RESEARCH IN PROCESS

FSMA agricultural-water die-off compliance provisions benefit from condition-specific modifiers

SUMMARY

The FSMA Produce Safety Rule includes a provision stating that if *E. coli* levels exceed a certain threshold in agricultural water, produce growers must wait a set period from water application to harvest according to a die-off rate of 0.5 log CFU/day. Validation is needed to confirm the accuracy of this die-off rate and to evaluate die-off of *E. coli* as an indicator for die-off of pathogens. This study will track the die-off of generic *E. coli* and attenuated *Salmonella* on baby spinach and baby lettuce in replicated, controlled field trials in Davis, CA, Freeville, NY, and Murcia, Spain. An irrigation event using water contaminated above the set threshold will be simulated using a cocktail of *E. coli* and *Salmonella* strains. Survival of these strains on the produce will be enumerated at several time points following application. This data will be used to evaluate the accuracy of the FDA estimated die-off rate and to identify factors that affect the die-off rate.

OBJECTIVES

1. Estimate die-off rates of indicators and attenuated pathogens on baby spinach and baby lettuce in a replicated controlled trial under field conditions in 3 different climatic regions.
2. Develop a predictive model of pathogen die-off under relevant environmental conditions and industry practices and use the model to evaluate the FSMA agricultural water matrix.

METHODS

Three replicated trials will be performed in each study location. In each trial, four 1.5m x 4m plots will be planted with baby spinach and four plots with baby lettuce (Figure 1). At ~40 days after planting, the produce will be spray inoculated with a cocktail of three rifampicin (rif)-resistant generic *E. coli* strains and two rif-resistant attenuated *Salmonella* strains to reach a target concentration of 10^5 CFU/g of produce (Figure 2 and 3). At 0 – 96h after inoculation, 5 samples of produce will be collected per plot and enumeration of rif-resistant *E. coli* and rif-resistant *Salmonella* will be performed (Figure 4). If no growth is observed, enrichment will be performed to test for the presence of viable cells below the detection limit. Additionally, for one trial per location, 3 lettuce and 3 spinach samples will be collected at 0h, 24h, 48h, 72h, and 96h after inoculation; PMA-qPCR will be performed on these samples to test for the presence of VBNC cells. Weather characteristics will be recorded in all locations from the time of planting to the end of sample collection for each trial.

RESULTS TO DATE

To date, representatives from the laboratory groups in each study location met in Davis, CA, to plan all experimental procedures to be used in the study. Specifically, it was determined that 1,680 data points would be collected per location for die-off calculations (3 trials × 2 produce types × 4 plots per produce type × 2 bacteria × 7 time points × 5 samples); this equates to 5,040 data points in total (1,680 per location ×3 locations). The laboratory in Spain has planted their first trial.

BENEFITS TO THE INDUSTRY

This project will produce a science-based microbial die-off rate for management of non-complying agricultural water and prediction of microbial die-off on produce. In other words, this project will strengthen the current FSMA die-off rule or indicate how it should be modified. Broadly speaking, the produce industry will benefit from the project, as the findings will provide for scientifically justified risk management of agricultural water application, which has direct implications for contamination risks throughout the produce supply chain and public health. Growers will be better able to predict when it is safe to harvest, which has the potential to increase their financial returns. This project will also provide a validated mathematical model of produce contamination following application of non-complying agricultural water, which could be used for prediction of pathogen die-off under diverse conditions. The model will be made available to the stakeholders for updating as more data become available.

