Quantifying risk associated with changes in EHEC physiology during post-harvest pre-processing stages of leafy green production

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Summary
The goal of the project is to determine if the time between harvest and end use of romaine lettuce impacts *E. coli* O157:H7 pathogenicity and detectability resulting in increased health risks. The study is being implemented in two broad phases. The first phase entails laboratory-scale experiments that will determine the changes in *E. coli* O157:H7 stress tolerance and virulence in romaine lettuce. In the second phase, primary data including temperature and supply chain logistics of commercial romaine handling from industry partners will be incorporated in a quantitative microbial risk assessment model. The resulting quantitative tool will be publicly available and allow for growers and producers to determine any practices that should be implemented to reduce the potential for O157 transmission on romaine lettuce.

Objectives
The overall goal of the project is to characterize physiological changes in the population of *E. coli* O157:H7 cells on leafy greens during pre-processing handling and refrigerated storage that may impede detectability and increase stress resistance and/or virulence.
The specific research objectives of the project are:

1. Determine the impact of pre-processing time delays on the physiological state of *E. coli* O157:H7 on romaine lettuce.
2. Evaluate the impact of physiological state on detectability of *E. coli* O157:H7 on romaine lettuce.
3. Quantify changes in *E. coli* O157:H7 risk over pre-processing cut-to-cool and refrigerated transport times.

Methods
Systematic review and modeling. A systematic review was conducted, and 205 datasets were identified that met the inclusion criteria of a minimum of 4 data points and that could be fitted with nonlinear inactivation (decay) models. Decay models were evaluated to determine those that best fit the data sets.

Methods (continued)
Quantifying survival, persisters, and chlorine tolerance on romaine lettuce. Greenhouse-grown romaine lettuce was spray inoculated with 2 strains of *E. coli* O157:H7. Inoculated plants were held at 17°C for 24 hours and then harvested. Harvested lettuce was placed in plastic lined bins and stored at 17°C for 4 hours before forced air cooling to 2.5°C. Lettuce was sampled at the time of harvest (0 h), before cooling (4 h), and every day after cooling for 5 days (24, 48, 72, 96, and 120 h). Collected samples were evaluated for culturable cells, persister cells, and

Results to Date
Most datasets identified were from lettuce, and from the following points in the production chain: primary production environment, processing, and storage (**Table 1**). The model that best fit the largest proportion of the datasets was the log-logistic model by Juneja and Marks (**Figure 1**). This model will be used for further analysis of the datasets to identify conditions that influence rates of inactivation.

Levels of *E. coli* O157:H7 culturable cells decrease over time on romaine lettuce during cold storage (**Figure 2A**). While variable, the mean percentage of cells in the persister state increase over time during cold storage (**Figure 2B**). So far, this increase in percentage of cells in the persister state does not appear to be associated with significant changes in chlorine tolerance. On average, log reduction due to chlorine exposure remained similar over time (**Figure 2C**).

Benefits to the Industry
The data analyzed so far will be used to determine which conditions influence pathogen inactivation parameters. Those that are significant will be included in the quantitative microbial risk assessment models. This quantitative tool will be available to the public to allow for growers and producers to determine any practices that should be implemented to reduce the potential for *E. coli* O157 transmission on romaine lettuce.
The study will detail the post-harvest pre-processing handling and timing practices that lower the risk of contamination as adaptable practices within the produce processing industry to reduce the potential for *E. coli* O157 transmission on romaine lettuce.

Variable		Frequency (n = 205)	Percentage of datasets in each category
Type of vegetable	Lettuce	172	83.90
	Spinach	31	15.12
	Cabbage	2	0.97
Stage of the value chain	Primary production	72	35.12
	Processing	35	17.07
	Transport	1	0.49
	Storage (consumer & retail)	97	47.32
Decontamination treatment	None	144	70.24
	Chlorine wash	43	20.98
	Cold nitrogen plasma	13	6.34
	Organic acids	3	1.46
	Irradiation	2	0.98

Table 1. Summary of *E. coli* O157:H7 inactivation datasets under different conditions

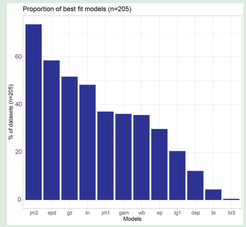

Figure 1. Proportions of datasets best fit by the different models. Models are as follows: bi- biphasic exponential, bi3- biphasic (3 parameters), dep- double exponential, ep- first order exponential decay, epd- Exponential damped , gz- Gompertz-3, gam- Gamma, jm1- two-stage model Juneja and Marks, JM2-log-logistic model Juneja and Marks, lg1- general logistic model, ln- Log-normal, and wb- Weibull.

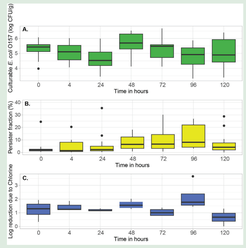

Figure 2. Distributions of culturable *E. coli* O157:H7 cells on romaine lettuce (A), percentage of E. coli O157:H7 cells in the persister state (B), and log reduction of *E. coli* O157:H7 on lettuce washed with 20 ppm free chlorine for 30 seconds (C). Time points are the time of harvest (0 h), prior to cooling (4 h), then storage at 2.5°C for 5 days (24, 48, 72, 96, and 120 h).