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 E. coli strains and two rif-resistant cocktail of three rifampicin (rif)-resistant generic E. coli 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.

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.

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 x 2 produce types x 4 plots per produce type x 2 bacteria x 7 time points x 5 samples), which equates to 5,040 data points in total (1,680 per location x 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 and 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.

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