2026 CLIMAS Environmental & Society Fellowship Final Report

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Introduction. In May 2024, the U.S. Food and Drug Administration (FDA) published revisions to Subpart E of the Food Safety Modernization Act (FSMA) Produce Safety Rule (PSR). The PSR establishes mandatory standards for the safe production and harvest of fresh fruits and vegetables. These updates specifically address pre-harvest agricultural water— water used for growing activities that is intended or likely to contact produce, such as water used for irrigation or crop protection sprays. The revisions shift from a testing-based to a systems-based approach, introducing requirements of a **pre-harvest agricultural water assessment**.

Historically, growers primarily relied on numerical water testing results for total coliforms and generic *Escherichia coli* (*E. coli*) to comply with bacterial limits set by the PSR. Now, they must evaluate factors related to pre-harvest agricultural water to identify potential food safety hazards (e.g., *E. coli, Listeria monocytogenes, Salmonella*) and their associated risk. Factors include, but are not limited to, the water source, water application method, crop characteristics, and environmental factors. Based on the assessment, science-based mitigation measures may be necessary to reduce identified risks, such as increasing the time interval between final water application and harvest. It is well established that elapsed time and environmental conditions, such as UV radiation and temperature, can reduce potential pathogen levels on produce. However, the extent of this decline under real-world conditions and across commodities is not always well understood.

Research Objectives. This study aimed to quantify the survival of pathogen surrogate *E. coli* on romaine lettuce and lemon fruits following contaminated water applications in Arizona to provide fresh produce growers with scientific support for risk-based decision-making.

Methods. Romaine lettuce heads (N = 150) and whole lemons (N = 205) were inoculated with *E. coli* contaminated water under field conditions. Samples were collected post-inoculation up to 14 days (lettuce) and 1 day (lemons) across two trials per commodity. For romaine, whole-head and composite (i.e., leaf pieces from several heads) samples were collected, and for lemons, whole-fruit. Trial 1 of lettuce used a high inoculum (~4.40 log CFU/mL), and Trial 2 a low inoculum (~2.69 log CFU/mL). Lemons were inoculated at a consistent level (~4.00 log CFU/mL). *E. coli* prevalence on produce was assessed using culture-based techniques, including spread plating, most probable number, membrane filtration, and enrichment.

Key Research Findings & Takeaways

- **Sample type impacted detection in romaine lettuce trials.** Whole head samples generally exhibited lower *E. coli* concentrations compared to composites, and/or *E. coli* was detectable for a shorter duration in whole head samples. This indicates that if preharvest testing occurs, composites may better represent contamination in the field.

- **Higher contamination levels impacted die-off.** When a higher bacterial load was assessed, minimal *E. coli* die-off was observed on romaine lettuce up to 14 days post-application, but at lower levels, decreased concentrations were observed within 1-day post-application. The high levels tested are unlikely to occur naturally in the growing environment, indicating that research conditions may need to be carefully assessed when used by a grower for decision-making.
- **Die-off patterns differed between tested commodities.** The spray treatment had minimal impact on bacterial contamination of lemon fruits compared to romaine lettuce under field conditions, indicating a commodity-specific difference in survival dynamics or die-off. For example, whole lemon fruits fell below the limit of detection (<2 CFU/fruit) by 2.5 hours, and romaine lettuce composite concentrations did not decrease by 14 days.
- **Survival is variable.** Bacterial survival is influenced by many factors, including initial contamination load, growing environment, weather conditions, and commodity type. Careful consideration is required for the scientific justification used in a Subpart E Pre-harvest Agricultural Water Assessment.

Key Outcomes and Accomplishments. New die-off data on romaine lettuce and lemons were formulated to better assist fresh produce growers with scientifically justifying decision-making regarding a pre-harvest agricultural water assessment. This project is a part of a greater effort funded by the Center for Produce Safety, with collaborations with Arizona State University and Drexel University. All field trial results combined with historical data have been incorporated into a Quantitative Microbial Risk Assessment (QMRA) Tool to assist growers with assessing mitigation measures. This tool allows users to customize factors regarding pre-harvest agricultural water, such as the water source, water application method, crop type, and time interval between final water application and harvest, and outputs infection risk based on the selected factors. This tool is in the final stages of development and will incorporate industry feedback to ensure applicability.

Research thus far has been shared across extension workshops, technical talks, and poster presentations. The relationships built with growers and industry professionals through these meetings have been invaluable and an extreme benefit to this project. Future efforts will continue to focus on educating the industry about these findings, including on how to utilize the risk tool and through developing written resources like fact sheets.

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