CENTER for PRODUCE SAFETY

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FINAL PROJECT REPORT

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

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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.*

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FINAL REPORT

Abstract

The development of effective preharvest, field-level produce sampling strategies is a key challenge facing the produce industry. Current industry practices involve collecting and compositing 60 preharvest produce items from a given field, often using a pre-determined sampling pattern, and subjecting that sample to laboratory testing for pathogens. Because existing good agricultural practices, fortunately, control foodborne pathogens to relatively low prevalence and level, preharvest tests are rarely positive – yet this presents a challenge for further sampling plan improvement because it is difficult to draw conclusions from experimental data when virtually all experimental tests would be negative. Therefore, this project sought to simulate preharvest produce sampling using computer models to allow for *in silico* experimentation in sampling for defined pathogen contamination as realistically low prevalence, level, and extent. The project built a generic in-field produce preharvest sampling model, validated the model against literature and newly collected field trial data, and used the model to simulate sampling of fields representative of major commodities in CA, AZ, NY, and VA. The preharvest sampling simulation model was validated against data in the academic literature, reproducing results from Xu and Buchanan (2019) for contamination in an 18 x 15 m^2 field with 1–10 contamination points, and results for taking 30 grab-sample composites by random, stratified random sampling, and systematic sampling. The sampling simulation was also validated against work in two fields, each inoculated for conditions representing a point source, systematic, or sporadic contamination using rifampicin-resistant *E. coli* and sampled by high-resolution stratified random sampling (n=228 samples per field). Our model simulated results of these six inoculated field trials and reproduced ranges of plausible positive samples that, in all cases, included the observed number of positive samples (3 and 7 for point source contamination, 139 twice for systematic contamination, and 2 and 18 for sporadic contamination). Use of the model found that current industry sampling practices are likely powerful enough to reliably detect large-scale, systematic contamination events. A generic 1-acre field simulation found that a 60 random sample composite of 3-g grabs would almost always detect uniform contamination at −1 log(CFU/g) (equivalent to 1 CFU per 100 g of product). Representative systematic contamination events including contaminated irrigation water and fertilization with improperly composted manure were modeled with intermediate levels of −3 log(CFU/g) (0.5 CFU per lb) and −0.7 log(CFU/g) (1 CFU per 5 g), with intermediate detection probabilities. Use of the model also found that current industry sampling practices are not likely powerful enough to reliably detect point source contamination events. A generic 1-acre field simulation found that a 60 random sample composite of 3-g grabs would fail 80% of the time to detect contamination from a fecal pellet with a 1.9 m radius spread. Representative field simulation supported these conclusions from the generic field, additionally finding even lower detection probabilities in scenarios where point source contamination does not spread, such as bird droppings contaminating tomatoes. Overall, results from this project that suggest that currently sampling practices are primarily useful to verify lack of major food safety failures. But, it is not likely possible to test individual fields for safety without significant, likely impractical, increases in sample sizes, or development of alternative sampling technologies.

Background

The development of effective preharvest, field-level produce sampling strategies is a key challenge facing the produce industry as communicated to us by our industry partners. For example, current industry practice involves collecting and compositing 60 preharvest produce items (e.g., 60 spinach leaves) from a given field (also known as n60 sampling) using a random sampling scheme or a pre-determined sampling scheme (e.g., Z-patterns). However, according to our industry partners, this approach typically yields few positive samples (e.g., 1 out of every 5,000 composite samples tested positive). While alternatives to preharvest field sampling exist, they cost more and suffer from the same drawback, a low prevalence of positive samples. For example, at least some industry partners use a 2-stage sampling scheme where positive fields are subject to re-testing with a larger sample size (e.g., at least 10-fold more samples). Partners report most fields re-test negative. Only rarely do re-tests yield additional positive results, which can be used to identify practices or events that may have been responsible for contamination. The produce industry also performs finished product testing, which is substantially costlier than in-field sampling and has similarly low prevalence of positive samples. This leads to broad questions of interest and relevance to industry:

1. What are the relative merits of different preharvest and finished product testing programs?
2. Do the initial sampling results detect anything more than random contamination?
3. Can the initial, or re-test, sampling be designed to identify systematic food safety risks?
4. Can a sampling plan demonstrate a given producer's food safety practices ensure a given pathogen has a prevalence of no greater than X?

To answer these questions the project proposed to develop simulation tools for produce field sampling. These tools will allow users, including growers, to directly compare the results of alternative preharvest sampling programs to determine which are most appropriate to their operation.

<u>Theoretical challenges to random sampling for pathogens in produce fields</u>

Development of effective plans for sampling preharvest produce to detect preharvest pathogens is difficult since contamination typically occurs at low levels. Reliable detection of contaminated produce during routine preharvest produce testing would require collection of large composite samples [1]. For example, when 1% of product is contaminated, a composite sample consisting of 60 units would detect a foodborne pathogen 45% of the time. Thus, current strategies, such as n60 sampling, do not have sufficient power to detect contaminated produce [2, 3]*;* a negative result following n60 sampling is not a reliable indication of the absence of contaminated product within the tested field. Instead, a composite sample of 299 produce items would be needed to have a 95% probability of detecting contamination within a field where 1% of the produce is microbially contaminated (and 3,000 samples for a 0.1% prevalence) [4, 5]. These calculations assume uniform contamination; however, produce fields are often contaminated in ways that produce heterogeneous contamination, which require additional sample numbers to detect.

<u>Not all sampling plans are appropriate to detecting preharvest contamination by all mechanisms</u>

There are many different mechanisms whereby preharvest produce may become contaminated by foodborne pathogens. For example, splash from in-field, wildlife feces may contaminate only those produce items within a set distance from the feces (e.g., 2 m), whereas irrigation with contaminated water may uniformly inoculate a field with the pathogens present in the water [6]. We refer to the former scenario as point-source contamination, and the latter as systematic contamination. In addition, produce items may be contaminated by pathogens endemic to the field environment, which could result in sporadic, low-level contamination within a field. The most efficient sampling strategy is likely to depend on which type of contamination has occurred [7]. For example, systematic sampling (e.g., Z-sampling) is most efficient to detect point sources of contamination, e.g., splash from in-field feces, but could miss sporadic, low-level contamination

[8]. As such, sampling plans should be developed that account for contamination events that are likely for the given operation.

Thus, the produce industry has a **research need** for tools to facilitate the development and evaluation of different approaches to preharvest field sampling. This project will meet this need by developing and validating a produce field simulation model, which can be used to evaluate different approaches for produce field sampling (e.g., n60 sampling, risk-based sampling). The results of these models will provide key data that end-users (e.g., the produce industry, extension agents) can use to develop preharvest sampling plans optimized to detect specific types of field contamination. Since physically sampling every type of field, for each produce commodity, and testing each sample for each pathogen of concern is prohibitively expensive and unrealistic, the use of *in silico* analyses (e.g., simulations) is necessary to produce results that are useful for industry stakeholders.

Research Methods and Results

Objective 1 Methods:

California and Arizona produce fields simulation

Leafy-green production field parameters were obtained by expert elicitation and observation during site-visits by the PI Matthew Stasiewicz and graduate students Jorge Quintanilla and Gustavo Reyes to commercial farms in California in September, 2019. Our industry partner has strong presence and production in both states; therefore, the fields were modeled similarly. As described in **Table A**, Iceberg and Romaine lettuce and Spinach are three major commodities grown in these regions and were selected after site-visit conclusion. Given the commercial status of the fields, the sampling standard switched from acre-based to pounds (#) per lot of production. For these regions, a production lot was defined as 100,000# of produce. This parameter determined the dimensions of the simulated fields that are needed to grow the lot.

California and Arizona fields – food safety hazard

The main food safety hazard of concern our industry partners is Shiga toxin–producing *E. coli* (STEC). Historic data regarding pathogen detection and contamination levels were shared in compiled spreadsheets and documents, respectively. The information was presented as an attempt to optimize pathogen sampling by modelling contamination challenges with specific sampling scenarios at different stages during farm-to-fork process. Preharvest contamination challenges were extracted and analyzed prior to transformation to inputs of the simulation model. These contamination loads were presented as clustered across the field in small, big and no cluster, matching the contamination scenarios of point source, systematic and sporadic contamination, respectively.

New York produce field simulation

Parameters for fresh produce production fields in New York were obtained from academic literature, e.g., Cornell Cooperative Extension, and official documents, e.g., Census of Agriculture. The selection of the products was made by reviewing official documents, and by meeting the following criteria: (1) consumption, (2) acres harvested on year of census, and (3) number of farms and monetary value. Once the product was determined, its production parameters were obtained by further reviewing official documents and extension literature. The main parameters obtained from these documents were row spacing, in-row spacing, rows per bed, yield, irrigation, and fertilization methods. The fields were standardized to 1-acre field, with dimensions of 153.4 x 26.58 m (500 x 87 ft).

New York fields – food safety hazard

The food safety hazard of concern is STEC. This was defined by consultations with academic partners and academic literature. Multiple studies had evaluated the survival of STEC in

improperly composted manure and fecal pellets in fresh produce production. After the literature review, the following three scenarios were selected as ways of fresh produce contamination: (1) contamination from fecal pellet, (2) widespread contamination from fertilization with improperly composted manure, and (3) endemic bacteria present in field.

Virginia produce field simulation

Parameters for fresh produce production fields in Virginia were obtained from academic literature, e.g., Virginia Cooperative extension, and official documents, e.g., Census of Agriculture. The selection of the products was made by reviewing official documents, and by meeting the following criteria: (1) consumption, (2) acres harvested on year of census, and (3) number of farms and monetary value. Once the product is determined, its production parameters were obtained by further reviewing official documents and extension literature. The main parameters obtained from these documents were row spacing, in-row spacing, rows per bed, yield, irrigation, and fertilization methods. Row spacing was used to determine the number of rows per acre, given that production was standardized to 1-acre field, with dimensions of 153 x 26.6 m (500 x 87 ft).

Virginia fields – food safety hazard

The food safety hazard of concern is *Salmonella.* This was defined by reviewing the State Department of Health, and academic literature. Multiple studies had evaluated the survival of *Salmonella* in fresh produce and its prevalence in Virginia [9-11]. After the literature review, the following three scenarios were selected as ways of fresh produce contamination: (1) contamination from fecal pellet, (2) widespread contamination from irrigation with contaminated water, (3) endemic bacteria present in field. Expert elicitation was necessary to determine the spread of these contamination events.

Objective 1 Results:

California and Arizona Food Safety Hazard in Production Fields

Based on the site visit to CA, both CA and AZ fields were modelled as one location because of similar large-scale production practices. Field dimensions were calculated by using specific production parameters, e.g., number of beds per acre of crop production. Iceberg lettuce commercial production lot was calculated to grow in 2.2 acres, Romaine lettuce in 5.14 acres and Spinach in 12.5 acres (**Table A**). A microbial load of 50,000 CFU per field was selected to represent an extreme contamination event, as expressed by our industry partners.

