RESEARCH IN PROCESS

A systematic review of *Listeria* growth and survival on fruit and vegetable surfaces: responding to critical knowledge gaps

SUMMARY

There are critical knowledge gaps regarding the risk of *Listeria monocytogenes* on intact fruit and vegetable surfaces. Within the last decade, *L. monocytogenes* has been associated with outbreaks linked to contaminated intact produce, including cantaloupe, stone fruit, and caramel apples. While *L. monocytogenes* has been isolated from a wide variety of environments, including produce production and urban, natural, processing, and retail environments, nearly all (99%) listeriosis infections are attributed to contaminated food. For that reason, controlling the growth or survival conditions is essential to minimize contamination events as food moves throughout the supply chain. This is especially important for produce that is often consumed raw, or with minimal processing. Since it is widely accepted that *L. monocytogenes* may be present in produce production environments, data is needed on *L. monocytogenes* behavior on whole produce when handled/stored at typical and abuse conditions over the typical shelf life.

OBJECTIVES

1. Conduct a systematic review to identify and characterize published data on the growth and survival of *L. monocytogenes* on intact fruit and vegetable surfaces.
2. Perform *L. monocytogenes* growth and survival experiments on intact fruit and vegetable commodities at selected conditions to fill critical data gaps.
3. Develop risk models for a sub-set of fruit and vegetable storage or handling conditions shown to display growth or enhanced survival of *L. monocytogenes*.

METHODS

Systematic Review

Searches were conducted using the following Medical Subject Heading (MeSH) terms: Listeria monocytogenes, growth, survival, intact, and produce. All MeSH search terms were modified for each database and exploded to find all related subheadings. To be eligible for inclusion, literature had to show sufficient information on methodology, experimental conditions, and quantitative growth and survival data over time. Two of the authors (CM and JZ) independently reviewed all relevant literature based on title and abstract. Discrepancies were resolved by discussion and consensus with another reviewer (LS). Information regarding relevant experimental conditions were extracted and entered into a standardized electronic format. When studies did not provide numerical data, images of data visualizations were reverse-engineered to extract the underlying numerical data using a semi-automated Web Plot Digitizer. Also, growth rates were obtained through linear regression models. Two reviewers (LS and DS) conducted a quality assessment on data extraction.

RESULTS TO DATE

The search identified 3,459 citations. After the data mining process, such as removal of duplicate data and review of relevant articles that met the inclusion criteria, a total of 29 articles were included (**Figure 1**). The 29 prospective studies included 21 commodities. In general, the studies suggest that *L. monocytogenes* growth and survival on surfaces of intact produce differ substantially by commodity. Furthermore, handling and storage parameters influenced *L. monocytogenes* growth and survival on produce surfaces:

- Contaminated produce held at ambient temperatures (≥20°C) had higher growth rates compared with contaminated produce held at lower temperatures (4±2°C, 10±2°C) (**Table 1**).
- Roughness (↑), nutrient availability (↑), and competitive background microflora (↓) all influenced growth/survival.
- At cooler storage temperatures (≤10°C), relative humidity influenced growth/survival, where low RH limited survival.
- Large shifts in CO_2 and O_2 concentrations within storage containers may suppress the growth/survival of *L. monocytogenes* on produce surfaces.
- Pathogen carrying capacity influenced growth/survival of *L. monocytogenes* on produce surfaces.

BENEFITS TO THE INDUSTRY

It is critical for the produce industry to understand *L. monocytogenes* growth/no growth conditions on intact fruits and vegetables to establish parameters during handling and storage that can be applied to reduce *L. monocytogenes* proliferation or long-term survival. The knowledge collected on which whole produce commodities support *L. monocytogenes* growth and/or long-term survival at various handling and storing conditions observed along the supply chain will assist industry professionals in managing their risk. Also, this will be key to assisting the industry at establishing optimal parameters along the supply chain for intact produce.

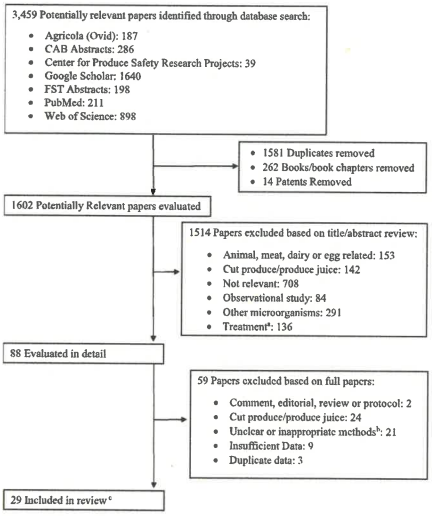

Figure 1. Schematic representation of study selection

[a] e.g., UV, chemical sanitizer

[b] Dip inoculation was excluded as it may promote internalization and therefore is out of the scope of this review

[c] Studies had to include sufficient information (i.e., enough information for studies to be reproduced) on methodology (e.g., inoculation methods), experimental conditions (e.g., storage temperatures), and quantitative growth and survival data (e.g., log CFU/g) over time to be eligible for inclusion. For studies in which the control group fit or partially fit the selection criteria, the pertinent, corresponding data were included for review. Conference abstracts, reports (e.g., CPS final grant reports), and dissertations were also reviewed for selection as long as data were not duplicated in research manuscripts.

Table 1. Growth[a] or Survival[b] Rate (log CFU/g/day) and Standard Deviations[c] of *L. monocytogenes* for the 21 Produce Commodities

Commodity	Growth Rate at Refrigeration Temperature (4°C ± 2 °C)	Growth Rate at Display Case Temperature (10°C ± 2 °C)	Growth Rate at Ambient Temperature (≥ 20°C)
Apple	-0.012	—[d]	-0.013 ± 0.01
Asparagus	-0.041 ± 0.04	0.104 ± 0.08	0.614
Avocado	—	—	-0.155
Blueberries	-0.050	-0.110	—
Canary melon	0.358	—	7.228 ± 6.23
Cantaloupe	-0.070 ± 0.17	0.092 ± 0.30	0.127 ± 0.38
Celery	-0.196 ± 0.05	0.119 ± 0.04	0.463 ± 0.12
Cranberries	-0.130	—	—
Cucumbers	0.132 ± 0.00	0.127 ± 0.09	0.434 ± 0.54
Jalapenos	—	0.089	—
Kale	0.187	—	—
Lettuce	—	-0.113 ± .007	-0.397 ± 0.09
Mango	-0.083 ± 0.02	0.008	-0.004 ± 0.11
Mushroom	-0.072	-0.002	—
Nectarine	—	—	-0.034 ± 0.26
Peaches	—	—	0.034 ± 0.16
Persimmons	—	0.492 ± 0.10	3.148 ±1.86
Raspberries	-0.007	—	0.533
Spinach	0.096 ± 0.08	0.243 ± 0.23	3.732 ± 3.15
Sprouts	-0.071 ± 0.06	0.028 ± 0.03	—
Strawberries	-0.340 ± 0.08	—	-1.246 ± 0.30

[a] Positive rate of change

[b] Negative rate of change

[c] Growth/survival rate is average based on all studies/replicates

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CPS CENTER for PRODUCE SAFETY

RESEARCH IN PROCESS

Significance of sanitizers on induction of viable but non-cultivable (VBNC) foodborne bacteria and their survival and resuscitation in fresh produce

SUMMARY

Sanitizers are needed to maintain the microbial quality of process wash water and prevent cross-contamination of the product. The efficacy of sanitizers on bacterial inactivation is conventionally determined using standard plate count procedures. However, foodborne pathogens are able to develop stress resistance mechanisms that enable them to enter into a temporary state of low metabolic activity—called the viable but non-cultivable (VBNC) state—whereby bacteria are viable but are not able to grow on agar plates. This project focuses on the ability of sanitizers to induce the VBNC state of foodborne pathogens and the consequences of underestimating the number of viable bacteria in process wash water. The ability of foodborne bacteria in the VBNC state to attach and survive in fresh produce also will be evaluated. Data obtained will allow us to understand if sanitizers induce the VBNC stage and if these foodborne bacteria are able to survive and grow on fresh produce.

OBJECTIVES

1. Estimation of the microbial inactivation and the induction of VBNC state of foodborne pathogens in process wash water (PWW) due to the action of commercial chemical sanitizers (sodium hypochlorite, calcium hypochlorite, chlorine dioxide and peroxyacetic acid) in the processing facilities of industry collaborators.
2. Establishment of the ability of foodborne bacteria in the VBNC state, present in the PWW, to attach to the surface of fresh produce during washing.
3. Evaluation of the conditions needed for VBNC foodborne bacteria, attached to fresh produce, to survive and recover from VBNC to culturable state during storage and distribution mimicking the conditions of the cold chain.
4. Performance of challenge tests to assess the growth potential of *Listeria monocytogenes* in fresh produce under foreseeable conditions of transportation, distribution and storage using molecular techniques able to differentiate between viable-culturable (VC) and VBNC.

METHODS

The work performed during the first trimester focused on Objective 1 through two types of experiments:

- **Optimizing the protocols to quantify total, viable, VBNC, culturable, and dead bacteria in broth and process wash water (PWW)** based on: (1) quantitative PCR (qPCR) combined with membrane impermeant dye (propidium monoazide) (PMA-qPCR); (2) PMA-qPCR combined with deoxycholate treatment (DC-PMA-qPCR); (3) Flow cytometry using a Live/Dead bacterial viability kit and flow cytometry analysis (**Figure 1**); and (4) ATP analysis.
- **Assessing the efficacy of commercial sanitizers to inactivate foodborne bacteria present in the PWW and induce a VBNC state in bacteria cells**. Studies were performed in a commercial peeled-garlic facility of an industrial collaborator to evaluate two commercial sanitizers: sodium hypochlorite (chlorine) and peroxyacetic acid (PAA). PWW samples were taken after 30 min and 2 h from the beginning of the washing. The proportions of culturable, VBNC, and viable bacterial cells were determined.

