pathogen presence, persistence, and survival in water, on produce, and in soil and how that ultimately may impact human health risk with QMRA.

Figure 1. UA research team member inoculating *E. coli* TVS353 on romaine lettuce

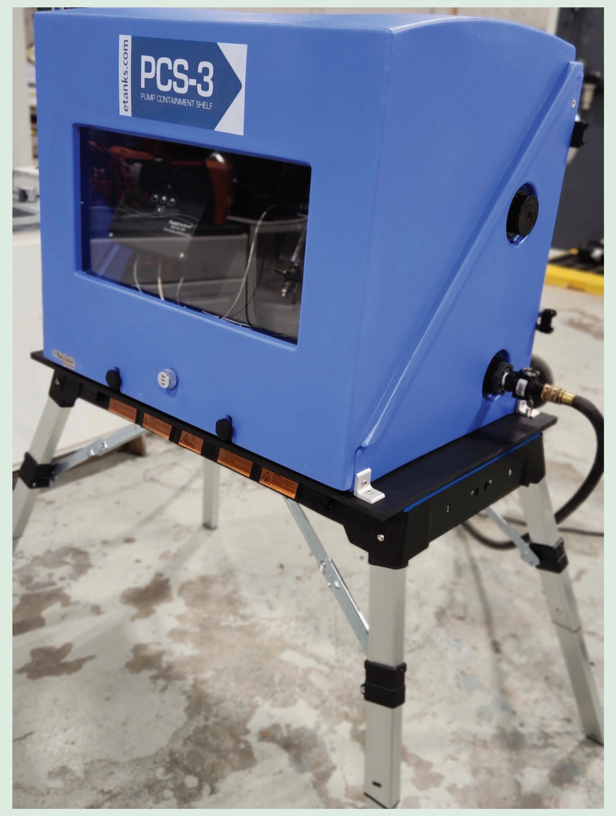

Figure 2. Portable in-line sensor suite "Beatrice the Blue Box"

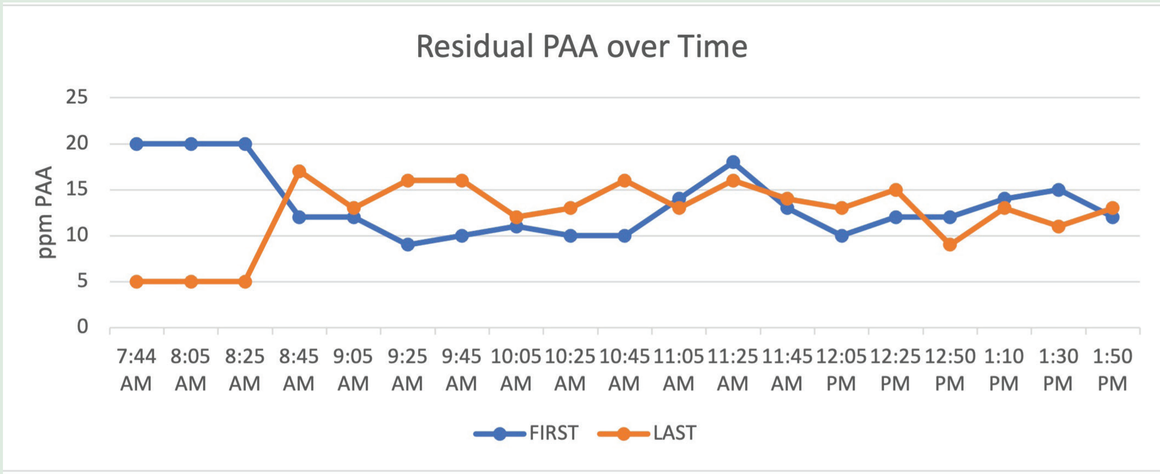

Figure 3. Residual PAA over time at first and last sprinkler heads

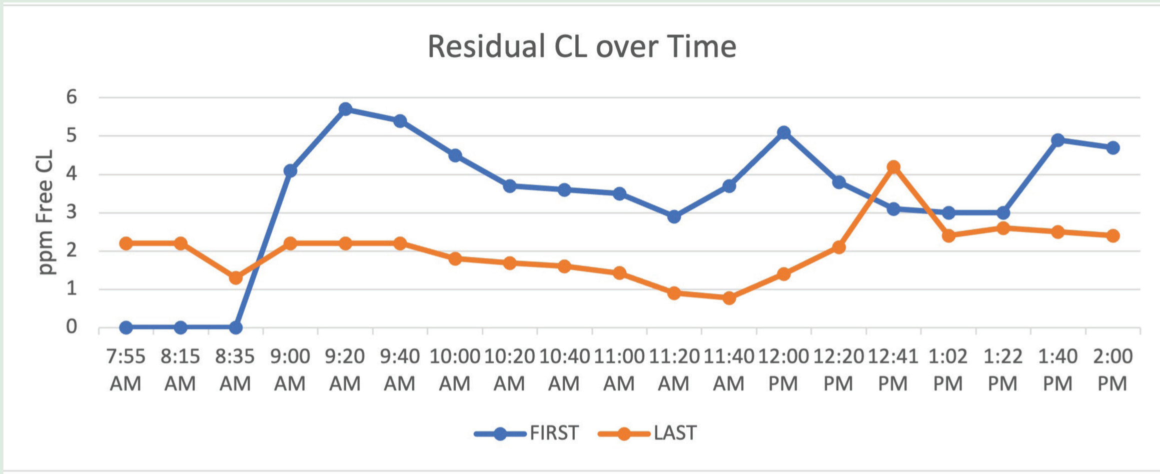

Figure 4. Residual chlorine over time at first and last sprinkler heads