For point-source contamination (small cluster in field), the microbial load was adjusted given the weight of produce grown in an area of r= 1.9 m [7, 8] located in the field. For Iceberg and Romaine lettuce the contamination level was -0.053 LogCFU/g (sd: 0.1), and 0.311 LogCFU/g (sd: 0.1), respectively. For spinach, the level was adjusted to 0.69 LogCFU/g. Systematic contamination was represented as a large contamination event covering 10% of the production lot (large cluster). The microbial load was adjusted for 10,000 pounds of product, in a similar fashion as point source, yielding a contamination level of -1.958 LogCFU/g (sd:0.1) for all commodities, with variations on the area affected. Sporadic contamination was set at -5.66 LogCFU/g (sd:0.1), expressed as the background level.

Fresh Produce Production Parameters in New York

Cabbage, Broccoli and Lettuce were selected as three common fresh produce types grown in New York [12]. Row spacing was determined as 2.5 ft for cabbage and broccoli [13], and 1.25 ft for lettuce [14], being 35 and 70 rows per acre, respectively (**Table E**).

The food safety hazard is STEC. Point-source contamination was defined as fecal contamination produced by cattle intrusion. Systematic contamination was defined as fertilization with improperly composted manure. Sporadic contamination was defined as endemic bacteria in the field in compliance with the Produce Safety Rule. For cabbage and lettuce, the spread was fixed at an

area of 1.9 m radius. Systematic and sporadic contamination was assumed to cover the entire field uniformly.

For point-source contamination, bacterial concentration in fresh cattle feces was determined as 3.61 LogCFU/g (sd: 1.38) [15].This concentration was adjusted by calculating the concentration per plant using the following formula: $Log\ CFU/g * grams\ of\ feces * g/plant$, where the weight of fecal pellet was 3.02 kg (estimated by 36.3 kg of feces produced per dairy cow per day [16, 17]), and the weight of the plants in the affected area was 61 kg for cabbage and broccoli and 56.8 kg for lettuce. The concentrations were 2.30 LogCFU/g (sd: 1.38) for cabbage and broccoli, and 2.33 LogCFU/g (sd:1.38) for lettuce. For systematic contamination, the concentration of *E. coli* in the worst-case scenario was 0.33 LogCFU/10g (sd: 0.24) found in soil with improperly composted manure at day of harvesting [18, 19]. This concentration was assumed to be found in produce at the same level. For sporadic contamination, the microbial load was defined as the same background level of the simulation model, simulating compliance with the non-objected 120-day time frame after fertilizing with raw manure [20].

Fresh Produce Production Parameters in Virginia

Tomatoes, Lettuce (Crisphead) and Cabbage were selected as three common fresh produce types grown in Virginia. Row spacing was determined as 5 ft for tomatoes, 2.5 ft for lettuce and 3 ft for cabbage, being 17, 35 and 29 rows per acre, respectively. For tomatoes, stake culturing was considered an additional key factor in produce simulation, staking at every 5 ft (**Table H**).

The food safety hazard is *Salmonella.* Point-source contamination was defined as fecal contamination produced by bird droppings. Systematic contamination was defined as irrigation with pond water. Sporadic contamination was defined as endemic bacteria in the field. To define the extent of point-source contamination in tomatoes, a study report shared by Michelle Danyluk from University of Florida suggested that *Salmonella* on visible fecal contamination in tomatoes does not spread to the adjacent tomatoes [21]. Therefore, the extent was defined as the area of a medium tomato (r= 0.03 m). For cabbage and lettuce, the spread was adapted to the diameter of medium size produce. Thus, the contamination level was adjusted to their weight and dimension. For Crisphead lettuce the diameter was set to 15 cm, with a weight of 907.18 g [22]; for cabbage the diameter was set to 16 cm, with a weight of 1360.77 g [23, 24]. Systematic and sporadic contamination was assumed to cover the entire field uniformly. Gulls (*Larus delawarensis)* are wild birds present in Virginia [10], producing an estimated 14.4 g/day of feces [25]. *Salmonella* concentration in bird droppings was determined as 0–1 LogCFU/g [26] and was adjusted to the amount of feces produced and the mass of produce affected.

For point source contamination, the concentration was -1.14 to -0.14 LogCFU/tomato, and -1.79 to -0.79 LogCFU/g and -1.975 to -0.975 LogCFU/g for lettuce and cabbage, respectively [27]. For systematic contamination, the concentration of *Salmonella* found in the rhizosphere of a tomato plant irrigated with pond water was determined to be 12 MPN/kg (95% CI: 5.4–25). This statistic was further analyzed and converted to a normal distribution of -1.92 LogMPN/g (sd: 1.85). The sporadic contamination level was defined as the same background level of the simulation model (**Table I**).

Objective 2 Methods:

A 2D simulation model in R was adapted from the model in Cheng and Stasiewicz (2021, Risk Analysis, https://doi.org/10.1111/risa.13721) to simulate taking samples from a contaminated 2D field and testing samples for pathogens. The simulation model consists of four major modules (**Fig. A**). The first module is the hazard simulation module where either point-source or area-based contamination is simulated. The end user can define the pathogen concentration distribution and the decaying pattern in concentration. The second module is the sampling module that determines the sampling locations in a contaminated field using either simple random sampling (SRS), stratified random sampling (STRS), or k-step systematic sampling (SS). The end

user may customize the number of samples under certain constraints. Once the contamination profile and the sampling locations are determined, the assay module will simulate the plating or enrichment process to quantify the number of positive samples. In this module, the end user may choose 15 different attribute sampling plans, depending on the severity of the hazard. With the number of positive samples, the decision-making module will decide whether to accept or reject the entire field. The simulation model can be iterated multiple times and is designed to output the detection probability of the acceptance probability. In this study, the detection probability is defined as the probability to have at least one sample located within any contaminated zone, regardless of the sample assay results. Meanwhile, the acceptance probability is defined as the probability of accepting a contaminated field given a specific sampling plan, where the assay results will play a role. Detailed definitions for all the input parameters are listed in **Table L**.

To demonstrate using the simulation model to address realistic sampling questions, four simulation experiments were conducted in 1-acre generic fields using the input values specified in **Table M**. The first simulation experiment focused on the effect of sampling strategy and prevalence level. In this case, we assumed point-source contamination existed in a 1-acre field ($64 \times 64\ m^2$) and compared the sampling performance between SRS, STRS, and k-step SS under scenarios with 1–6 contamination points that corresponded to 6 prevalence levels (0.3%, 0.6%, 0.8%, 1.1%, 1.4%, 1.7%). The second simulation experiment was designed to investigate the effect of individual sample mass and sampling strategy on sampling performance. We assumed the 1-acre field had area-based contamination, and compared the acceptance probability under the three sampling strategies and four different sample masses (2.5, 5, 10, 20 g). Based on the results of the first two experiments, we further designed the next experiments to investigate the effect of sample size and contamination level using SRS only. Both experiments focused on five different sample sizes (60, 120, 300, 600, 1200 grabs of 3 g). While the third experiment was conducted under the assumption of point-source contamination (0.3% prevalence) with fixed contamination level (3 log CFU/g (sd:1), the fourth experiment examined four different contamination levels (-1, -2, -3, -4 log CFU/g) in an area-based contamination scenario. For all the experiments, each sampling scenario was iterated 10,000 times.

Representative fields sampling simulation

Based on Objective 1 results, generic and convenience sampling strategies were adapted to each of fresh produce production fields representing the four regions and simulated. The simulations evaluated three different sample sizes (60, 300, 600 grabs of produce) and compared the sampling performance between STRS, SRS, and SS. Base sampling was fixed to a n60 composite. For STRS, the stratifications must match the number of samples input in the simulation. The division of fields depended on the number of beds or rows needed for the production lot.

California and Arizona

Given that the production lot involved partial acres, the total number of beds was rounded to the next higher number. Iceberg lettuce production is based on 10 beds per acre, Romaine lettuce on 6 and spinach on 11 beds per acre; this resulted in 22, 31 and 138 beds per production lot (**Table A**). All the fields were divided into 20 rows x 3 columns, which is about 3 samples per bed in Iceberg lettuce production, 3 samples within 1.5 beds for Romaine lettuce production, and 3 samples across 7 beds in spinach production. The sample mass was determined based on-site visits and conversations with industry partners. Sample mass for both Iceberg and Romaine lettuce was 300 g (5 g per specimen), while 150 g (2.5 g per specimen) for spinach.

New York

Based on conversations with Cornell's Agritech Executive Director, Elizabeth Bihn, if fresh produce sampling is performed, it would be upon customer, i.e., retailer, requirement. Hence, the sampling parameters are estimated based on previous knowledge from site visits to other regions.

The stratification of New York production fields from Objective 1 was adapted depending on the number of rows contained per acre and presented in **Table E**. For cabbage and broccoli, the field containing 35 rows per acre was divided into 30 rows x 2 columns, which might translate into about 1–2 samples per row. For lettuce, the field was stratified into 60 rows x 1 column, which might translate into about 0–1 samples per row. All sample masses were determined to be 300 g (5 g per specimen).

Virginia

The stratification of the production fields in Virginia from Objective 1 was not only adapted depending on the number of rows contained per acre, but in column divisions. For lettuce the field containing 35 rows per acre was divided into 30 rows x 2 columns, which might translate into about 1–2 samples per row. Cabbage fields containing 29 rows per acre were divided similarly. All sample masses were determined to be 300 g (5 g per specimen) (**Table H**). Based on conversations with Michelle Danyluk, bird droppings tend to happen close to the stakes in tomato production. Therefore, the sampling in tomato fields revolved around the number of stakes per row instead of the number of rows per acre. Tomato production field with 100 stakes per row was divided into 1 row x 6 columns. The sample consisted of an n6 composite sample [28], assumed to be ready to harvest medium tomatoes (200 g) [29] and analyzed as LogCFU/whole fruit. To fit the simulation, the point-source contamination concentration was randomly picked from a uniform distribution.

Objective 2 Results:

One-Acre Generic Fields

The first experiment compared the three sampling strategies with varying prevalence levels in a point-source contamination scenario (**Fig. B**). Overall, the acceptance probability would decrease as the prevalence level increased from 0.3% to 1.7%. Specifically, SRS and STRS exhibited similar trend, with the median acceptance probability decreasing from around 90% to 60%. Both sampling strategies resulted in relatively small variation in acceptance probability, suggesting stable sampling performance. By contrast, k-step SS had a distinct decreasing trend, with the median acceptance probability decreasing from around 100% to 75%. While this range was seemingly higher than that of SRS or STRS, k-step SS exhibited a substantially larger variation especially when the prevalence level was above 0.8%, indicating highly unstable sampling performance. In conclusion, SRS or STRS may be better than k-step SS for sampling in a point-source contamination scenario due to high stability in performance.

The second experiment focused on area-based contamination and compared the three sampling strategies under different total sample masses (150, 300, 600, 1200 g) (**Fig. C**). By visual inspection, the three sampling strategies had highly similar performance, with the median acceptance probability decreasing from around 85% to 30% as the total sample mass increased. In addition, all of them had relatively small variation in acceptance probability, suggesting that under area-based contamination, sampling performance was dependent on the total sample mass, regardless of sampling strategy.

Based on the first two experiments, it is evident that SRS and STRS may have equivalent or superior sampling performance compared to k-step SS. For simplicity, we decided to use only SRS in the subsequent experiments.