RESULTS TO DATE

- **Optimization of the protocols**: Initial trials were performed using pure cultures of *Listeria monocytogenes* in BHI broth and PWW. The enumeration of cells at the different bacterial stages using PMA-qPCR, DC-PMA-qPCR and flow cytometry was not significantly different among the methods (**Figure 2**). However, the loads of viable cells obtained using ATP analysis were significantly lower compared with the rest of the methodologies. Experiments using inoculated PWW reflected the complexity of the matrix (e.g., background microbiota, solid particles), which caused interferences when using the flow cytometer. Based on the results, the PMA-qPCR and DC-PMA-qPCR methodologies were selected as the reference methods to be used for monitoring.
- **Commercial sanitizers**: Sodium hypochlorite (**Figure 3**) and PAA (**Figure 4**) significantly reduced the levels of culturable bacteria, but the number of viable and VBNC cells were significantly higher. These results showed that the use of culturable methods overestimates the antimicrobial efficacy of these sanitizers.

BENEFITS TO THE INDUSTRY

These results highlight the need to analyze bacterial viability and physiological state by using nongrowth-dependent methods to quantify VBNC cells instead of the inactivation of foodborne bacteria using plate count procedures. Selection of the appropriate methodology capable of distinguishing between the different physiological stages of the bacteria will allow the industry to monitor fresh produce washing and the efficacy expected from sanitizers. The selected methodologies tested in commercial operations emphasize the great benefit that the produce industry can obtain if the effectiveness of sanitizers can be better established. The project is timely because it will be able to corroborate previous recommendations of the optimum sanitizer dose of approximately 10 ppm of hypochlorous acid at optimum pH (6.5 to 7.0) to avoid cross-contamination during washing, not only based on culturable bacteria but also taking into account the viable cells that still may be pathogenic.

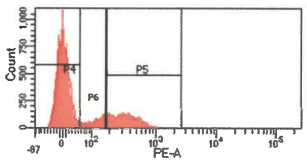

Figure 1. Histogram from a mix of viable (50%) and dead (50%) cells of a 3-strain cocktail of *Listeria monocytogenes* overnight culture, obtained using the Live/Dead flow cytometry analysis. The x-axis shows the intensity of the red fluorescence. The vertical lines separate viable bacterial cells (P4) from damaged cells (P6) and dead cells (P5). The cytometer used for the analysis was a LSR-Fortessa X-20.

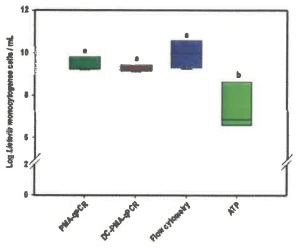

Figure 2. Comparison between the enumerations of viable cells obtained using different methodologies, including PMA-qPCR, DC-PMA-qPCR, flow cytometry, and ATP analysis, in a 3-strain cocktail of *Listeria monocytogenes* overnight culture in broth. Data obtained with the flow cytometer was transformed to log cells/mL based on the enumeration of total bacteria obtained using qPCR. Different letters denote significant differences ($P < 0.05$).

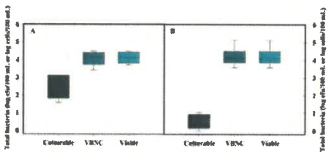

Figure 3. Populations of total bacteria (log cfu or cells/100 mL) in process wash water treated with sodium hypochlorite used for washing peeled garlic, after 0.5 h (A) and 2 h (B) from the beginning of the washing. Levels of culturable bacteria were obtained by plate count, levels of viable bacteria by PMA-qPCR, and VBNC by the differences between viable and culturable bacteria.

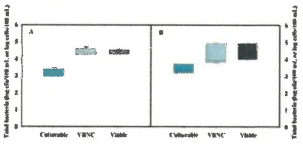

Figure 4. Populations of total bacteria (log cfu or cells/100 mL) in process wash water treated with peroxyacetic acid used for washing peeled garlic after 0.5 h (A) and 2 h (B) from the beginning of the washing. Levels of culturable bacteria were obtained by plate count, levels of viable bacteria by PMA-qPCR, and VBNC by the differences between viable and culturable bacteria.

Reference: FreshPlaza.com (23 January 2019). China: Demand surges for fresh garlic cloves in the European market. https://www.freshplaza.com/article/9064688/china-demand-surges-for-fresh-garlic-cloves-in-the-european-market/. Accessed May 2019.

Figure 5. Peeled garlic processing plant (FreshPlaza.com, 2019)

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CPS CENTER for PRODUCE SAFETY

RESEARCH IN PROCESS

Preventive sanitation measures for *Listeria monocytogenes* biofilms in critical postharvest sites

SUMMARY

Surfaces in the packinghouse can have intrinsic properties, making them difficult to sanitize. The main goals of this project are to identify these surfaces, characterize the surface roughness and hydrophobicity, use these surfaces for biofilm formation in the laboratory, and design methodologies for biofilm inactivation. In a previous CPS-funded project we assembled a biofilm apparatus with flow-through cells, where *Listeria monocytogenes* (*Lm*) biofilms are grown under laminar flow conditions. In this project to date, wood coupons were mounted on the flow cells and biofilms were grown on the coupons for 48 hours and then treated with 200 ppm chlorine. The wood coupons also were characterized for their surface properties. Results indicated that surfaces with increased roughness/waviness require longer contact time with the sanitizer for microbial inactivation.

OBJECTIVES

1. Construct testing beds from fabricated surfaces in the laboratory to replicate real-life conditions found in the packinghouse, by identifying areas, materials, specific environmental conditions, and microflora in the packinghouse that can harbor *Lm*. These test beds will be validated with conditions found in the packinghouse.
2. Determine cleaning and cleaning-and-sanitizing procedures for biofilms (*Lm* single culture and in co-culture with the resident microflora) grown in conditions simulating the packinghouse. Compare and validate efficacy of the tested sanitizers against *Lm* biofilms with the EPA requirements for hard surface (porous and non-porous) sanitizers.

METHODS

Construct test beds with fabricated surfaces to replicate real-life conditions found in the packinghouse:

- Materials (wood coupons) were obtained from a peach packinghouse.
- Coupons were characterized for surface roughness, sterilized with 75% ethanol and used to grow *L. monocytogenes* biofilms on their surface; some samples were treated with mineral oil to simulate a soiling event.
- *L. monocytogenes* Scott A was transformed with plasmid pNF8 to constitutively express green fluorescent protein (GFP) – the plasmid encodes for erythromycin resistance.
- Coupons were mounted on the biofilm apparatus (flow-through enclosures with laminar flow) and biofilms were grown for 48 hours.
- Samples were treated with 200 ppm free chlorine for 5 minutes and then 20 minutes.
- Control samples were treated only with phosphate buffer.
- Chlorine was inactivated at the end of the treatment, and the surviving population was determined by plating on tryptic soy agar supplemented with erythromycin.

RESULTS TO DATE

- Biofilm growth was conducted in a flow-through apparatus (constructed in our previous CPS-funded project), consisting of a media reservoir connected through tubing and connectors to a pump and then to a flow-break/air bubble trap device (**Figure 1**). Bacterial inoculum is introduced through a different port, with a sterile syringe that can be clamped on/off. Microorganisms are allowed to attach for 1 hour in the flow cells and then media is pumped through to the waste reservoir. Test surfaces are sandwiched between a machined silicone gasket and a sterile glass slide in the flow cells.
- Wood coupons were characterized for surface roughness (**Figure 2**).
- *L. monocytogenes* can form biofilms on less-than-optimal surfaces such as wood.
- Surfaces with increased roughness promote bacterial attachment.
- Surfaces with crevasses and pits should be exposed to sanitizer (chlorine) for longer time for microbial inactivation.
- Soiling of wood surfaces reduces sanitizer (chlorine) efficacy (**Figure 3**).

BENEFITS TO THE INDUSTRY

- Identification of critical microenvironments for a seek-and-destroy approach, where pathogens are eliminated before posing risks for product contamination.
- Outcomes of this project will be communicated with equipment manufacturers for better equipment design.
- Characterization of the conditions favorable for *L. monocytogenes* contamination of stone fruit in the packinghouse and design of measures to reduce pathogen survival and avoid cross-contamination.

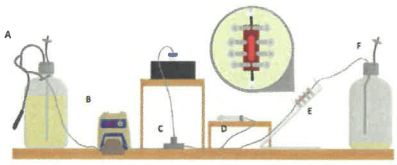
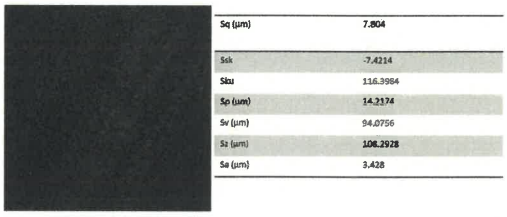


Figure 1. Diagram of the biofilm apparatus assembled with flow-through enclosures. (A) Media reservoir, (B) pump, (C) flow break/air bubble trap, (D) port with sterile syringe for microbial inoculation, (E) flow cell, and (F) waste reservoir. Insert shows a flow cell.