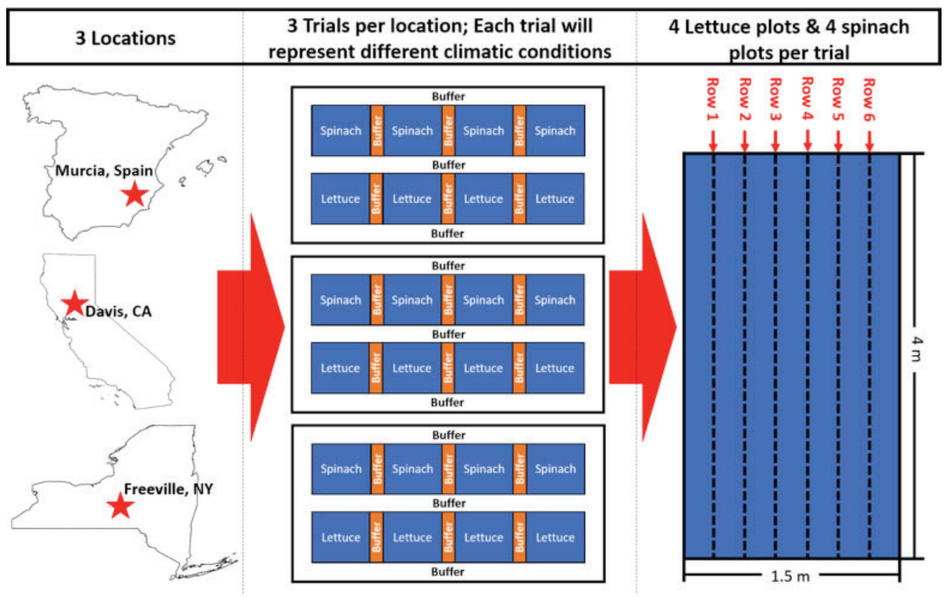

Figure 1. Field set-up to be used in each location for each trial.

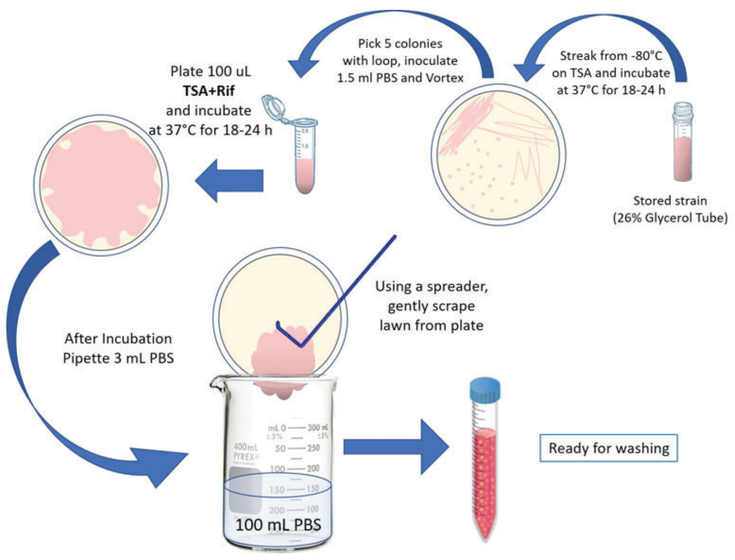

Figure 2. Diagram of inoculum preparation.

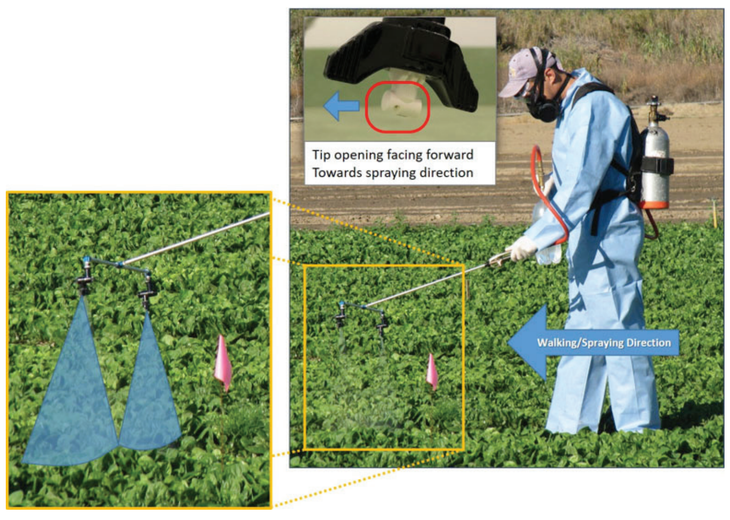

Figure 3. Example of in-field inoculation of baby spinach to simulate an irrigation event with contaminated water.

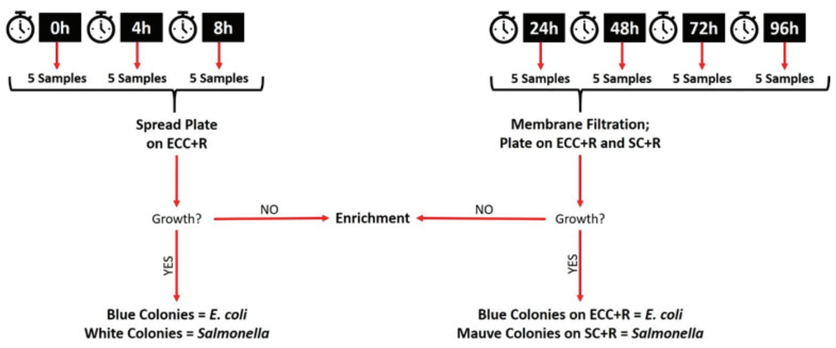

Figure 4. Sampling scheme and sample processing flow diagram.

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