The third experiment examined the effect of sample sizes (60–1200 grabs of 3 g) on sampling performance in a low-prevalence (0.3%) point-source contamination scenario (**Fig. D**). As the sample size increased, the median acceptance probability would decrease from around 85% to 5%. The variability in acceptance probability remained fairly constant across different sample sizes. Our finding suggests that when the prevalence level is low, the commonly used n60 sampling plan may not be powerful enough to detect contamination and it would require a substantial number of samples to reject a contaminated field.

To better understand the interaction between contamination level and sample size, we conducted the fourth experiment where four contamination levels (-1, -2, -3, -4 log CFU/g) were investigated in an area-based contamination scenario. The same five sample sizes used in the previous experiment (60–1200 grabs of 3 g) were also adopted in this experiment. Evidently, taking more samples would decrease the acceptance probability. When the contamination level was high (i.e., -1 log CFU/g), the n60 sampling plan would be sufficient to detect contamination and reliably reject the field. As the contamination level was reduced, the acceptance probability of n60 sampling plan would drastically increase, suggesting that lower contamination level may hinder pathogen detection using the N60 sampling plan. This could be remedied by increasing sample size, but when the contamination level was extremely low, the benefit of taking extra samples may be diminished. For example, taking 1200 samples in a field with -3 log CFU/g contamination would result in acceptance with a chance of 5%. Meanwhile, taking the same number of samples in a field with -4 log CFU/g contamination would lead to around 67% acceptance.

California and Arizona Fields

The simulation evaluated three different sampling strategies and compared it across three sample sizes. Overall, the median probability of accepting an Iceberg lettuce field (**Table B**), given a single point-source contamination, is 99%, 98% and 2% for SS, while the remaining strategies yielded slightly lower values, with a median of 90%, 68% and 45% for SRS and 91%, 66% and 45% for STRS, when taking one, five and 10 composite samples respectively. For large-scale contamination events, the median probability of accepting a field behaved similarly across STRS and SRS, ranging from 76% to 6% when taking one, five, and 10 composite samples. Low-level contamination showed overall higher probabilities of accepting the field. For STRS, the median probability of accepting a field was 99% across all sample sizes. For SRS, the median probability of accepting a field decreased from 99% to 97% for one and 10 composites taken. Similarly, SS yielded 99%, 98% and 96% median probabilities of accepting a field when taking one, five and 10 composite samples.

Similarly, the performance STRS and SRS in Romaine lettuce fields showed similarities in probability of acceptance across all sample sizes. As described in **Table D**, the median probability of acceptance for point-source contamination ranged from 95% to 69% in both sampling strategies. On the other hand, SS shows higher, steadier probability of acceptance when taking one and five composites, before decreasing to 76% when taking 10 composites. However, the variability increases dramatically when multiple composites are taken. Systematic contamination has higher chances of being detected by all sampling strategies. The median probability of acceptance when taking one composite sample ranges from 74–73% for all strategies, multiple composite samples decrease the probability of acceptance as low as 3% (STRS) when taking 10 composite samples. Sporadic contamination reflected a similar trend across the three sampling strategies, yielding 99–97% across the three sampling strategies and sample sizes.

For spinach, the median probability of accepting a field (**Table C**), given a single point-source contamination using STRS or SRS, behaved similarly, with values ranging from 98% to 86% when taking one and 10 composite samples, respectively. Performing SS yielded higher median probabilities, however, the variability in 95% CI is drastically higher. For large-scale contamination events, the median probability and variability showed similar behavior across sampling strategies, ranging from 86% (all) to 20% (SS), when taking one and 10 composite samples. Low-level contamination showed overall higher probabilities of accepting the field. Across all sampling strategies, the median probability was 99% and 99% when taking one and five composites, respectively, and taking 10 composites yielded a median of 98%, while showing steady variability.

New York Fields

Overall, the median probability of accepting the production field behaved similarly for the three commodities. For broccoli and cabbage (**Table F**), the median probability of accepting a field given a single point-source contamination, STRS and SRS showed a similar behavior, with values

of 85%, 44% and 17%, and 84%, 44% and 18%, respectively, while taking one, five and 10 composite samples. However, SS showed drastic changes when increasing the number of composites, yielding 100%, 13% and 0% when taking one, five and 10 composite samples, respectively. For large-scale contamination events, the median probability of accepting a field was 0% across all sampling strategies and number of composites taken. Low-level contamination showed overall higher probabilities of accepting the field. Across all sampling strategies, the median probability ranged between 99% and 97% when taking one and five composites, while taking 10 composites yielded a median probability of acceptance range of 96% and 94%. As described in **Table G**, STRS and SRS show a similar probability of acceptance in lettuce fields when taking one composite, at 85% and 88%, respectively. Increasing to five and 10 composites, STRS shows lower probability of acceptance than SRS. In parallel to broccoli and cabbage, SS shows identical drastic changes in probability of accepting a lettuce field by increasing the sample size. For large-scale contamination events, the median probability of accepting a field was 0% across all sampling strategies and number of composites taken. Low-level contamination showed a generally high acceptance probability, varying from 99%, 97% and 95% by taking one, five and 10 composites, respectively, across all sampling strategies.

Virginia Fields

The median probability of accepting either tomato, lettuce and cabbage fields with a single point-source contamination when taking a single composite sample was 99–98%. When taking multiple composites, lettuce (**Table J**) and cabbage (**Table K**) behave similarly, with high probabilities of acceptance when taking five and 10 composites. Sampling in tomato fields showed a slightly lower acceptance when taking 10 composites, with a value as low as 88% (STRS) (**Table I**). Based on this result, sampling strategies are not likely to detect spreading of fecal contamination, hence, evident contaminated fruit should not be harvested.

For tomatoes, low-level contamination is usually not likely to be detected, given that the median probability of acceptance when taking one, five and 10 composites ranged from 99% to 82% across sampling strategies. However, the 95% CI shows a drastically wider range across all sample sizes evaluated in each sampling strategy. On the other hand, the sampling strategies had variable performance in detection of large systematic failures, i.e., irrigating the production field with pond water, yielding median probability of acceptance as low as 15% (SS) when taking a single composite, but with a wide 95% CI, ranging from 0% up to 77%.

The probability of accepting a lettuce or cabbage field after a large contamination event is highly variable, as shown in the 95% CI at every sample size in all sampling strategies. However, the median suggests that large contamination events are likely to be detected by the sampling strategies at a single composite, yielding a probability of acceptance of 43%. The probability of acceptance of the fields of both cultivars at low-level contamination behaved similarly, showing high probability of acceptance and high variability at every sample size and strategy evaluated.

Objective 3 Methods:

Validating the simulation model with literature data involves three steps: (1) extracting the input values from the literature data and mapping them to the input parameters of the simulation model; (2) iterating the simulation model a sufficient number of times; and (3) comparing the simulation output with the literature output and determining whether the literature output falls within the simulation output range. Specifically, our simulation model is considered validated when the detection probability from the literature data falls within the range between the 2.5^{th} and 97.5^{th} percentile simulated detection probability, assuming that the model is sufficiently iterated to exhaust all possible values of detection probability.

The literature study by Xu and Buchanan (2019) [30] was utilized as the validation source. This study simulated the sampling process in an 18×15 m^2 field with 270 subplots of 1×1 m^2 and reported the detection probability under a combination of inputs, including four different numbers

of sample (5, 10, 15, 30) and 10 different numbers of contaminated subplot (from 1 to 10). Successful detection was defined as sampling at least one of the contaminated subplots in a field. Since our simulation model describes the sampling process in a continuous two-dimensional space instead of a grid system, the first step to validation was to map the input values from the literature study to the input parameters of our simulation model. To create an equivalent contaminated subplot in our system, we set the spreading radius to $\sqrt{\frac{1}{\pi}}$ m so that each contaminated subplot would have an area of 1 m^2. After parameter mapping, we iterated the model 10,000 times to calculate the detection probability.

Objective 3 Results:

Comparison between the simulated detection probability and the theoretical probability from the literature was conducted to validate our simulation model (Fig. E). By visual inspection, the increasing trend of detection probability in our simulation matched that of the literature study for all the sample sizes examined (5, 10, 15, 30), with 30 samples resulting in the steepest curve and 5 samples resulting in the most gradual curve. A closer examination revealed that all the detection probabilities from the literature study successfully fell within the corresponding 2.5th -97.5th percentile simulated range, indicating that our simulation model can faithfully represent the produce sampling process and accurately predict the detection probability. In conclusion, our simulation model was successfully validated with literature data.

Objective 4 Methods:

Field setup

Four approx. 20 m by 30 m experimental fields were planted with baby spinach (Seaside F1, treated seeds; Harris Seeds, Rochester, NY) in Freeville, NY. Four rows of spinach were planted at a seeding rate of approx. 2 cm. Irrigation, fertilization, and herbicide application were performed as needed. Each field was made up of four beds, which were approx. 1.5 m wide and 30 m long; the beds were spaced approx. 1.5 m apart. Each bed was split into 57 identical plots, such that each plot was 1.5 m wide and 0.5 m long; there were 228 total plots across the four beds. Each plot was then split into eight approx. equal sized subplots.

Inoculum and controlled contamination

Two of the four fields were used to investigate sampling plans for point-source contamination and the other two fields were used to investigate systematic and sporadic contamination events. In total, six trials were performed: two point-source, two systematic, and two sporadic contamination trials. For all contamination patterns, inoculation was performed 25 to 35 days following planting. Each field was inoculated with a three-strain cocktail of rifampicin-resistant generic *E. coli* (TVS 353, TVS 354, and TVS 355) (r*E.coli)*; all strains were provided by Trevor Suslow. Each of the strains was streaked onto separate tryptic soy agar plates supplemented with 0.1 g/L rifampicin (TSA+R) to form a confluent lawn and incubated at 37°C for 18–24h. Following incubation, each plate was flooded with 10 mL of phosphate buffered saline (PBS), the cells were resuspended using a sterile loop, the resuspended cells were transferred into a sterile bottle containing 90 mL of PBS, and the suspension was vortexed; each strain was transferred to a separate bottle. In duplicate, 10 mL of each bacterial suspension was transferred to separate 15-mL conical tubes. The tubes were centrifuged at 4,000 RPM for 10 minutes, then the supernatant was pipetted off. The cells were then washed twice with PBS using the same centrifuge conditions as described above. The optical density (600 nm) of each suspension was measured to ensure the suspensions were at approx. log 9 CFU/mL PBS using a standard curve. The suspensions were then stored at 4°C overnight. The following morning, 4 mL of each strain's suspension were combined, and serial dilutions were performed to reach the final concentration for inoculation.