Sq (µm)	7.804
Ssk	-7.4214
Sku	116.3984
Sp (µm)	14.2174
Sv (µm)	94.0756
Sz (µm)	108.2928
Sa (µm)	3.428

Figure 2. Confocal microscopy and surface roughness measurements of wood samples collected from a local packinghouse. Images and roughness measurements were obtained with an Olympus LEXT OLS4100 digital microscope, magnification 10X. The following parameters were measured and represent the average of 5 samples (measurements taken for each sample in 5 different spots): Sq, root mean square height; Ssk, skewness; Sku, kurtosis; Sp, maximum peak height; Sv, maximum pit height; Sz, maximum height; and Sa, arithmetical mean height.

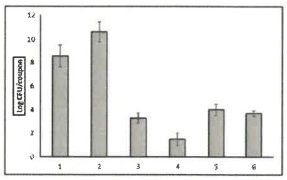


Figure 3. *L. monocytogenes* Scott A biofilm formation on wood coupons and inactivation by 200 ppm chlorine. (1) Wood coupon controls; (2) wood coupons soaked briefly in mineral oil control, before inoculation; (3) Wood treated with chlorine for 5 min, (4) wood treated with chlorine for 20 min, (5) wood coupon with mineral oil treated with chlorine for 5 min, and (6) wood coupon with mineral oil treated with chlorine for 20 min.

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CPS CENTER for PRODUCE SAFETY

RESEARCH IN PROCESS

Non-fouling food contact surfaces – prevention of biofilm and surface-mediated cross-contamination

SUMMARY

Post-harvest preventive control is a critical research topic for the produce industry because post-harvest handling is an essential stage in the food supply chain. A practical preventive control will not only impact public health but also the produce industry's long-term profitability. Despite the previous interest in fouling of mainstream FDA-approved food contact substances (FCS), there has not been a comprehensive evaluation of non-fouling properties of any FCS or of the practicality of industrial sanitization procedures for the produce industry. The major aim of this project is to develop an applicable post-harvest preventive control approach to enhance the non-fouling properties of FDA-approved FCS against *Listeria monocytogenes* biofilms. Project outcomes will provide scientific information that will support the sanitary design of packing, holding, and processing equipment/devices, coatings, and coating modifications to simplify cleaning/sanitization, and to prevent pathogen attachment and biofilms on FCS for new and retrofitted equipment.

OBJECTIVES

1. Evaluate the baseline non-fouling properties of existing FDA-approved FCS.
2. Enhance FCS performance by simple and cost-effective physical/topographical modification without altering the chemical composition.
3. Evaluate whether the top-performing FCS are compliant with sanitary designs for the fresh produce industry.
4. Validate the findings at a fresh-cut processing pilot plant.

METHODS

The project will evaluate standard and modified coupons of stainless steel 304 (SS-304) and plastic. Only FDA-approved FCS (approved for multiple uses as substrate coatings) will be evaluated for their non-fouling properties again *Listeria monocytogenes* (*Lm*) biofilm formation.

Topographical modifications are simple and cost-effective approaches to significantly improve the non-fouling properties of a surface without changing its chemical composition. Protocols will be developed, for example by combining chemical and electro-etching of SS-304 to generate topographical roughness for the applied FCS. FDA-approved FCS coatings can be applied on modified SS-304 at suppliers' facilities. Modified SS-304 can also work as the master mold for thermal molding of plastic FCS.

This project also includes an assessment of the non-fouling properties of modified FCS by growing biofilms that contain a cocktail of *Lm*, *E. coli* O157:H7, *Pseudomonas fluorescens* (*Pf*) and/or *Ralstonia insidiosa* (*Ri*), which have been identified as top biofilm formers among microbiota of leafy greens.

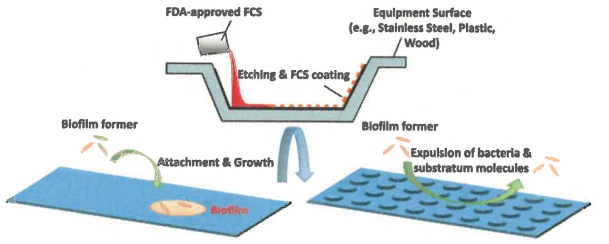

Figure 1. Topographical modification for FCS on a surface, without altering the chemical composition.

RESULTS TO DATE

We developed a protocol that combines chemical and electro-etching of SS-304 to generate roughness, and an FDA-approved FCS can be applied to the topographically modified surface (**Figure 1**).

Biofilm formation was confirmed on blank coupon controls by crystal-violet (CV) staining, and preliminary results show reduced biofilm formation on SS-304 coupons with Dursan® coating (FDA-approved FCS) (**Figure 2**) and on micro-patterned high density polyethylene (HDPE) coupons (**Figure 3**).

BENEFITS TO THE INDUSTRY

- This project will provide preventive control solutions to suppress fouling of food contact surfaces by bacterial biofilms – this is especially important in mitigating risks in produce operations with *Lm* biofilm formation and cross-contamination.
- Developed coatings could enhance sanitary design by reducing FCS bio-fouling, which will simplify cleaning and sanitization.
- A simple, cost-effective approach to enhance the non-fouling properties of existing FDA-approved FCS, without chemical modification that would require regulatory approval – this approach is significant in that it provides an opportunity to retrofit legacy equipment and improve future equipment design.
- One direct outcome would be mitigating *Lm* biofilm formation in packinghouses and fresh-cut processing facilities. Upon application of the proposed coatings, we anticipate there will be significant reductions in *Lm* biofilm formation.

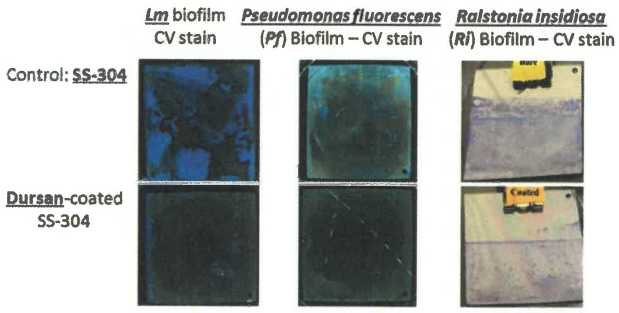

Figure 2. Crystal violet (CV) staining of biofilm formation on control (SS-304) and Dursan-coated SS-304 coupons (6 × 6 cm). Dursan coating significantly reduced biofilm formation.

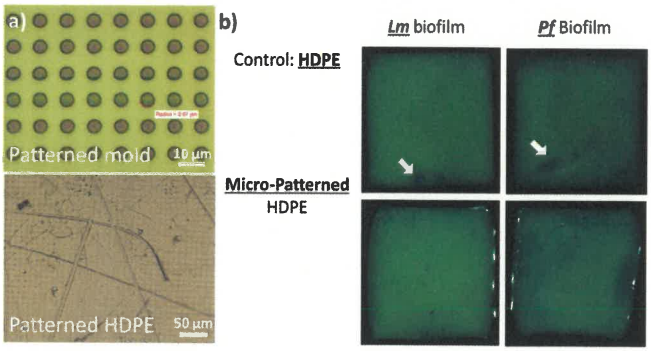

Figure 3. a) Fabricated mold with micropillar patterns (top) and thermal molded HDPE (bottom) coupons (5 × 5 cm). **b)** CV staining of *Lm* and *Pf* biofilm formation on the control

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CPS CENTER for PRODUCE SAFETY

RESEARCH IN PROCESS

Modeling tools for design of science-based *Listeria* environmental monitoring programs and corrective action strategies

SUMMARY

Well-designed environmental monitoring programs for *Listeria* as a strategy to identify and eliminate *Listeria monocytogenes* risks are essential for the produce industry, and are increasingly mandated by both regulatory agencies and buyers. The industry also needs science-based tools to evaluate responses to *Listeria* detection that are both appropriate for a specific facility and its unique processes and effective in reducing risk of contaminated products. As it is not practical to test out different corrective actions and sampling strategies in a given facility, our objective is to use computer modeling to identify the optimal approaches for a particular setting. Specifically, a model we have previously developed is being adapted to fresh produce processing facilities and will be validated with sampling data collected through an on-going project. This project will provide industry with science-based resources for selecting appropriate corrective action approaches and demonstrating the equivalency of different sampling strategies in their unique facilities.

OBJECTIVES

1. Develop a series of computer models, representing different produce processing facilities, to be validated with industry data collected through an on-going complementary USDA Specialty Crop Research Initiative (SCRI)–funded project at Cornell University, as well as historical industry data where applicable.
2. Evaluate differential corrective actions in response to *Listeria* spp. and environmental monitoring plans in the modeled fresh produce processing facilities.

METHODS

Four produce operations were selected for this project: two packinghouses and two fresh-cut facilities.

The production schedule and routine events in each operation are being mechanistically modeled using NetLogo 6.0 to simulate *Listeria* transmission and cross-contamination over time.

The model prioritizes the observed features of a food production environment that are relevant to the behavior and persistence of *Listeria* contamination. Initial visits to produce processing facilities involved detailed measurement/observation of structures (floor, walls, and ceiling), equipment, personnel, and conditions (water, temperature, traffic, and workflow) that have been previously identified as important predictors of risk by many published studies.

For this project, major emphasis is placed on discretizing the floor and equipment in Zones 2 and 3, and potential sources and niches in Zone 4, and identifying contamination routes to Zone 1 surfaces.