For the simulated systematic and sporadic contamination events, the inoculum was diluted into 12 sanitized 2-L bottles to reach a final concentration of log 4 CFU/mL PBS; 5 mL from each bottle was saved to confirm the inoculum concentration. Inoculation was performed using a CO_2 powered backpack sprayer with the pressure between 27–30 psi and two Turbo TeeJet (tip #8) nozzles spaced 38 inches apart. The inoculum was applied to the field at a rate of 2 L/ 30.5 m.
For the simulated point-source contamination event, the inoculum was diluted into three 1-L spray bottles with final concentrations of log 0 CFU/mL, log 3 CFU/mL, and log 5 CFU/mL; a 10-mL aliquot from each bottle was saved to confirm the inoculum concentration. To inoculate the field, a subplot within the field was randomly selected using a random number generator. A 2-m buffer area was marked around the center of the selected subplot, and the 2-m buffer was split into three 0.67-m rings. Using the individual spray bottles, the innermost ring was spray inoculated with 2.8 mL of the log 0 CFU/mL inoculum, the middle ring was inoculated with 2.8 mL of the log 3 CFU/mL inoculum, and the outermost ring was inoculated with 2.8 mL of the log 5 CFU/mL inoculum. The inoculum was only sprayed on plants (i.e., no bare soil).

Sample collection

From the fields used for the simulated systematic and sporadic contamination events, two samplings were performed; the systematic sampling was conducted 1 d following inoculation and the sporadic sampling was conducted 6 d following inoculation in the first trial and 5 d following inoculation in the second trial. From the fields used for point-source contamination, one sampling event was performed; the sampling was conducted 1 d following inoculation.
At each respective sample collection time, 228 samples were collected. Each sample consisted of all spinach plants in 1 pre-selected subplot per plot in the field; a random number generator was used to identify which subplot within each plot was collected. The spinach samples were collected by cutting each plant approx. 2 cm above the soil line using sanitized scissors. All plants within a single sample were transferred to a single Whirl-Pak bag (Nasco Sampling, Fort Atkinson, WI). The sample collectors' gloves were changed, and the scissors were sanitized between each sample. All samples were stored at 4°C and transferred to the lab for processing.

Sample processing

All samples were processed with 24 h of collection. Each sample was weighed and 225 mL of tryptic soy broth (TSB) was added. Each sample was hand massaged for 1 min, then incubated at 37°C for 18–24 h. After incubation, 50 µL of each enrichment was streaked for isolation onto *E. coli* CHROMagar (supplemented with 0.1 g/L rifampicin) plates (ECC+R; DRG International, Springfield, NJ). The plates were incubated at 37°C for 18–24h. ECC+R plates with blue colonies after incubation were recorded as being positive for rifampicin-resistant *E. coli*.

Strain identification

To determine if any r*E. coli* isolated from the spinach was naturally occurring (i.e., not one of the inoculum strains), the PCR protocol described by Belias et al. (2020) [31] for differentiating the three inoculum strains (TVS 353, TVS 354, and TVS 355) was performed (i.e., the TVS PCR protocol). For those isolates that match the banding pattern of one of the inoculum strains according the TVS PCR protocol, *clpX* PCR and subsequent sequencing was performed for further differentiation (Belias et al., 2020). Those isolates that did not match the TVS PCR banding pattern and did not have the same *clpX* allelic type were considered naturally occurring r*E. coli* and excluded from analyses.
For the two simulated point-source contamination events, up to two isolates per positive sample were tested according to the above PCR and sequencing protocols. For the two simulated systematic contamination events, two isolates from 13 randomly selected positive samples (i.e., to confirm <20% of the positive samples were positive due to naturally occurring rifampicin-resistant *E. coli* at a 0.05 significance level) were tested according to the above PCR and

sequencing protocols. For the two simulated sporadic contamination events, up to two isolates per positive sample were tested according to the above PCR and sequencing protocols.

Die-Off in @Risk

To build the die-off model, all data cleaning, modelling and analyses were performed in Palisade @Risk version 8.0. The die-off model represented a biphasic change in bacterial concentration following certain time after inoculation. The model is based on a shared dataset developed by Belias et al. in a similar study [31]. For each field trial, the following statistics were extracted, log-transformed and used as the predictors for the final concentration at day of sampling: (i) Sprayed Concentration, (ii) Concentration after Adhesion, (iii) Segment 1 die-off rate, (iv) Break point, (v) Segment 2 die-off rate.

Relationships were observed between predictors Segment 1, Segment 2, and Break point, leading to the inclusion of Spearman's correlation coefficient in the model. To simulate the bacterial die-off in the field trials, the predictors and relationships are described in **Table O** were used as inputs of the model. Given that during this study, the concentration loss due to spraying was not measured, the total concentration adhered to the tissue was tuned after back calculating the concentration in field needed to recover n positive samples. For this, the following formula was used: $E(n_+) = n_{sp} \times CFU_i = n_{sp} \times m_{sp} \times C_i$, where n_+ follows a binomial distribution, where $n = n_{sp}, p = CFU_i$, $E(n_+)$ is the total number of positive samples; n_{sp} the total number of samples; $CFU_i = m_{sp}$ the mass of the sample, and C_i the concentration, when sampling one day following inoculation. The parameters described in **Table N** represent the inputs for each contamination scenario in the field trials. All simulations were iterated 10,000 times. These scenarios were developed to represent estimates of die-off rates believed to influence bacterial behavior from inoculation spray through the day of sampling.

Validating the simulation model with the experimental data involves four steps: (1) extracting the parameters of the outcome distributions from the die-off model and mapping them to be inputs of the simulation model; (2) extracting the field and sampling parameters from the field trials and mapping them to be input parameters of the simulation model; (3) iterating the simulation model for sufficient number of times; and (4) comparing the simulation output with the field trials output and determining whether the field trial results fall within the simulation output range. Specifically, our simulation model is considered validated when the total number of positive samples from each trial fall within the range of the boxplot minimum and maximum whiskers. Since the field trials are the validation source for the simulation model, field parameters and sampling strategies were extracted and used as static inputs of the model, while parameters inherent to the food safety hazard, such as concentration at day of sampling, geometry of the contamination, and decay functions varied depending on the field trial, i.e. point-source contamination in the field covers an area of 1.9-m radius and the concentration decays over distance from contamination point, while with systematic contamination, we assume the contamination covers the entire field uniformly.

Objective 4 Results:

Field Trials

For the point-source contamination trials, r*E. coli* was recovered from 17/228 and 7/228 samples, for the first and second trial, respectively. In the first trial (PS1), naturally occurring r*E.coli* was identified in 14/17 samples, while 3/17 matched one of the inoculum strains and were confirmed as positive. For the second trial (PS2), all samples matched one of the inoculum strains and were confirmed as positive.

The recovery of r*E. coli* following both systematic contamination trials was observed in 139/228 samples per trial (SS1 and SS2). Given the high number, the purpose of PCR confirmation was to determine if less than 20% of spinach samples where r*E.coli* was recovered, were positive due to naturally occurring r*E.coli.* For both trials, 13/13 samples matched one of the inoculum strains,

and we had confidence less than 20% of the samples were positive due to naturally occurring r*E.coli.*

For sporadic contamination trials, r*E. coli* was recovered from 10/228 and 18/228 samples, for the first and second trial, respectively. In the first trial (SP1), naturally occurring r*E.coli* was identified in 8/10 samples, while 2/10 matched one of the inoculum strains and were confirmed as positive. For the second trial (SP2), all positive samples matched one of the inoculum strains and were confirmed as positive.

Die-Off Model - Tuning for concentration adhered to leaf

With an initial assumption for high systematic contamination to be $E(n_+) = n_{sp}$ and uniformly spread across the field, all samples taken are expected to become positive. Yet both systematic contamination trials showed fewer positive samples than expected ($n_+ = 139$). Since the assumption was not met, to account for the 89 negative samples, inoculum adhered to the plant and bacterial die-off in field were modeled.

The calculated concentration adhered to the plant was -1.47 LogCFU/g. Hence, inoculum reduction due to spraying was tuned to -2.98 LogCFU (sd= 0.1) to reach the calculated concentration as median of the values at sampling day.

Die-off rates of inoculated r*E. coli* in spinach were extracted from Belias dataset to fit a biphasic pattern to each field trial. Overall, the die-off pattern modeled for the field trials shows a drastic reduction in inoculum concentration after spraying, followed by a rapid decay that transitions to a steadier change in concentration at the breaking point (**Figure G**). Relationships between variables are described in **Table O**, where high rates of Segment-1 die-off are followed by small values of breaking points. The concentrations at day of sampling for trial PS1 was 0.401 (95% CI: -3.37, 0.747) LogCFU/g, and 0.429 (95% CI: -2.27, 1.91) LogCFU/g. Concentrations of the widespread contamination trials sampled one day following inoculation were -1.806 (95% CI: -5.83, 0.0117) LogCFU/g for SS1, and -1.58 (95% CI: -5.37, -0.00471) LogCFU/g for SS2. Low-level widespread contamination trial concentrations were -3.30 (95% CI: -7.22, 1.23) LogCFU/g for SP1, and -2.81 (95% CI: -6.61, 0.861) LogCFU/g for SP2. The fitted distributions to the output data are shown in **Table P**.

Simulation Outcome for Validation

Comparison between the samples confirmed positive for r*E. coli* and the outcome of detected samples from the simulation model was conducted for validation. The simulation model shows a range of estimated positive samples given the mapped inputs from field trials, i.e., variability in concentration at day of sampling and field and sampling parameter inputs. The simulation model output ranged from 0 (min) to 22 (max) positive samples, with an IQR from 9 to 15 positive samples for point-source contamination; from 0 (min) to 228 (max) positive samples, with an IQR from 20 to 228 positive samples for systematic contamination; and from 0 (min) to 228 (max) positive samples, with an IQR from 0 to 45 positive samples for sporadic contamination. Because all experimental data fell within the predicted range, the model was considered valid.

Outcomes and Accomplishments

- This project developed an in-field produce preharvest sampling simulation model based on a generic 2D food safety sampling simulation model.
- The preharvest sampling simulation model was validated against data in the academic literature – Objective 3. Specifically, the model accurately reproduced results from Xu and Buchanan (2019) for contamination in an 18 x 15 m^2 field with 1–10 contamination points, and results for taking 30 grab-sample composites by random, stratified random, and systematic sampling. Our model reproduced trends in median detection probability and variance for both random and stratified random sampling. Our model simulated a different convenience sampling pattern (systematic, serpentine sampling, rather than Z-pattern sampling) and also found increased variance with convenience sampling.
- The sampling simulation was validated against newly collected inoculated field trial data – Objective 4. Specifically, in this work, two fields each were inoculated for conditions representing a point-source, systematic, or sporadic contamination using rifampicin-resistant *E. coli* and sampled by high-resolution stratified random sampling. Our model simulated results of these six inoculated field trials and reproduced ranges of plausible positive samples that, in all cases, included the observed number of positive samples.
- The project used the model to simulate sampling a generic 1-acre field by simple random sampling (SRS), stratified random sampling (STRS), and systematic sampling (SS) for detection of point-source, systematic, and sporadic contamination. Results found that for major systematic contamination the sampling pattern does not matter to detection probability, but that the total sample mass has a large impact on detection. Conversely, for point-source detection, randomized sampling is more powerful, and likely requires taking more grab samples than the current 60-sample composites. *This outcome was an additional achievement outside the scope of the original objectives.*
- The project modeled fields representative of three major produce commodities in CA, AZ, NY, and VA, subject to contamination realistic to those locations, and sampling plans appropriate to those commodities and consistent with current industry best practices (if applicable). These representative fields are characterized systematically by mathematical descriptions of input parameters. This was the goal of Objective 1.
- The project then used the representative fields to simulate sampling, including simple random sampling, stratified random sampling, and systematic sampling appropriate to each field, and a range of one, five, or 10 composite samples per field. Results for individual simulations are collected in a series of sampling plan performance tables. Broadly, results found that existing composite sampling can detect systematic failures. In contrast, composite sampling will not likely detect point-source contamination, even at 5 to 1- fold more sampling than is currently used. This was the goal of Objective 2.
- The project developed the sampling simulation as a publicly available web application in the open-source language RShinny. Currently the application is hosted by Shinnyapp.io and available at the URL https://go.illinois.edu/foodsafetysampling2 which redirects to the hosting server. When the current contract expires for professional hosting, the PI will ensure the sample URL redirects to an Illinois-based server host for continued stakeholder access.