Once the models for the four facilities are parametrized to reflect the facility-specific features, they will be validated with historical and prospectively collected *Listeria* contamination data. Facility relevant corrective actions will be coded in the model to test their effectiveness for the given facility.

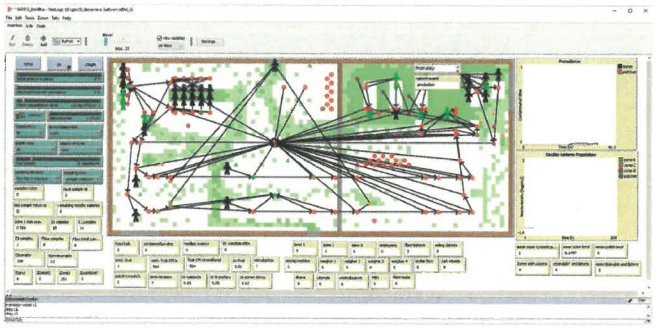

Figure 1. Model interface for a facility as viewed in NetLogo, displaying topography, agents (e.g., employees, equipment), and features of the preliminary agent-based model developed.

RESULTS TO DATE

As this project involves the development of computer models, main results are expected towards the project end. So far, a preliminary version of the first model has been constructed. **Figure 1** shows the screenshot of the model interface. **Figures 2–4** show equipment, traffic, and water maps underlying the model. Also, several activities are being pursued and accomplished that have led to interesting case studies. For example, observations at Facility 1 revealed that there was standing water nearly covering the entire floor of the production area, which may have a significant effect on model predictions for this facility as this was not the case in the facility modeled in our previously developed agent-based model (EnABLe). It was also observed that at certain times during production there are extra employees present, and therefore it will be of interest to evaluate the effect of additional employees on the model's prediction of *Listeria* dynamics.

BENEFITS TO THE INDUSTRY

Currently, there are few studies reporting surveillance of *Listeria* spp. in U.S. fresh produce processing facilities and no studies on optimal environmental monitoring strategies. Similarly, there are no computer modeling tools available to industry or researchers for microbial dynamics in fresh produce processing facilities that we are aware of. The goal of this project is to provide data and tools that will help fresh produce processing facilities (i) optimize corrective actions in response to presence/absence test results in various product handling areas, and (ii) identify alternative routine environmental monitoring programs that provide equivalent risk reduction.

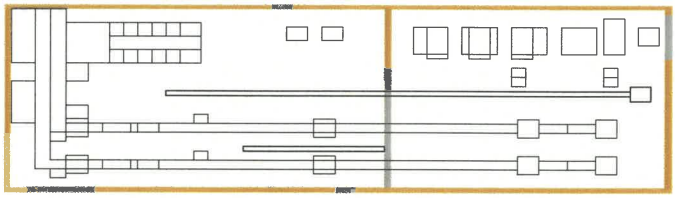

Figure 2. Floor map of Facility 1, with outline of equipment, walls, drains, and doors. Locations and dimensions based on measurements taken during initial observation visit.

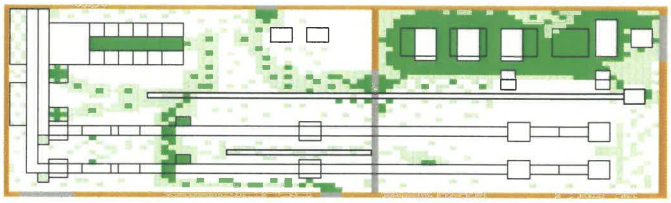

Figure 3. Traffic map of Facility 1, with darker green representing high-traffic areas and white representing low-traffic areas.

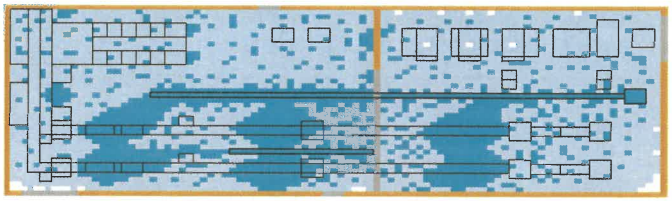

Figure 4. Water map of Facility 1, with darker blue representing areas with standing water, light blue representing areas where moisture was observed, and white representing dry areas.

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LENGTH OF FUNDING January 1, 2019 – December 31, 2019

CPS CENTER for PRODUCE SAFETY

RESEARCH IN PROCESS

Managing *Listeria* risk in fresh produce using predictive models

SUMMARY

Many foods are perishable and require time/temperature control throughout their shelf life. In many cases this control is required to ensure quality, but in an uncertain number of situations, it may also be required for food safety (to control foodborne pathogen growth).

This issue is often discussed by fresh produce producers and buyers and is now exacerbated by several federal regulations and policies, including the Preventive Controls Rule and Sanitary Transportation Rule.

There is an urgent need for short-term science-based parameters on this topic. This project focuses on the pathogen most likely to grow at the temperature range of interest (*Listeria monocytogenes*) and uses "off-the-shelf" computer models in the form of ComBase Predictor (https://browser.combase.cc/).

OBJECTIVE

The objective of this small project is to produce a report with a series of time and temperature tables, comparing relative risk of *Listeria monocytogenes* growth for different conditions to guide science-based risk management decisions.

METHODS

Schaffner worked in collaboration with a small team of produce industry experts to define times (days or weeks) and temperatures (e.g., 40–55 °F) that are relevant to the storage of fresh produce. The modeling predictions include constant temperature conditions as well as representative examples of changing temperature conditions that are relevant to fresh produce storage. Although pH values in the range common in fresh produce are unlikely to have a dramatic effect on predicted *L. monocytogenes* growth rate, this factor was included as well.

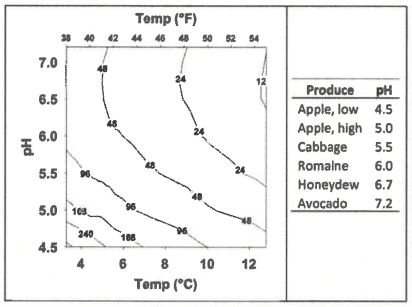
The modeling assumptions make the models highly conservative, and thus quite robust and able to withstand scrutiny from regulatory agencies or over-zealous inspectors. It is quite likely that actual pathogen growth on specific produce commodities is much less than the model predictions, and in some cases no growth or slow decline would actually be observed in the real world.

RESULTS TO DATE

Figure 1 shows the relative importance of assumed pH and temperature on the time required in hours for a 1-log increase in the concentration of *L. monocytogenes*.

One way to determine the relative risk of holding food out of temperature control would be to look at currently allowed practices, determine the risk of those practices, and then look for equivalent risk for other time/temperature conditions. **Table 1** assumes a pH of 6 and a very high water activity (0.997).

Figure 2 uses actual time and temperature data from a produce shipment (sent in the summer of 2018). The top panel shows the temperature profile in degrees Fahrenheit over the course of the shipment. Shipment dates and times are shown on the X axis. The bottom panel shows predictions for *L. monocytogenes* growth, assuming the same conditions as above (pH 6.0, water activity 0.997).



Produce	pH
Apple, low	4.5
Apple, high	5.0
Cabbage	5.5
Romaine	6.0
Honeydew	6.7
Avocado	7.2

Figure 1. Time required for a 1-log increase in *L. monocytogenes* as a function of pH and temperature, assuming a permissive water activity (0.997).

BENEFITS TO THE INDUSTRY

It's clear from the data presented that the primary driver for *Listeria* growth is time. For example, when evaluating *Listeria* growth for the shipment lasting 5 days, it would be instructive to look at **Table 1** to get an idea of the growth that would occur over 5 days at 38°F, or 40°F, and then look at the relative difference in growth for the shipment in question.

Table 1 is available as an Excel spreadsheet, and will automatically change color with the input of different values for "green light" and "yellow light." The information is currently only available for pH 6, but it would be possible to develop a similar tool which allows input of the pH value.

The assumptions make the models highly conservative, and thus quite robust and should withstand scrutiny from regulatory agencies or over-zealous inspectors.

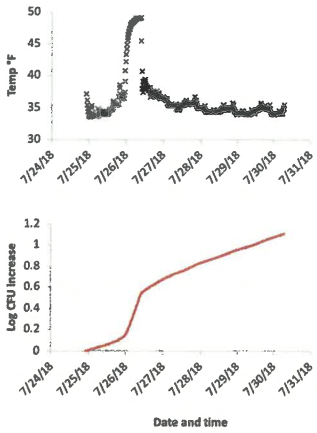


Figure 2. Actual time and temperature data from a produce shipment sent in the summer of 2018 (top panel). The bottom panel shows predictions for *L. monocytogenes* growth, assuming the same conditions (pH 6.0, water activity 0.997).

Table 1. Log CFU increases for *L. monocytogenes* as a function of time and temperature relative to 17- and 21-day shelf life at 38 or 40 °F.