Summary of Findings and Recommendations

Simulation can provide valid results for in-field microbiological sampling. The model was considered valid because it was used to simulate experiments in both existing academic literature and newly collected inoculated field trial data, and in both cases the model results gave confidence intervals that included experimental results. This validation justified use of the model for studying the power of low prevalence, low concentration contamination scenarios in industry-relevant 1-acre and larger fields.

Results confirmed that randomized sampling plans such as simple random sampling (SRS) and stratified random sampling (STRS) outperform convenience sampling plans (here we evaluated systematic, serpentine sampling) by having lower variance in the power to detect point source hazards.

Current industry sampling practices are likely powerful enough to reliably detect large-scale, systematic contamination events. A generic 1-acre field simulation found that a 60 random sample composite of 3-g grabs would almost always detect uniform contamination at −1 log(CFU/g) (equivalent to 1 CFU per 100 g of product).

- Conversely, a lower-concentration systematic contamination of −4 log(CFU/g) (equivalent to 1 CFU per 10 kg of product) was rarely detected.
- Representative systematic contamination events, including contaminated irrigation water and fertilization with improperly composted manure, were modeled with intermediate levels of −3 log(CFU/g) (0.5 CFU per lb) and −0.7 log(CFU/g) (1 CFU per 5 g), with intermediate detection probabilities.

Current industry sampling practices are <u>not</u> likely powerful enough to reliably detect point-source contamination events. A generic 1-acre field simulation found that a 60 random sample composite of 3-g grabs would fail 80% of the time to detect contamination from a fecal pellet with a 1.9-m radius spread.

- Representative field simulation for CA, AZ, and NY confirm this failure to detect.
- Representative field simulation for VA tomatoes, where point sources of contamination have been shown to have little spread, found very little detection of point-source contamination.

Current industry sampling practices are <u>not</u> likely to detect sporadic contamination from endemic soil bacteria at very low levels (defined as −5 log(CFU/g) (1 CFU per 100 kg). Therefore, preharvest false positives are not likely.

These three points, taken together, suggest that currently sampling practices are primarily useful to verify lack of major food safety failures.

- Further, it is not likely possible to test individual fields for safety without significant, likely impractical, increases in sample sizes, OR alternative sampling technologies.

APPENDICES

Publications and Presentations

Publications

A manuscript discussing the findings for this entire project will be submitted with the working title of: An experimentally validated simulation model for in-field produce safety sampling to characterize the power of sampling plans to detect realistically low prevalence and level foodborne pathogens. Our goal is to target Applied and Environmental Microbiology with the rough outline of: Produce field simulation model results for a generic field, simulation model literature validation, inoculated field trial validation, then commercially representative fields.

Presentations

Quintanilla-Portillo, J., Belias, A., Cheng, X., Weller, D., Wiedmann, M., Stasiewicz MJ. 2020. Validation of an in-field produce sampling simulation using experimental data. International Association for Food Protection Annual Meeting. Poster Presentation. October, 2020.

Stasiewicz MJ. 2020. Simulation analysis of in-field produce sampling for risk-based sampling plan development. Center for Produce Safety Annual Research Symposium Webinar Series I. Oral Presentation. June, 2020.

Budget Summary

Total funds award to this project were $249,210. All funds will be expended by the end of the budget term. The primary expense for the project was graduate student stipends to carry out the representative field parameterization and modelling (Objectives 1–2), and validation using literature data (Objective 3) and inoculated field trial data (Objective 4, largely the subcontract to Cornell University). Other major expenses included the materials and supplies for the inoculated field trials, travel to required CPS Research Symposium for reporting in 2019, and funds for the Shinnyapp.io hosting of the sampling application for the first year of the project. The team feels they had adequate funds to fully implement the project, and appreciates the flexibility of CPS to accommodate a no-cost extension to recover from COVID-19-related delays.

Tables A–P and Figures A–J

Table A. Representative Fields – California/Arizona produce fields parameters

California and Arizona				
	Description	Iceberg Lettuce	Spinach	Romaine Lettuce
Field Input/ Parameter		Value	Value	Value
Field dimensions	Area Length x Width (m)	2.2 acres 200 x 45 m	12.5 acre 200 x 256 m	5.14 acre 200 x 104 m
Beds	Defined space where produce grow/ Field division for sampling	10 beds/acre	11 beds/acre	6 beds/acre
Space between rows	Distance between two rows of produce (including drainage) (m & inches)	0.76m 30 inches	0.38m 15 inches	0.76m 30 inches
Field Contamination Scenarios				
Point Source (point)	Contamination focused on a specific area (r= 1.9 m)	-0.053 LogCFU/g (sd: 0.1)	0.69 LogCFU/g (sd:0.1)	0.311 LogCFU/g (sd: 0.1)
Systematic (Area)	Contamination across entire field with irrigation water	-1.958 LogCFU/g (sd: 0.1)		
Sporadic (area)	Low level contamination/ Endemic soil bacteria across the field	-5.66 LogCFU/g (sd:0.1)		
Sampling				
Sample Size	Total number of samples/composite samples collected	1 n60	1 n60	1 n60
Sample mass	Weight of individual samples (g)	300 (5g per grab)	150 (2.5g per grab)	300 (5g per grab)
Stratified Random Sampling (STRS)	Sampling the field in pre-defined strata	2D(20(x),3(y)) in field: 3 samples per bed	2D (20(x),3(y)) in field: 3 samples taken across 7 beds	2D (20(x),3(y)) in field: 3 samples across two beds
Systematic Sampling (SS)	Sampling the field in specific order	Sampling at every k-row	Sampling at every k-row	Sampling at every k-row

Table B. Representative Fields Simulation Summary – Iceberg Lettuce in California and Arizona

State	Commodity Field (acres, L x W (m))	Contamination Scenario	Contamination Sites	Contamination Load (logCFU/g)	Affected Area	Generic Sampling Strategy	Composite Sample Mass (g)	Probability of Acceptance (median %, CI 5%-95%)		
								1 composite	5 composites	10 composites
CA	Iceberg Lettuce (2.2, 200 x 45)	Point Source	1	-0.053 (sd:0.1)	11.34m^2 (r=1.9 m)	Stratified Random Sampling	300	91 (90-92)	66 (64-75)	45 (44-56)
						Random Sampling	300	90 (90-92)	68 (66-75)	45 (44-58)
						Systematic Sampling	300	99 (32-99)	98 (28-98)	2 (0-94)
		Systematic failure	1	-1.958 (sd:0.1)	10% of the Field	Stratified Random Sampling	300	74 (65-86)	25 (12-22)	6 (1-22)
						Random Sampling	300	76 (65-87)	25 (12-47)	6 (2-23)
						Systematic Sampling	300	63 (44-95)	23 (10-45)	5 (2-23)
		Sporadic	1	-5.66 (sd:0.1)	Entire Field	Stratified Random Sampling	300	99 (99-99)	99 (80-99)	99 (85-99)
						Random Sampling	300	99 (99-99)	98 (98-98)	97 (97-98)
						Systematic Sampling	300	99 (99-99)	98 (97-98)	96 (95-96)

Table C. Representative Fields Simulation Summary – Spinach in California and Arizona

State	Commodity Field (acres, L x W (m))	Contamination Scenario	Contamination Sites	Contamination Load (logCFU/g)	Affected Area	Generic Sampling Strategy	Composite Sample Mass (g)	Probability of Acceptance (median %, CI 5%-95%)		
								1 composite	5 composites	10 composites
CA	Spinach (12.5, 200 x 252.93)	Point Source	1	0.69 (sd:0.1)	11.34m^2 (r=1.9 m)	Stratified Random Sampling	150	98 (97-98)	93 (92-94)	86 (85-87)
						Random Sampling	150	98 (98-98)	92 (91-93)	87 (85-87)
						Systematic Sampling	150	99 (49-99)	99 (41-99)	98 (42-99)
		Systematic failure	1	-1.958 (sd:0.1)	10% of the field	Stratified Random Sampling	150	86 (79-92)	45 (34-69)	23 (11-53)
						Random Sampling	150	86 (79-92)	47 (33-69)	22 (12-50)
						Systematic Sampling	150	86 (79-92)	47 (32-67)	20 (20-45)
		Sporadic	1	-5.66 (sd: 0.1)	Entire Field	Stratified Random Sampling	150	99 (99-99)	99 (99-99)	98 (98-98)
						Random Sampling	150	99 (99-99)	99 (99-99)	98 (98-98)
						Systematic Sampling	150	99 (99-99)	99 (99-99)	99 (99-99)

Table D. Representative Fields Simulation Summary – Romaine Lettuce in California and Arizona

State	Commodity Field (acres, L x W (m))	Contamination Scenario	Contamination Sites	Contamination Load (logCFU/g)	Affected Area	Generic Sampling Strategy	Composite Sample Mass (g)	Probability of Acceptance (median %, CI 5%-95%)		
								1 composite	5 composites	10 composites
CA	Romaine Lettuce (5.14, 200 x 104)	Point Source	1	0.311 (sd:0.1)	11.34m^2 (r=1.9 m)	Stratified Random Sampling	300	95 (95-95)	84 (82-85)	69 (66-76)
						Random Sampling	300	95 (94-96)	85 (84-88)	69 (67-72)
						Systematic Sampling	300	99 (99-99)	98 (32-99)	76 (1-97)
		Systematic failure	1	-1.958 (sd:0.1)	10% of the field	Stratified Random Sampling	300	73 (62-87)	19 (9-47)	3 (1-21)
						Random Sampling	300	74 (63-86)	22 (11-51)	5 (1-23)
						Systematic Sampling	300	73 (60-85)	20 (8-49)	5 (1-22)
		Sporadic	1	-5.66 (sd:0.1)	Entire Field	Stratified Random Sampling	300	99 (99-99)	99 (99-99)	98 (98-98)
						Random Sampling	300	99 (99-99)	99 (99-99)	97 (97-98)
						Systematic Sampling	300	99 (99-99)	99 (99-99)	98 (98-98)