	Temp (°F)	38.0	40.0	41.0	42.0	44.0	45.0	50.0	55.0
	Temp (°C)	3.3	4.4	5.0	5.6	6.7	7.2	10.0	12.8
Time (d)	Time (h)								
0	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	24	0.3	0.4	0.4	0.5	0.6	0.6	1.1	1.7
2	48	0.6	0.7	0.8	0.9	1.2	1.3	2.1	3.4
3	72	0.9	1.1	1.2	1.4	1.7	1.9	3.2	5.1
4	96	1.2	1.4	1.6	1.9	2.3	2.6	4.3	6.8
5	120	1.5	1.8	2.1	2.4	2.9	3.2	5.4	8.6
6	144	1.7	2.2	2.5	2.8	3.5	3.8	6.4	
7	168	2.0	2.5	2.9	3.3	4.1	4.5	7.5	
8	192	2.3	2.9	3.3	3.8	4.6	5.1	8.6	
9	216	2.6	3.2	3.7	4.2	5.2	5.8		
10	240	2.9	3.6	4.1	4.7	5.8	6.4		
11	264	3.2	4.0	4.5	5.2	6.4	7.0		
12	288	3.5	4.3	4.9	5.6	7.0	7.7		
13	312	3.8	4.7	5.3	6.1	7.5			
14	336	4.1	5.0	5.7	6.6	8.1			
15	360	4.4	5.4	6.2	7.1				
16	384	4.6	5.8	6.6	7.5				
17	408	4.9	6.1	7.0	8.0				
18	432	5.2	6.5	7.4					
19	456	5.5	6.8	7.8					
20	480	5.8	7.2						
21	504	6.1	7.6						
17 days	Green light	6.1							
21 days	Yellow light	7.6							

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LENGTH OF FUNDING February 1, 2019 – May 30, 2019

RESEARCH IN PROCESS

Development of a model to predict the impact of sediments on microbial irrigation water quality

SUMMARY:

The concentrations of *Escherichia coli*, *Salmonella*, *Listeria monocytogenes*, and viruses are higher in sediments than in the overlaying water in irrigation canals and river systems. Resuspension of these microorganisms from the sediments can result in the rapid increase in their concentration in the overlaying water. The overall goal of this project is to determine how the type of sediment, the pathogen species, and water flow properties impact the microbial quality of irrigation waters. This will be done in a series of experiments by identifying flow/velocity on the resuspension of sediment-bound bacteria and viruses in the overlaying water. The data will then be used to develop a predictive model on the degree of resuspension based on environmental factors. The project objectives will be accomplished through a series of laboratory experiments using a hydrologic flume and field studies.

OBJECTIVES:

1. Identify factors which would result in the resuspension of sediment-bound bacteria/viruses in irrigation channels, specifically *E. coli*, *L. monocytogenes*, and MS-2 and phiX-174 viruses.
2. Quantify the impact of resuspension of different levels of these bacteria and viruses on the quality of the overlaying water.
3. Suggest guidelines for growers and producers to minimize the occurrence of pathogenic bacteria and viruses in irrigation water.

METHODS:

The project objectives will be achieved through a series of laboratory experiments in a hydraulic flume (**Figure 1**) and through a field study. Sediments of different composition (sand, silt, clay) will be used in flume experiments in which the flow rate and velocity can be controlled. The degree of study organisms attached to the sediment will then be determined and the impact of changing environmental conditions on their resuspension determined. (See **Figure 2** for conceptual model of microorganism in sediment transport). A predictive model will be developed using the laboratory data. These relationships will be verified using field measurements at various locations in irrigation canals in Arizona (see **Figure 3** for typical irrigation canal). The field measurements will include flow velocity, flow depth, water temperature, suspended sediment concentration, bacterial counts in the water, bed sediment size gradation, bacterial counts in the bed sediment, and bed load transport rate.

RESULTS TO DATE:

Experiments are currently being conducted with a hydraulic flume comparing the impact of flow rate, velocity, and sediment type on the resuspension of MS-2 and phiX-174 viruses. These experiments will be completed by the middle of June and incorporated into a predictive model.

Figure 1. Hydraulic flume

BENEFITS TO THE INDUSTRY:

This study will aid in the management of irrigation waters used for food crops by providing guidelines to quantify bacterial/virus counts in irrigation waters based on flow rates and microbial concentrations in the sediments, recommending maximum irrigation flow rates to minimize bacteria/virus resuspension, and providing data to risk models that can guide farmers to identify bacterial/virus contamination on produce. The model will aid in the assessment of the impact of extreme events (e.g., above normal rainfall) on the microbial quality of the irrigation water, help in the design of sampling programs to maximize contaminant detection, and facilitate integration with microbial risk assessment models.

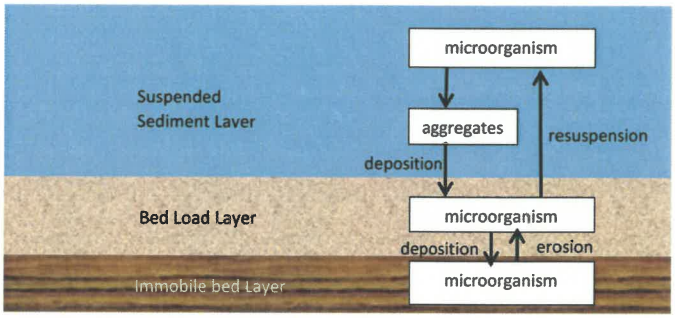

Figure 2. Conceptual model of microorganism in sediment transport

Figure 3. Irrigation canal in Arizona

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CPS CENTER for PRODUCE SAFETY

RESEARCH IN PROCESS

Exploring the relationship between product testing and risk

SUMMARY

Risk to consumers is directly related to prevalence and concentration of pathogens in products. Sampling to determine if levels of pathogens are at acceptable levels is one approach adopted to manage the consumer risk. However, the relationship between different sampling options, and the reduction in risk provided by implementing those options has not been well described. In this project we will develop a sampling-risk model that quantifies the relationship between product testing and the risk to consumers. This model will consider factors such as sample size (mass), number of samples, lot size, and many others. These analyses can be used to explore the efficacy of alternate risk management strategies, and help answer questions such as "If I increase the sample mass what is the impact on risk?" or "If I sample at point X instead of point Y, what will the benefit be?".

OBJECTIVES

1. Develop a sampling-risk model that quantifies the relationship between product testing, lot rejection rates, and risk (which is directly related to prevalence and concentration in products post testing).
2. Provide detailed, fully documented, analyses of the relationship between product sampling variables driving the risk.
3. Support risk reduction initiatives through analyses that explicitly enable the exploration of risk management options, facilitating selection of actionable sampling strategies that have the biggest impact on risk reduction.

METHODS

Risk model development: We are developing a quantitative model that links the frequency of contamination and concentration to lot rejection considering multiple sampling plan options (sample location, sample mass and number, proportion of lots tested, etc.). We will link this sampling model to a supply chain model to predict concentration and prevalence of microbial hazards post sampling to estimate risk to the consumer, and explore the residual risk post sampling (model framework is shown in **Figure 1**).

Model analyses: We will use the sampling-risk model to explore the impact of the sampling options and incorporate factors that will further impact the risk post-testing (e.g., possible pathogen growth) and develop real-world case studies.

Stakeholder engagement: The project stakeholder group is involved throughout the project, including an online workshop to discuss the early findings and the sampling options that are most (and least) influential on lot rejection and residual risk.

RESULTS TO DATE

Model development has begun. To date we have explored the current knowledge base with respect to the underlying statistical properties of sampling plans and have identified the key components required by the mathematical model. The underlying statistics behind the basic properties of sampling plans are being implemented in the modelling system to describe the relationship between sample plan options and the risk remaining post sampling. Both two-class and three-class sampling types are being implemented in a way that allows easy exploration of the impact of the inputs (number of samples, sample mass, etc.). **Figure 2** and **Figure 3** show examples of the types of relationships being explored.

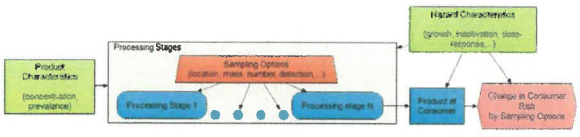

Figure 1. Model framework for the sampling-risk model linking sampling options to consumer residual risk.

To support the case study development we are researching the source of outbreaks and recalls (related to microbial contamination) to determine candidates for the case studies that are recognized as part of the current produce risk issue.

BENEFITS TO THE INDUSTRY

- Provide industry with a modelling approach that facilitates exploration of the benefit of various sampling strategies to reduce risk to the consumer
- Enable industry to explore the efficacy of alternate risk management strategies, helping answer questions such as "If I increase the sample mass what is the impact on risk?" or "If I sample at point X instead of point Y what will the benefit be?"
- Access to a detailed report, including a series of tables and charts that describe the relationship between the factors and risk
- A series of analyses relevant to a range of produce items (e.g., baby greens, whole head greens, cole crops, tomatoes, onions, peppers, celery, pome fruits, stone fruits, and tropical fruits).

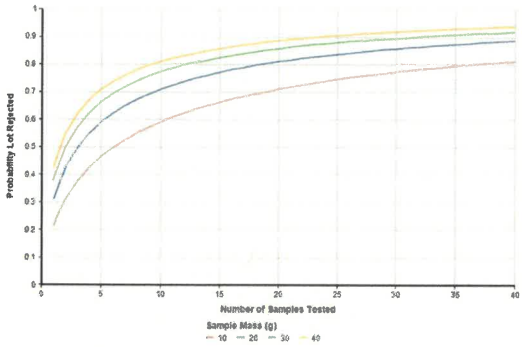

Figure 2. Relationship between the number of samples tested, the sample mass, and the probability a lot is rejected based upon a hypothetical contamination scenario.

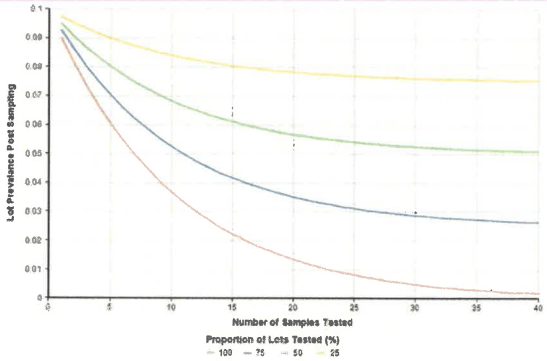

Figure 3. Relationship between the prevalence of contaminated lots post sampling, the percentage of lots tested, and the number of samples tested per lot (based upon a hypothetical contamination scenario).

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LENGTH OF FUNDING January 1, 2019 – December 31, 2019

CPS CENTER for PRODUCE SAFETY

RESEARCH IN PROCESS

Identifying competitive exclusion microorganisms against *Listeria monocytogenes* from biological soil amendments by metagenomic, metatranscriptomic, and culturing approaches

SUMMARY

Listeria monocytogenes is a leading foodborne pathogen that can contaminate fresh produce at both farm and processing environments. Fully understanding the ecology of *L. monocytogenes* in biological soil amendments is essential to reduce produce contamination with this pathogen. Next generation sequencing approaches are powerful tools for understanding microbial composition and functions at the metagenomic level in complex samples. In considering compost as a rich source of microorganisms with a diversity of species, this project will identify compost-adapted competitive exclusion microorganisms against *L. monocytogenes* using 16S/18S rRNA and metatranscriptomic sequencing approaches along with culturing methods. Preliminary sequencing analysis indicated incubation time and moisture level as the main factors driving variation in compost microbial community composition. Analysis by denaturing gradient gel electrophoresis revealed that *L. monocytogenes* should be inoculated at 7 log CFU/g or higher for 72 h in compost before sequencing analysis. Also, multiple competitive exclusion strains with anti–*L. monocytogenes* activities have been isolated.

OBJECTIVES

1. Use 16S rRNA and 18S rRNA sequencing to profile the microbial communities of biological soil amendments.
2. Analyze functional metatranscriptomics of *L. monocytogenes* interactions with indigenous microorganisms in composts.
3. Optimize culturing conditions to isolate and validate competitive exclusion (CE) microorganisms with antagonistic activities against *L. monocytogenes*.

METHODS

For a preliminary study, turkey litter compost was adjusted to moisture levels of 40 and 60%, and inoculated with *L. monocytogenes* strain LCDC 81-861 (~5 log CFU/g) or not. DNA was extracted from compost samples after propidium monoazide (PMA) treatment with different incubation conditions. High-quality sequenced reads were analyzed using a custom modified QIIME analysis pipeline. Alpha diversity, principal component analysis, and canonical correspondence analysis were performed with PAST3 software. The significance of different environmental factors was tested by analysis of similarity and permutational multivariate analysis of variance, and the top genera driving variation in the microbial community were identified by similarity percentage (SIMPER) and Random Forest analysis.

Additionally, the inoculation level and incubation conditions of *L. monocytogenes* in the biological soil amendments were optimized by denaturing gradient gel electrophoresis (DGGE).

Potential CE strains against *L. monocytogenes* from three biological soil amendments were isolated and characterized.

RESULTS TO DATE

- The top ten taxonomically assigned families, namely Flavobacteriaceae, Trueperacea, Halomonadaceae, Balneolaceae, Pseudomonadaceae, Alteromonadaceae, Alcaligenaceae, Xanthomonadaceae, Bacillaceae, and Sphingobacteriaceae, were observed in all turkey litter compost samples (**Figure 1**).
- The top five genera in turkey litter compost that were found to be most impacted by *L. monocytogenes* inoculation included KSA1, B-42, Halomonas, Marinimicrobium, and Gillisia (belonging to the families Balneolaceae, Trueperaceae, Halomonadaceae, Alteromonadaceae, and Flavobacteriaceae, respectively), as screened by similarity percentage (SIMPER) and Random Forest analysis (**Figure 2**).
- The results from DGGE indicated that *L. monocytogenes* should be inoculated at 7 log CFU/g or higher and incubated for 72 h in compost prior to metagenomic analysis (**Figure 3**).
- A total of 58 isolates were confirmed with various levels of anti–*L. monocytogenes* activities (**Figure 4**).

BENEFITS TO THE INDUSTRY

This project will directly impact the fresh produce industry and the compost industry in California and nationwide as well as impact consumers of these products. Water, soil, and compost are three of the major inputs in the production of fresh produce. As California produces nearly half of U.S.-grown fruits, nuts and vegetables, it is imperative that California's produce industry can verify the absence of human pathogens including *L. monocytogenes* in these inputs that are used to produce their crops. This research on understanding compost microbial communities with the goal to identify competitive exclusion microorganisms as a biological control tool against *L. monocytogenes* will have direct impacts on the practices of biological soil amendments, and contribute to the safe production of fresh produce. Also, our research findings will help the compost industry to understand their products better at the microbial species and gene levels, and lead to an increase in the value of their products.

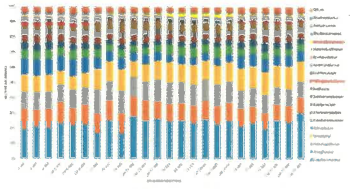
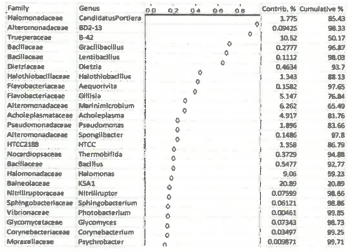


Figure 1. Relative abundances of family in each sample.



Family	Genus	Contrib. %	Cumulative %
Halomonadaceae	CandidatusPortiera	1.775	85.43
Alteromonadaceae	B02-13	0.09425	98.33
Trueperaceae	B-42	10.52	50.17
Bacillaceae	Gracilibacillus	0.2777	96.87
Bacillaceae	Lentibacillus	0.1112	98.03
Dietziaceae	Dietzia	0.4634	93.7
Halothiobacillaceae	Halothiobacillus	1.343	88.13
Flavobacteriaceae	Aequorivita	0.1582	97.65
Flavobacteriaceae	Gillisia	5.147	76.84
Alteromonadaceae	Marinimicrobium	6.262	65.49
Acholeplasmataceae	Acholeplasma	4.917	81.76
Pseudomonadaceae	Pseudomonas	1.896	83.66
Alteromonadaceae	Spongiibacter	0.1486	97.8
HTCC2188	HTCC	1.358	86.79
Nocardiopsaceae	Thermobifida	0.3729	94.88
Bacillaceae	Bacillus	0.5477	92.77
Halomonadaceae	Halomonas	9.06	59.23
Balneolaceae	KSA1	20.89	20.89
Nitriliruptoraceae	Nitriliruptor	0.07589	98.66
Sphingobacteriaceae	Sphingobacterium	0.06121	98.86
Vibrionaceae	Photobacterium	0.02661	99.85
Glycomycetaceae	Glycomyces	0.07343	98.73
Corynebacteriaceae	Corynebacterium	0.03497	99.25
Moraxellaceae	Psychrobacter	0.009871	99.71

Figure 2. Random Forest and SIMPER analysis.

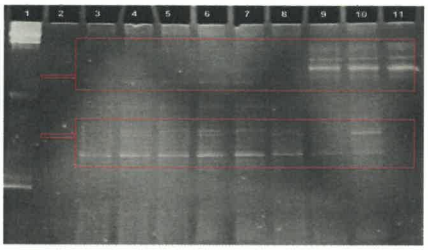


Figure 3. DGGE profiles of PCR-amplified 16S rDNA fragments from finished chicken litter compost with 80% MC. Lane 1, 1Kb Ladder; Lane 2, space lane without sample; Lanes 3-4, compost samples w/o *L. monocytogenes* inoculation after 0 and 72 h incubation, respectively; Lanes 5-6, compost samples with ca. 5 log *L. monocytogenes* inoculation after 0 and 72 h incubation, respectively; Lanes 7-8, compost samples with ca. 7 log *L. monocytogenes* inoculation after 0 and 72 h incubation, respectively; Lanes 9-10, compost samples with ca. 9 log *L. monocytogenes* inoculation after 0 and 72 h incubation, respectively; Lane 11 *L. monocytogenes* strain only.

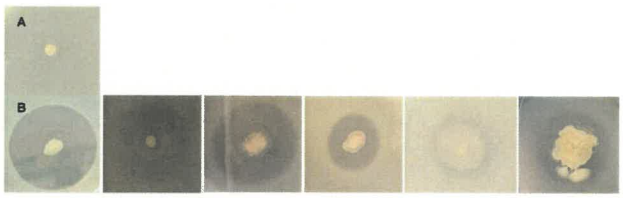


Figure 4. Selection of competitive exclusion microorganisms against *L. monocytogenes* from compost samples. The isolates showed no inhibition zone (A) and with various sizes of inhibition zones (B) on *L. monocytogenes* lawn.

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CPS CENTER for PRODUCE SAFETY

RESEARCH IN PROCESS

Illuminating the role of whole genome sequencing in produce safety

SUMMARY:

Whole genome sequencing (WGS) is rapidly becoming the gold standard for foodborne outbreak investigations by public health agencies around the world. Source-tracking investigations during an outbreak can often come down to the epidemiological data and a difference of just a few single nucleotide polymorphisms (SNPs) in the foodborne pathogen's genome (**Figure 1**). Investigations can be made even more challenging with highly clonal pathogens that lack high levels of genetic diversity (**Table 1**). Therefore, the goal of this project is to provide data to public health agencies to help improve the use of WGS as an outbreak investigation tool. Furthermore, this project can be used by the produce industry to implement WGS for internal source tracking to identify "resident" versus "transient" pathogens, sources of contamination for either, and a better overall understanding of the breakdowns or gaps in prevention, thus improving produce safety by closing these gaps.