Table E. Representative Fields – New York produce field parameters

New York				
Field Input/ Parameter	Description	Cabbage Value	Lettuce Value	Broccoli Value
Field dimensions	Area Length x Width (m)(ft)	1 acre 152.4 x 26.58 m 500 x 87 ft	1 acre 152.4 x 26.58 m 500 x 87 ft	1 acre 152.4 x 26.58 m 500 x 87 ft
Rows	Defined space where produce grow/ Field division for sampling	35 rows [13]	70 rows [14]	35 rows [13]
Space between rows	Distance between two rows of produce (including drainage) (m & inches)	0.76 m [13] 30 inches	0.38 m [14] 15 inches	0.76 m [13] 30 inches
Field Contamination Scenarios				
Point Source (point)	Contamination from fecal pellet focused on a specific area (r= 1.9 m)	3.61 ± 1.38 LogCFU/ml [15]		
Systematic (Area)	Contamination across large portions of the field with untreated manure/slurry	0.33 ± 0.24 LogCFU/10g [18]		
Sporadic (area)	Low level contamination/ Minimum compliance with Produce Safety Rule	< 0 LogCFU/g (-5 LogCFU/g $\pm$ 1) [32]		
Sampling				
Sample Size	Total number of samples/composite samples collected	1 n60	1 n60	1 n60
Sample mass	Weight of individual samples (g)	300 (5g per grab)	300 (5g per grab)	300 (5g per grab)
Stratified Random Sampling (STRS)	Sampling the field in pre-defined strata	2D (30(x),2(y)) in field: 1-2 samples per row	2D (60(x),1(y)) in field: 0-1 samples per row	2D (30(x),2(y)) in field: 1-2 samples per row
Systematic Sampling (SS)	Sampling the field in specific order	Sampling at every row	Sampling at every row	Sampling at every row

Table F. Representative Fields Simulation Summary – Cabbage and broccoli in New York

State	Commodity Field (acres, L x W (m))	Contamination Scenario	Contamination Sites	Contamination Load (logCFU/g)	Affected Area	Generic Sampling Strategy	Composite Sample Mass (g)	Probability of Acceptance (median %, CI 2.5%-97.5%)		
								1 composite	5 composites	10 composites
NY	Cabbage and Broccoli (1 acre, 152.48 x 26.58)	Point Source	1	2.30 (sd=1.38)	11.34m^2 (r=1.9 m)	Stratified Random Sampling	300	86 (85-88)	44 (39-57)	17 (16-32)
						Random Sampling	300	84 (82-88)	44 (39-56)	18 (16-34)
						Systematic Sampling	300	100 (63-100)	13 (0-82)	0 (0-0)
		Systematic failure	1	-0.67 (sd 0.24)	Entire Field	Stratified Random Sampling	300	0 (0-0)	0 (0-0)	0 (0-0)
						Random Sampling	300	0 (0-0)	0 (0-0)	0 (0-0)
						Systematic Sampling	300	0 (0-0)	0 (0-0)	0 (0-0)
		Sporadic	1	-5 (sd: 1)	Entire Field	Stratified Random Sampling	300	99 (63-99)	98 (25-98)	96 (20-97)
						Random Sampling	300	99 (63-99)	97 (25-97)	96 (20-97)
						Systematic Sampling	300	99 (66-99)	97 (25-97)	94 (20-95)

Table G. Representative Fields Simulation Summary – Lettuce in New York

State	Commodity Field (acres, L x W (m))	Contamination Scenario	Contamination Sites	Contamination Load (logCFU/g)	Affected Area	Generic Sampling Strategy	Composite Sample Mass (g)	Probability of Acceptance (median %, CI 5%-95%)		
								1 composite	5 composites	10 composites
NY	Lettuce (1 acre, 152.48 x 26.58)	Point Source	1	2.33 (sd=1.38)	11.34m^2 (r=1.9 m)	Stratified Random Sampling	300	85 (84-88)	43 (40-60)	18 (17-35)
						Random Sampling	300	88 (83-92)	50 (41-59)	25 (19-49)
						Systematic Sampling	300	100 (60-100)	13 (0-82)	0 (0-0)
		Systematic failure	1	-0.67 (sd; 0.24)	Entire Field	Stratified Random Sampling	300	0 (0-0)	0 (0-0)	0 (0-0)
						Random Sampling	300	0 (0-0)	0 (0-0)	0 (0-0)
						Systematic Sampling	300	0 (0-0)	0 (0-0)	0 (0-0)
		Sporadic	1	-5 (sd: 0.0001)	Entire Field	Stratified Random Sampling	300	99 (64-99)	97 (25-98)	95 (20-97)
						Random Sampling	300	99 (64-99)	97 (25-98)	95 (20-97)
						Systematic Sampling	300	99 (66-99)	97 (25-98)	95 (20-97)

Table H. Representative Fields – Virginia produce field parameters

Virginia				
Field Input/ Parameter	Description	Tomatoes Value	Crisphead Lettuce Value	Cabbage Value
Field dimensions	Area Length x Width (m)(ft)	1 acre 152.4 x 26.58 m 500 x 87 ft	1 acre 152.4 x 26.58 m 500 x 87 ft	1 acre 152.4 x 26.58 m 500 x 87 ft
Rows/Beds	Defined space where produce grow/ Field division for sampling	17 rows	35 rows	29 rows
Columns	Stake Culture	Stakes every 5 ft	NA	NA
Space between rows	Distance between two rows of produce (including drainage) (m & inches)	0.91 m [33] 3 ft	0.45 m [33] 18 inches	0.53 m [33] 21 inches
Field Contamination Scenarios				
Point Source (point)	Contamination from fecal pellet focused on a specific area	-1.14 - -0.14 Log [26] CFU/g (r=0.03m)	-1.79 - -0.79 [26] LogCFU/g (r=0.075 m)	-1.975 - -0.975 [26] LogCFU/g (r=0.08 m)
Systematic (Area)	Contamination across large portions of the field with contaminated irrigation water	-1.92 ± 1.858 Log CFU/g [11]		
Sporadic (area)	Low level contamination i.e. endemic soil bacteria across the field	-5 ± 1 LogCFU/g		
Sampling				
Sample Size	Total number of samples/composite samples collected	1 n6 [28]	1 n60	1 n60
Sample mass	Weight of individual samples (g)	Tomato fruit (200 g)	300 (5g per grab)	300 (5g per grab)
Stratified Random Sampling (STRS)	Sampling the field in pre-defined strata	2D (1(x),6(y)) in field: 6 tomatoes across field	2D (30(x),2(y)) in field: 0-1 samples per row	2D (30(x),2(y)) in field: 1-2 samples per row
Systematic Sampling (SS)	Sampling the field in specific order	Sampling in between stakes	Sampling at every row	Sampling at every row

Table I. Representative Fields Simulation Summary – Tomatoes in Virginia

State	Commodity Field (acres, L x W (m))	Contamination Scenario	Contamination Sites	Contamination Load (logCFU/g)	Affected Area	Generic Sampling Strategy	Composite Sample Mass (g)	Probability of Acceptance (median %, CI 2.5%-97.5%)		
								1 composite	5 composites	10 composites
VA	Tomatoes (1 acre, 152.48 x 26.58)	Point Source	1	-1.14 - -0.14	Single tomato (r=0.03m)	Stratified Random Sampling	1200	98 (98-98)	94 (93-94)	88 (87-88)
						Random Sampling	1200	98 (98-98)	94 (93-95)	89 (87-90)
						Systematic Sampling	1200	98 (98-99)	94 (94-95)	89 (88-90)
		Systematic failure	1	-1.92 (sd 1.858)	Entire Field	Stratified Random Sampling	1200	17 (0-76)	0 (0-24)	0 (0-5)
						Random Sampling	1200	18 (0-77)	0 (0-26)	0 (0-7)
						Systematic Sampling	1200	15 (0-76)	0 (0-27)	0 (0-5)
		Sporadic	1	-5 (sd: 1)	Entire Field	Stratified Random Sampling	1200	97 (26-98)	90 (19-94)	83 (15-88)
						Random Sampling	1200	97 (26-98)	90 (19-93)	84 (15-89)
						Systematic Sampling	1200	97 (27-98)	90 (19-94)	82 (15-87)

Table J. Representative Fields Simulation Summary – Lettuce in Virginia

State	Commodity Field (acres, L x W (m))	Contamination Scenario	Contamination Sites	Contamination Load (logCFU/g)	Affected Area	Generic Sampling Strategy	Composite Sample Mass (g)	Probability of Acceptance (median %, CI 2.5%-97.5%)		
								1 composite	5 composites	10 composites
VA	Lettuce (1 acre, 152.48 x 26.58)	Point Source	1	-1.79 - -0.79	Single head (r=0.075 m)	Stratified Random Sampling	300	99 (99-99)	98 (98-99)	97 (97-98)
						Random Sampling	300	99 (99-99)	98 (98-98)	97 (96-98)
						Systematic Sampling	300	99 (99-99)	98 (97-98)	97 (97-97)
		Systematic failure	1	-1.92 (sd 1.858)	Entire Field	Stratified Random Sampling	300	43 (0-93)	12 (0-72)	5 (0-49)
						Random Sampling	300	43 (0-93)	13 (0-70)	5 (0-52)
						Systematic Sampling	300	43 (0-93)	15 (0-70)	5 (0-51)
		Sporadic	1	-5 (sd: 1)	Entire Field	Stratified Random Sampling	300	99 (65-99)	98 (25-99)	96 (20-97)
						Random Sampling	300	99 (63-99)	98 (26-99)	96 (21-97)
						Systematic Sampling	300	99 (63-99)	98 (25-99)	96 (20-97)

Table K. Representative Fields Simulation Summary – Cabbage in Virginia

State	Commodity Field (acres, L x W (m))	Contamination Scenario	Contamination Sites	Contamination Load (logCFU/g)	Affected Area	Generic Sampling Strategy	Composite Sample Mass (g)	Probability of Acceptance (median %, CI 2.5%-97.5%)		
								1 composite	5 composites	10 composites
VA	Cabbage (1 acre, 152.48 x 26.58)	Point Source	1	-1.975 - -0.975	Single head (r=0.08 m)	Stratified Random Sampling	300	99 (99-99)	98 (98-99)	97 (97-98)
						Random Sampling	300	99 (99-99)	98 (98-98)	97 (97-98)
						Systematic Sampling	300	99 (99-99)	98 (97-98)	96 (95-97)
		Systematic failure	1	-1.92 (sd 1.858)	Entire Field	Stratified Random Sampling	300	42 (0-93)	15 (0-72)	5 (0-51)
						Random Sampling	300	42 (0-94)	15 (0-69)	5 (0-52)
						Systematic Sampling	300	42 (0-94)	15 (0-72)	5 (0-52)
		Sporadic	1	-5 (sd: 1)	Entire Field	Stratified Random Sampling	300	99 (65-99)	97 (26-98)	94 (20-97)
						Random Sampling	300	99 (64-99)	97 (25-98)	95 (20-96)
						Systematic Sampling	300	99 (65-99)	97 (25-98)	95 (20-96)

Table L. Simulation model input variable description.