OBJECTIVE:

1. Determine mutational rates of pathogens during persistent colonization in agricultural soil and irrigation water under distinctive geographical environmental conditions.

METHODS:

Irrigation water and soil samples will be collected from romaine lettuce fields in Arizona and California and transferred back to the Cooper laboratory. Individual agricultural samples along with buffered peptone water control will be inoculated with either *Salmonella*, *Listeria*, or *Escherichia coli* O157:H7, and then maintained under environmental conditions (light, humidity and temperature) for Yuma, AZ and Salinas, CA. Every two weeks for the first three months, and then monthly afterwards, each of the inoculated samples will be cultured for the corresponding inoculated pathogen, and five colonies of the pathogen will be selected for genome sequencing. Each of the five selected colonies will be whole genome sequenced and compared to the genome of the original pathogen inoculum to determine the number of mutations that have developed since the initial inoculation. The project will maintain the inoculated samples for up to one year to establish an annual mutation rate (**Figure 2**).

RESULTS TO DATE:

We are in the process of obtaining soil and irrigation water samples from the leafy greens fields in Arizona and California, and will be inoculating each agricultural sample with *Salmonella*, *Listeria*, and *Escherichia coli* O157:H7 for the long-term evolution studies under the different environmental conditions prior to the CPS Research Symposium. Due to the long-term nature of this study, initial mutational rates for the various pathogens will not be available until at least November or December 2019.

Table 1. ***Salmonella*** **Typhimurium strains from different sources and temporal isolation demonstrating the difficulty of source tracking**

Strain	Source	State or country	Isolation date	# of SNPs compared to reference strain 14028s[1]	# of SNPs compared to RM13677[2]
14028s (Reference strain)	Poultry	N/A	1960	N/A	13
RM8835	Celery	Washington	2002	9	4
RM8837	Cantaloupe	Washington	2002	9	4
RM10002	Pond Water	California	01/23/2009	12	5
RM13670	Clinical	Oregon	08/06/2009	11	4
RM13671	Clinical	Oregon	08/06/2009	10	3
RM13672	Clinical	Oregon	08/06/2009	9	4
RM13673	Clinical	Oregon	08/13/2009	10	3
RM13674*	Clinical	Oregon	08/14/2009	10	3
RM13675	Clinical	Oregon	08/22/2009	11	4
RM13676*	Clinical	Oregon	08/15/2009	10	3
RM13677	Clinical	Oregon	10/21/2009	13	N/A
RM14512	Clinical	Maine	08/06/2009	9	4
RM14513	Clinical	Pennsylvania	08/06/2009	10	3
LT2 (Outlier strain)	N/A	Sweden	1940s	3041	380*

*Samples isolated from the same person
[1]Number of SNPs in non-repeat regions compared to the complete reference genome of *S. Typhimurium* strain 14028s
[2]Number of SNPs in non-repeat regions compared to most distantly related of the 18 strains *S. Typhimurium* strain RM13677
*SNPs for *S. Typhimurium* str. LT2 are only the chromosome

BENEFITS TO THE INDUSTRY:

This project has direct and indirect benefits to the produce industry. The indirect benefits include an improvement of WGS as a tool for outbreak investigations, thus benefiting the industry by allowing for more accurate and rapid outbreak investigations. The project helps support the further development of the source tracking database, GenomeTrakr, especially the aspect of speeding up investigations using geographical or regional pathogen data. This benefits the industry by decreasing outbreak investigation times, removing contaminated products faster, preventing additional cases, and ultimately getting an outbreak declared over faster. The direct benefit of this project is that it improves the use of WGS as a tool that can be applied for internal source tracking of contamination in a facility. Moreover, using these mutation rates can identify the source of "resident" or long-term pathogen contamination by tracking the pathogen through a produce facility, as has been applied in other food industries, and identify and close gaps in prevention.

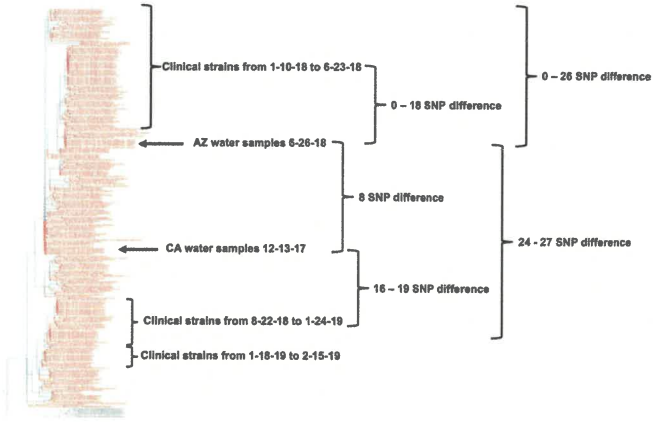

Figure 1. Phylogenic tree from the National Center for Biotechnology Information (NCBI) Pathogen Detection database, representing the complexity of WGS and source tracking. This tree shows single nucleotide polymorphism (SNP) differences among clinical and environmental *Escherichia coli* O157:H7 associated with the 2018 Arizona romaine lettuce outbreak. Results show up to 26 SNP differences between clinical strains and irrigation water strains that were isolated in Arizona during June 2018, whereas up to 27 SNP differences between irrigation water samples and clinical strains from as recently as January 2019. This example demonstrates the importance of the epidemiological data obtained during the outbreak investigation, while also the need to refine WGS as a tool to assist investigations.

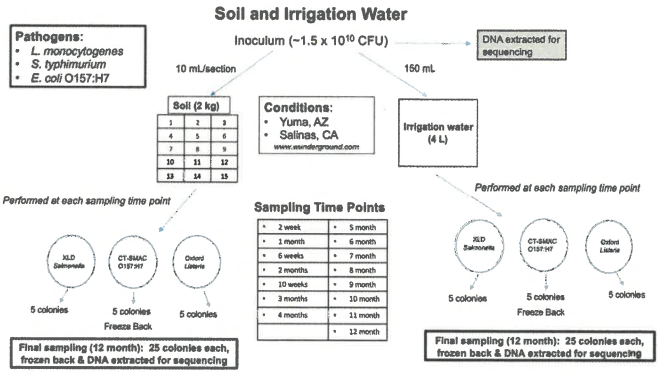

Figure 2. Flowchart of irrigation water and soil experiments for the entire project.

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CPS CENTER for PRODUCE SAFETY

RESEARCH IN PROCESS

Towards a decision-support tool for identifying and mitigating on-farm risks to food safety

SUMMARY

Contaminated produce continues to be a leading cause of foodborne illness. Yet farmers still lack the ability to predict when their crops are at risk and lack effective strategies to manage those risks. While evidence is accumulating regarding the efficacy of many practices, results are often not made available to growers in a useable way. We plan to develop data-driven, pre-harvest tools to help growers predict and mitigate risks associated with foodborne pathogens. This tool will be based on existing literature and will be customizable to each farmer's unique management practices. We also will explore novel methods for suppressing pathogens, when they do occur on farms. Specifically, we will study how soil amendments affect the ability of feces-feeding soil bacteria to suppress *Salmonella* and *Listeria*. Farmers, industry, and conservation organizations have expressed strong interest in making informed decisions about on-farm practices to improve produce safety without comprising environmental health. By combining literature syntheses with lab and field experiments, this project will provide growers with both new strategies for mitigating pathogen prevalence and an effective tool to assist growers in navigating decisions regarding the food safety/conservation "stale-mate."

OBJECTIVES

1. To develop a grower-motivated decision-support tool that synthesizes evidence for the efficacy, feasibility, and costs of food-safety practices.
2. To understand the community dynamics of feces-feeding bacteria in order to harness their activity to bolster food safety

METHODS

Literature review and expert assessment:
Based on a broad literature review and follow-up expert review scores, we will assess the efficacy, costs, and feasibility of on-farm food safety practices and landscape risk factors. As outlined in **Figure 1**, we will scour the literature for studies that quantitatively assess impacts of agricultural practices and the surrounding landscape on foodborne pathogen prevalence in North America. Systematically summarized paragraphs of each paper will then be distributed to an expert panel of scientists and growers. Experts will score the evidence for the efficacy of each practice by adapting Delphi technique. We will then compile results into an interactive web-application for growers.

Pathogen suppression study:
We are utilizing the UC Davis Russell Ranch farm management plots (directed by Co-PI Kate Scow) to evaluate the short-term and long-term effects of compost addition on pathogen suppression. Composite soil samples will be collected at 4 time points from 2 experimental fields—"Long Term Century Experiments" and "Short Term Dairy Manure Experiments"—as outlined in **Figure 2A** and **2B**, respectively. After quantifying soil physico-chemical properties and soil microbial diversity, we will inoculate soils with foodborne pathogens (**Figure 3**) and enumerate soils after 10 and 30 days to identify which soils are most suppressive. We predict soils with compost and manure additions will host more soil microbial diversity and suppress foodborne pathogens.

RESULTS TO DATE

To date, we have focused on building the project team and planning our research activities:

- Successfully recruited personnel to conduct experiments and accomplish the research goals.
- Systematically scheduled research activities by organizing regular meetings, cloud-based data management plans, and sampling protocols.
- Organized a science advisory team to lead the development of the decision support tool.
- By leveraging prior soil data and ongoing experiments, we have developed a concrete experimental design and sampling timeline; field sampling will begin in mid-April 2019.

BENEFITS TO THE INDUSTRY

Our overall goal is to develop an ecologically based, data-driven approach to reducing on-farm food-safety risk. We intend to create an open-source tool to help the produce industry understand how different farming practices affect food safety risk AND begin exploring new soil-based approaches for mitigating risk. We hope to create a decision-support tool that is immediately useful and relevant. Ideally, our work will serve the ~176,400 U.S. farmers that grow fresh produce that is vulnerable to foodborne pathogens, as well as the many auditors, buyers, and regulators involved in on-farm food-safety management.

In the longer term, we hope that our pathogen suppression experiment will identify paths forward for co-managing produce farms for food safety and soil quality. We will understand how historical farm management influences a soil bacterial community's ability to suppress foodborne pathogens and how these communities can be altered through soil management to maximize foodborne pathogen suppression. We also will determine which species of bacteria are most beneficial in suppressing selected foodborne pathogens.

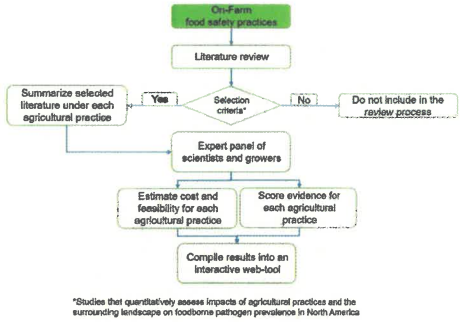

Figure 1. A graphical representation of the literature review procedure.

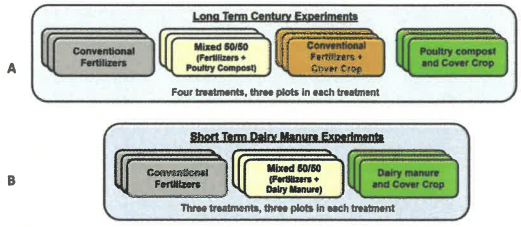

Figure 2. A graphical representation of the overall experimental design: A) long term century experiments, B) short term dairy manure experiments.

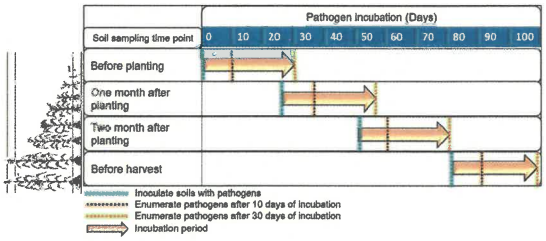

Figure 3. A graphical representation of the pathogen suppression experiment.

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CPS CENTER for PRODUCE SAFETY

RESEARCH IN PROCESS

Simulation analysis of in-field produce sampling for risk-based sampling plan development

SUMMARY

Effective preharvest, field-level produce sampling is challenging because current practices typically yield few positive samples with fields rarely re-testing positive. Statistical theory suggests one reason is that detecting rare contamination events would require 100s to 1,000s of random samples, or targeted sampling of higher risk locations in fields. This project will develop and validate tools for the produce industry to evaluate exiting and improved produce field sampling plans. Results will be used to communicate to growers the number and location of samples needed to achieve a known power to detect contamination. We will validate these simulations against academic literature, industry partner data, and field-trials of controlled contamination of spinach. Our project will provide growers with tools to (i) develop improved sampling plans, (ii) customize those plans to their individual fields, and (iii) quantify the performance and costs of the plan – all to better identify and manage preharvest food safety risks.

OBJECTIVES

1. Simulate contamination of produce fields that are representative of commercial fields in four produce-growing regions of the United States.
2. Evaluate convenience, improved generic, and risk-based sampling plans.
3. Validate simulations against data from industry partners and academic literature.
4. Validate simulations against field-trials of controlled contamination events.

METHODS

We will simulate (scope in **Table 1**) a **produce field** and **product**, contaminated by a **food safety hazard**. A **sampling plan** will pass said product to a laboratory **assay** to determine + and – **outcomes**. These simulation results will then be used to evaluate sampling plans, and be validated against existing industry data and experimental data.

To the *use* the generic simulation model, we will simulate produce fields representative of (i) the Central Valley, CA; (ii) Yuma, AZ; (iii) the Delmarva Peninsula; and (iv) Upstate NY, through site visits and expert elicitation. We will simulate typical contamination for each field, including: (i) point sources, e.g. fecal deposits; (ii) systematic sources, e.g. contaminated irrigation water; and (iii) sporadic contamination, e.g. endemic soil bacteria. Finally, we will validate these results against data, including from experimentally contaminated spinach fields subject to high-resolution sampling.

RESULTS TO DATE

The Illinois team has programmed the generic produce field simulation (**Figure 1**). With our existing generic field simulation we are able to evaluate generic sampling plans such as n_{60} composite random sampling, stratified random sampling, and composite sampling (**Figure 2**). We are currently developing code for convenience sampling plans (such as Z-pattern sampling) and risk-based sampling. The Illinois team's graduate student has begun a literature review to extract parameters relevant to risk-based sampling, including the impact on foodborne pathogen prevalence of water practices (irrigation, surface water, rainfall), soil (amendments, presence of indicators), and landscape features (proximity to natural areas or other agricultural production). Our industry partner connected us with a data analyst who has provided parameters for four typical contamination scenarios in their fields: background contamination, small cluster, larger cluster, and major events.

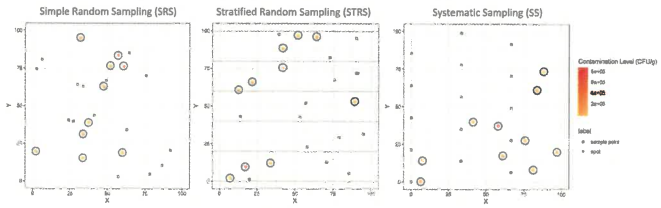

Figure 1. Example simulation of a generic 100-unit by 100-unit produce field with randomly located point sources of contamination, each with a 2-unit radius of spread, and 15 samples selected by different sampling schemes. The simulation predicts the contamination level at each sampling point based on the distance to contamination points (such as fecal droppings) and decay out to the indicated radius of spread. Then the individual sample points are composited for enrichment testing, and the simulation outputs if the hazard would be detected. Our next steps are to parameterize these simulations to represent the real geometry of fields, incorporate convenience (Z-pattern) and risk-based sampling, and represent likely hazards.

BENEFITS TO THE INDUSTRY

The key beneficiaries of this project are growers and those individuals who are responsible for pre-harvest produce sampling. Specifically, this project will provide beneficiaries with generic guidance for improving sampling to target specific types of contamination. We will also provide tools to help growers adapt generic guidance to field-specific, risk-based sampling plans. In the short-term, our findings will increase the understanding of the limitations of existing sampling plans – particularly since some current n_{60} composite plans are more a response to buyer requirements than to a science-based understanding of how to best detect contamination. It will also describe expected performance and resource requirements of improved sampling plans, which are critical to building the business case for investing additional resources in food safety risk management.

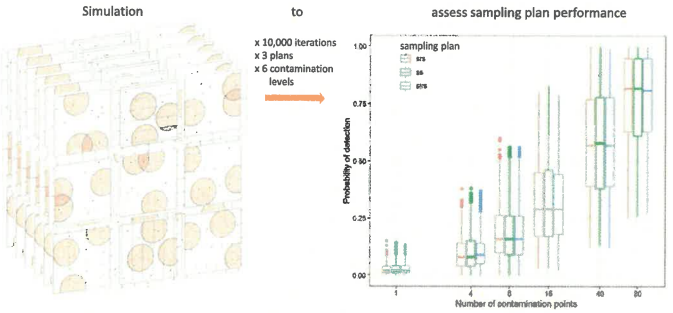

Figure 2. Here we (left) iterate a field model 10,000 times, each time simulating 3 different types of sampling plans and 6 different contamination levels, and then (right) compare the probability of detecting the hazard across these variables. In this example case, all three sampling plans perform equally well, and performance improves with the number of contamination points. Our next steps are to parameterize these simulations according to real-world scenarios to evaluate actual sampling plans of interest to the produce industry.

Simulation Domain	Parameters to Simulate	Variable Characteristics for Representative Fields
Produce Field	Geometry (field dimensions, field boundaries, slope) Risk Factors (landscape structure, non-crop buffers, irrigation system type and set-up, time since rain)	Field setup High- or low-risk features
Product	Location (seeding rate, row spacing) Plant characteristics (edible fraction, yield)	Major products (leafy greens, tomatoes); Planting patterns
Food Safety Hazard	Location and area contaminated Point source, e.g. feces Systematic source, e.g. irrigation Sporadic low-level, e.g. endemic soil bacteria Proportion of plants that are contaminated Pathogen concentration on contaminated plants	Pathogens of concern (e.g., *Salmonella* in tomatoes, STEC in leafy greens) Known contamination events Known research-testing results
Sampling Plan	Sampling Scope (number and size of composite samples) Sampling strategy Convenience: Z-shape Improved Generic: random, stratified, systematic Risk-Based: targeting field-specific high-risk areas	Typical sampling strategies Numbers Sizes Patterns
Assay *Performance*	Parameters (Limit of detection, sensitivity, specificity) Setup (Sample compositing, re-testing requirements) Inputs (Time per test, cost per test)	Typical assays Local assay costs, timing Retesting conditions, thresholds
Outcomes	Probability of detecting hazard above threshold Incremental cost for improved performance	Typical, ranges of sampling results Relative costs, efficacy

Table 1. Field simulation scope indicating a representative subset of the parameters that will be simulated.

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