Variable	Description	Value range / Distribution	Default value	Unit	Source
x_lim, y_lim	Dimensions of the field (length, width)	> 0	1, 1	m	User input
$geom$	Contamination geometry	point, area	point		User input
n_contam	Number of contamination points (only applicable when $geom$ = 'point')	positive integer	3		User input
$cont\_level$	Contamination level	log normal distribution (μ, σ)	μ = 3, σ = 1	Log CFU/g	Assumed
bg_level	Background level	> 0	0.00001	CFU/g	Assumed
$spread\_radius$	Spreading radius	> 0	1	m	Assumed
LOC	Limit of contamination contribution (modifying decay function shape)	0 ~ 1	0.001		Assumed
fun	Decay function of contamination level	exp, norm, unif	exp		User input
$method\_sp$	Sampling strategy	SRS, STRS, SS	SRS		User input
n_sp	Number of samples	5, 10, 15, 20, 30, 60, or other positive integers	60		ICMSF*
n_strata	Number of strata (for STRS)	positive integer	NA		User input
by	Sampling direction (for STRS and SS)	row, column, 2d	NA		User input
m_sp	Individual sample mass	> 0	25	g	ICMSF*
$method\_det$	Detection method	plating, enrichment	enrichment	CFU/g or CFU	User input
$case$	Attribute sampling plans	1 ~ 15	15		ICMSF*
m	Microbial count threshold (low)	≥ 0	0	CFU/g	ICMSF*
M	Microbial count threshold (high)	≥ 0	0	CFU/g	ICMSF*

*[34]

Table M. Simulation model input variable for generic one-acre field

No.	Contamination geometry ($geom$)	Number of contamination points (n_contam)	Contamination level ($cont\_level$) (log CFU/g)	Sample plan	Sampling strategy ($method\_sp$)	Sample mass (m_sp) (g)	Number of iterations per input combination
1	Point ($spread\_radius$ = 1.9 m)	1, 2, 3, 4, 5, 6	μ = 3, σ = 1	Case 14 (n = 30, c = 0)	SRS, STRS (n_strata = 5), SS	25	10,000
2	Area	NA	μ = -3, σ = 1	Case 15 (n = 60, c = 0)	SRS, STRS (n_strata = 5), SS	2.5, 5, 10, 20	10,000
3	Point ($spread\_radius$ = 1.9 m)	1	μ = 3, σ = 1	n_sp = 60, 120, 300, 600, 1200	SRS	3	10,000
4	Area	NA	μ = -4, -3, -2, -1, σ = 1	n_sp = 60, 120, 300, 600, 1200	SRS	3	10,000

Table N. Die-Off model input parameters for simulation in @Risk

Cell	Variable	Input Distribution Formula						Unit	Source
		PS1	PS2	SS1	SS2	SP1	SP2		
A1	Sprayed Concentration	=@Risk Normal (5.12, 0.1)	=@Risk Normal (5.15, 0.1)	=@Risk Normal(2.914, 0.339)	=@Risk Normal (3.13,0.05)	=@Risk Normal(2.914,0.339)	=@Risk Normal (3.13,0.05)	Log CFU/ml	This study
A2	Log Reduction in adhesion	=@RiskNormal(-2.975, 0.1)						LogCFU/plant	Calculated
A3	Concentration Adhered	=A1+A2						LogCFU/plant	Calculated
A4	Transformed Break Point	=RiskExtValue(-0.45175, 0.26206(RiskCorrmat(breakpoint_segments))						Log Days	[31]
A5	Break Point	=10^A4						Days	Calculated
A6	Transformed Segment 1 die off rate	=RiskTriang(-0.9704, 1.0179, 1.0179,(RiskCorrmat(breakpoint_segments))						Log CFU/day	[31]
A7	Segment 1 die off rate	= -1*10^A6						Log CFU/day	Calculated
A8	Transformed Segment 2 die off rate	=RiskNormal(-0.17916, 0.20036,(RiskCorrmat(breakpoint_segments))						Log CFU/g	[31]
A9	Segment 2 die off rate	= (10^A8)-1						Log CFU/day	Calculated
A10	Time following inoculation	1	1	1	1	6	5	days	User input
A11	Log Reduction at day of sampling	=IF(A11>A5, ((A7*A5) + (A10(A11-A5)), (A11*A7))						LogCFU	Calculated
A12	Concentration at day of Sampling	=A1 + A2 + A12						LogCFU/plant	

Table O. Spearman correlation matrix for Die-Off rates and breakpoint in simulation model

@Risk Correlations	Segment 1	Segment 2	Break Point
Segment 1	1		
Segment 2	-0.19	1	
Break Point	-0.431	0.593	1

Table P. Die-Off simulation model outputs and fitted distribution formula

Field Trial	Output Distribution Formula	Minimum	Maximum	2.5%	Median	97.5%	Mean	SD
PS1	@RiskExtvalueMin (0.98718, 0.9307)	-7.947	2.512	-3.374	0.747	1.987	0.401	1.409
PS2	@RiskExtvalueMin (1.0153, 0.92847)	-7.894	2.680	-2.266	0.781	1.907	0.429	1.409
SS1	@RiskExtvalueMin (-1.19, 1.0076)	-9.961	0.902	-5.825	-1.484	0.0117	-1.806	1.451
SS2	@RiskExtvalueMin (-0.99386, 0.92748)	-9.848	0.563	-5.374	-1.240	-0.00471	-1.580	1.409
SP1	@RiskNormal (-3.303, 2.093)	-9.987	8.012	-7.220	-3.434	1.232	-3.3030	2.0930
SP2	@RiskNormal (-2.8047, 1.8444)	-10.0	5.605	-6.607	-2.820	0.861	-2.805	1.8444

Figure A. schematic diagram of the simulation process

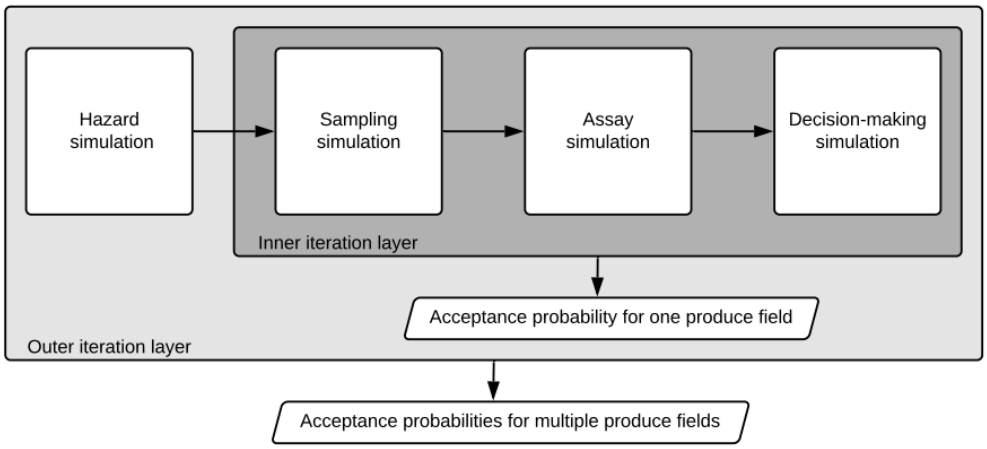

Fig. A. The schematic diagram of the simulation process adapted from Cheng and Stasiewicz, 2021; (1) simulating point- or area-based contamination in a produce field; (2) taking samples y a specific sampling strategy; (3) assaying the samples; (4) accepting or rejecting the field. The inner iteration layer produces an acceptance probability for one produce field and the outer iteration layer produces multiple acceptance probabilities, each corresponding to a different field.

Figure B. Theoretical probabilities of acceptance between sampling strategies one-acre generic fields

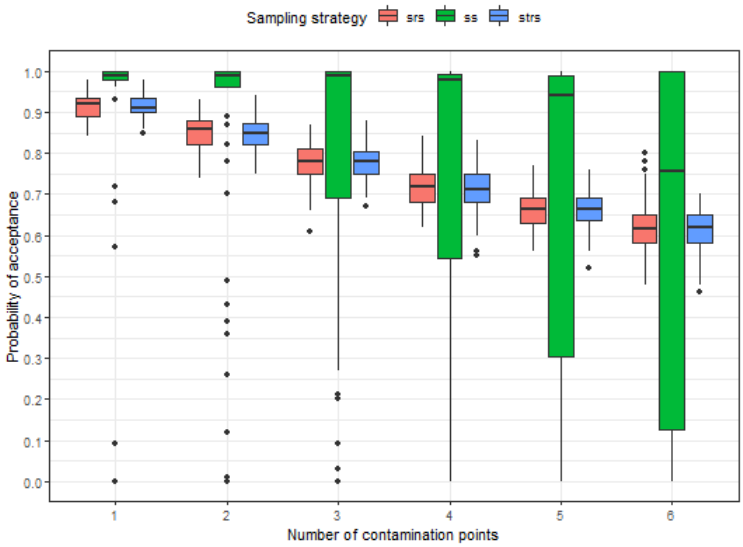

Fig. B. Probability of acceptance between three sampling strategies (SRS, STRS, k-step SS) in a point-source contamination scenario in a 1-acre field. The effects of 1 – 6 contamination sites (equivalent to prevalence level from 0.3% to 1.7%) were investigated. Each contaminated site's microbial concentration followed a normal distribution with mean at 3 log CFU/g and standard deviation at 1 log CFU/g. The N60 sampling plan was adopted and each sample was 25 g. Each scenario was iterated for 10,000 times.

Figure C. Probability of acceptance between sampling strategies and total sample mass

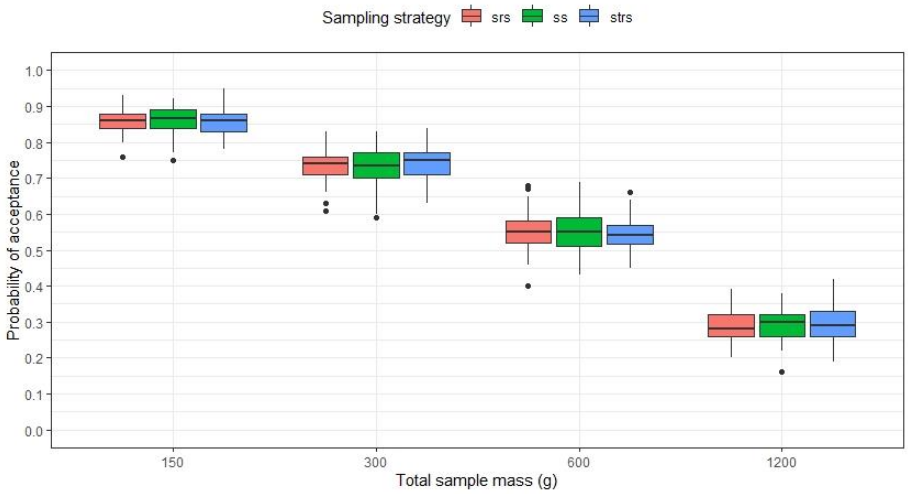

Fig. C. Probability of acceptance between three sampling strategies (SRS, STRS, k-step SS) in a systematic contamination scenario. The entire 1-acre field was contaminated with -3 log CFU/g of pathogen and the N60 sampling plan was adopted with different total sample masses (150, 300, 600, 1200 g). Each scenario was iterated for 10,000 times

Figure D. Theoretical probabilities of acceptance in contaminated one-acre generic fields

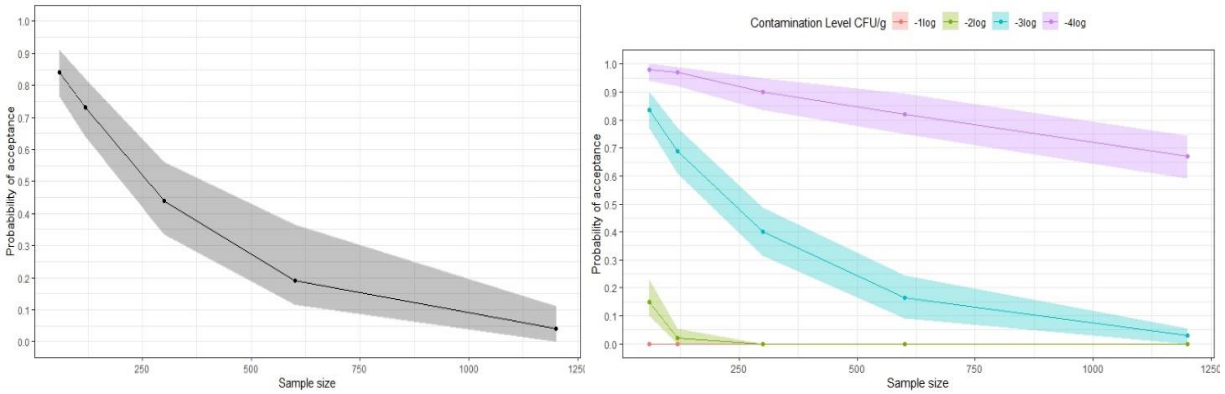

Fig. D. (Left) Theoretical probabilities of acceptance presented as medians and 2.5th – 97.5th quantile intervals for scenarios with a single fecal contamination and different composite sample sizes (60-1200) in one-acre generic fields**. (Right)** Probabilities of acceptance presented as medians and 2.5th – 97.5th quantile intervals with product uniformly contaminated with low level to high level contamination, and different composite sample sizes (60-1200) in one-acre generic fields. Sampling is performed by simple random sampling and each scenario is iterated for 10,000 times

Figure E. Simulation model validation against literature outcome

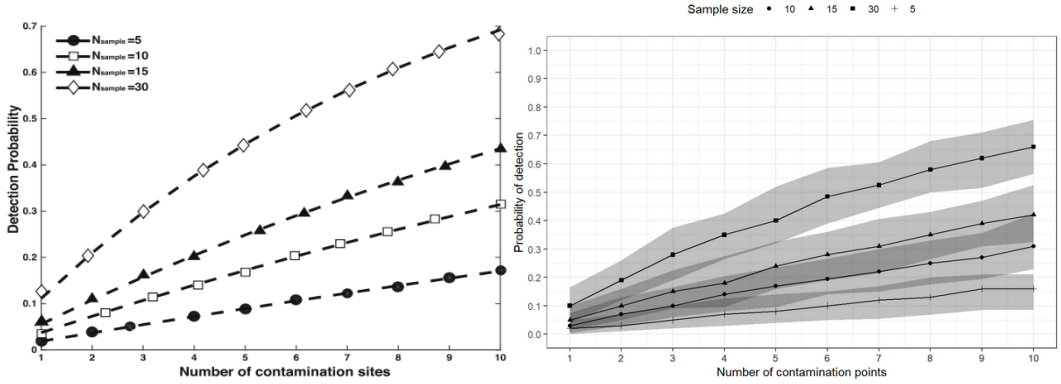

Fig. E. (Left) Validation of the simulation model with literature data (Xu and Buchanan, 2019) where the number of contamination points ranged from 1 to 10 in an 18 x 15 m^2 field (equivalent to prevalence level from 0.03% to 3.7%). SRS was implemented with 5, 10, 15, and 30 samples and each scenario was iterated with 10,000 times. Each point in the curve represents the median detection probability and the shade area represents the range between 2.5th and 97.5th percentile. **(Right)** Figure of theoretical detection probability retrieved directly from Xu and Buchanan, 2019.

Figure F. Simulation model validation against literature outcome

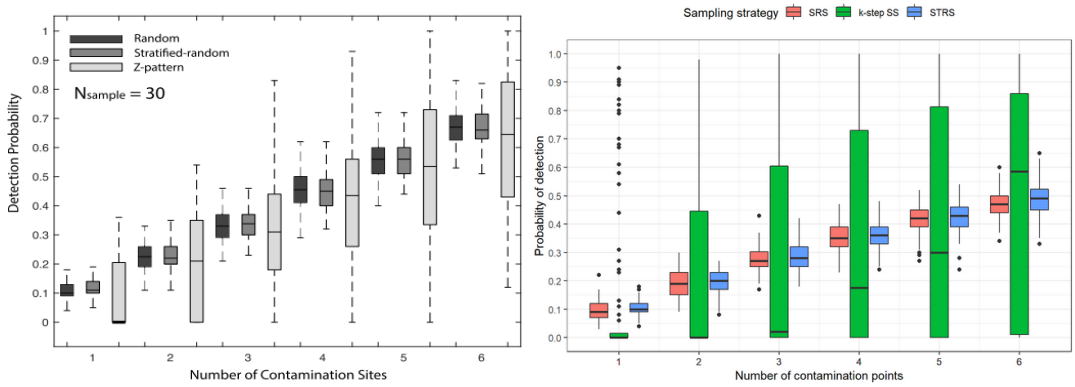

Fig. F. (Left) Detection probability versus number of contamination sites (1-6) with three sampling strategies (SRS, STRS, Z-pattern). Sample size is fixed at 30 (Xu and Buchanan, 2019). **(Right)** Probabilities of detection with a series of contamination points (1-6), a fixed sample size (30), and three sampling strategies (SRS, STRS, k-step SS). Sample size is fixed at 30

Figure G. Simulation pattern for a six-day die-off following inoculation

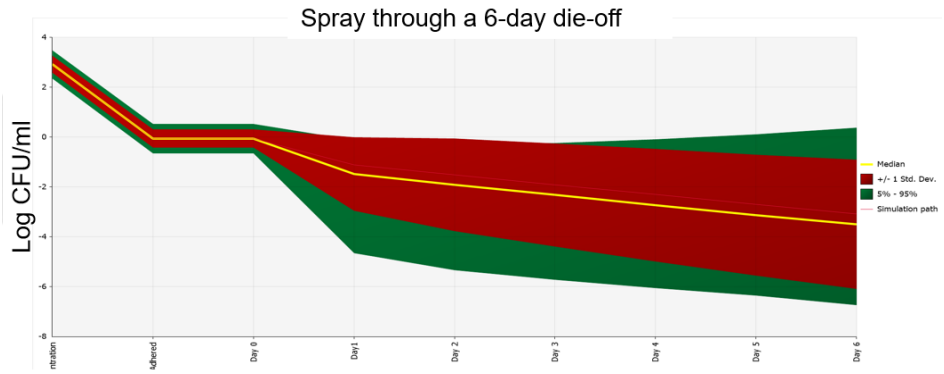

Fig. G. Six-day die-off simulation model. The graph shows the drastic reduction in bacterial concentration primarily due to adhesion parameter tuning, and the increasing variability as function of time.

Figure H. Point Source Contamination – Field trials and Simulation Validation

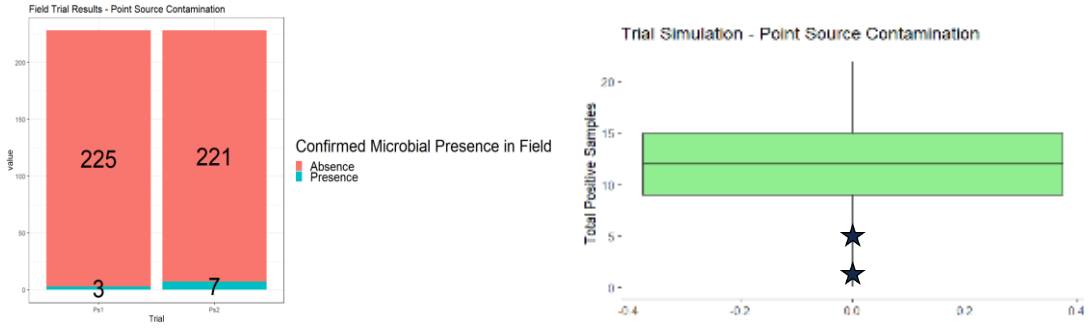

Fig. H. Point Source Contamination: Field trials and Simulation results comparison. Stars on the Simulation graph represent number of positive samples observed in the field trials

Figure I. Systematic Contamination – Field trials and Simulation Validation

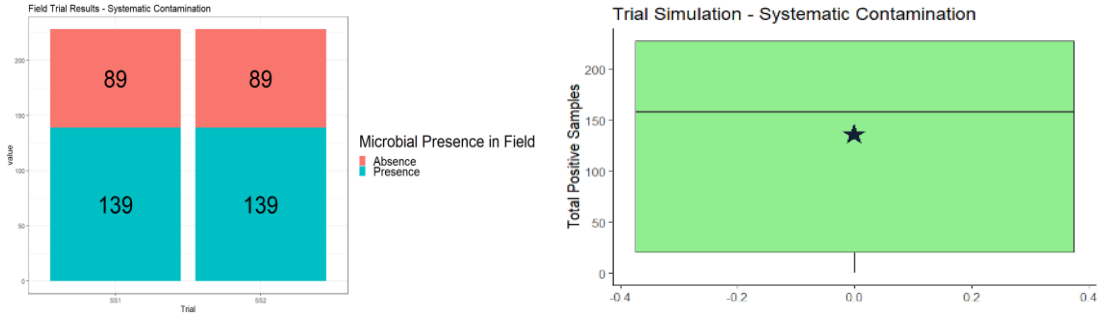

Fig. I. Systematic Contamination: Field trials and Simulation results comparison. Stars on the Simulation graph represent number of positive samples observed in the field trials

Figure J. Sporadic Contamination – Field trials and Simulation Validation

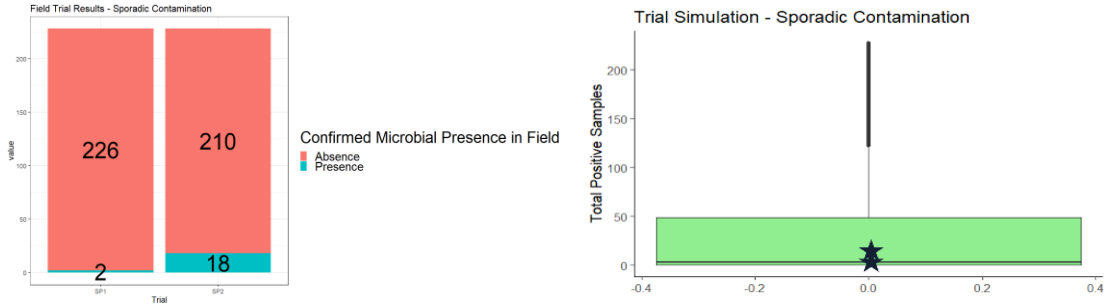

Fig. J. Sporadic Contamination: Field trials and Simulation results comparison. Stars on the Simulation graph represent number of positive samples observed in the field trials

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