CENTER FOR PRODUCE SAFETY

CPS 2023 RFP – FINAL PROJECT REPORT

Project Title:
Testing wetting agents for soil drag and bootie swabs and validating them in varied agricultural soils

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Objectives:

1. Test increasingly practical wetting agents for drag and bootie swabs.
2. Validate optimal wetting agent for the drag and bootie swabs on varying soil created by various stages of ground preparation for different commodities.

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FINAL REPORT

Summary of Findings and Recommendations

This work focuses on optimizing wetting agents for drags and booties used in collecting soil samples to improve preharvest produce safety management.

First, to identify practical wetting agents for drag and bootie swabs, five increasingly practical wetting agents were evaluated: skim milk, tryptic soy broth, buffered peptone water, phosphate buffered saline, and deionized water. A total of 484 samples were collected from fallow, row-crop fields where untreated animal manure was applied, and samples tested for aerobic bacteria, total coliforms, and generic *Escherichia coli.* Applying untreated animal manure was necessary to ensure recoverable levels of typically rare fecal indicator organisms. The results suggested that variations between wetting agents or sampling methods were small and biologically not meaningful (although some statistically significant differences were observed). Therefore, the choice of wetting agent can be guided by practicality, where simple, stable wetting agents like phosphate buffered saline may be preferred.

The next phase was to validate promising wetting agents for collecting soil samples from produce fields. The most promising wetting agents were buffered peptone water and phosphate buffered saline. A total of 240 samples (96 drags, 96 booties, and 48 soil grabs) were collected from a commercial melon farm, a small mixed produce farm (leafy greens, peppers, beets), and an apple orchard. Aerobic bacteria, total coliforms, and *E. coli* were enumerated, and samples were also enriched for *E. coli*. Overall results show little biologically meaningful variation between the performance of buffered peptone water and phosphate buffered saline for detecting any of the indicator microorganisms. Further, in some fields bootie and drag swabs, regardless of wetting agent, recovered higher levels of indicator bacteria than composite soil grabs, and, overall, more booties and drags than soil grabs tested positive for *E. coli*. Microbiome analysis revealed no major differences in diversity between samples collected with the two different hydration buffers.

These data confirm that bootie and drag swabs are a promising alternative to composite soil grab sampling, and that protocols can use whatever wetting agents are most practical.

Abstract

Drags and booties are of interest for produce soil sampling, however the common hydration buffer in animal production, skim milk (SKM), is inappropriate for produce soil sampling use. This study tested alternative wetting agents to replace SKM for hydrating drags and booties and validated their performance across diverse soil types. Tryptic soy broth (TSB), buffered peptone water (BPW), phosphate buffered saline (PBS), and deionized water (DI) were compared to skim milk by sampling in fallow fields with untreated subsurface-injected swine manure and surface-spread dairy manure applied. Aerobic plate counts (APCs), total coliforms, and generic *E. coli* were enumerated from 220 paired drags and booties collected along 100-meter paths. BPW and PBS were selected for validation in a melon farm, a mixed vegetable farm (leafy greens, peppers, apples), and an apple orchard. An additional 96 paired drags and booties were analyzed for the same indicators, with enrichment for generic *E. coli* and 16S V3-V4 microbiome analysis. For wetting agent selection, mean recovery differences across the five wetting agents ranged from 0.0-0.2 log(CFU/g) for APCs, 0.1-0.6 log(CFU/g) for total coliforms, and 0.1-0.4 log(CFU/g) for generic *E. coli*, across fields with both swine and dairy manure application. For validation in produce fields, mean difference of paired samples (BPW–PBS) were 0.14±0.11 and -0.02±0.09 log(CFU/g) for drags and booties, respectively, for APCs, and 0.26±0.07 and -0.02±0.09 log(CFU/g) for

drags and booties, respectively, for total coliforms. Enrichment data showed 88-94% of drags and booties hydrated with PBS or BPW tested positive for generic *E. coli* (no significant differences). Microbiome analysis revealed no major differences in alpha or beta diversity between PBS and BPW hydrated samples. Differences between wetting agents were minor and biologically insignificant, suggesting that wetting agent selection can be guided by compliance requirements and ease of use.

Background

Soil sampling is critical since soil is a potential reservoir of foodborne pathogens in the produce production environment, with potential contamination introduced by irrigation water, manure, animal grazing, municipal soil wastes, etc. The use of hydrated drags and booties in produce production systems represents an advancement over soil grab sampling. Compared to soil grab sampling, which is labor-intensive and less representative of the broader soil environment, hydrated drags and booties have potential to be more practical, efficient, and representative. Therefore, optimizing the use of hydrated drags and booties for soil sampling is essential for monitoring contamination risks and ensuring the safety of fresh produce.

Drags and booties have been used for environmental sampling in food safety research, often with the choice of different wetting agents. In animal production (e.g., poultry litter), skim milk (SKM) is the gold standard to hydrate drags or booties, however using milk in produce fields is problematic because it is allergenic, animal-sourced, and not shelf-stable. Researchers have not yet compared the performance of different wetting agents in hydrating drags or booties for produce safety sampling purpose. This leads to broad questions of interest and relevance to the industry well suited for academic study:

1. What is the optimal wetting agent for drag and bootie swabs? Where the “optimal” balances performance practical issues like avoiding animal-sourced ingredients.
2. How well do drag and bootie swabs hydrated with optimal wetting agents perform in various soils?

To answer the first question, we evaluated four alternative wetting agents to SKM for hydrating drags and booties used in soil sampling. Two of these agents, tryptic soy broth (TSB) and buffered peptone water (BPW), are nutritive wetting agents that require refrigeration to extend shelf life, and could be animal-sourced, but are usually non-allergenic. The other two agents, phosphate buffered saline (PBS) and deionized water (DI), are non-nutritive wetting agents that do not require refrigeration, are non-allergenic, and are not animal-sourced. In addition to evaluate the performance of the wetting agents, we assessed the efficiency and validity of hydrated drags and booties in comparison to grabs, which is a standard soil sampling method.

To answer the second question, PBS and BPW were selected as the optimal wetting agents to be used for the validation stage, to represent one nutritive and one non-nutritive wetting agent from the first objective. Hydrated bootie and drag swabs were brought to commercial fields that grow various commodities (melons, leafy greens, peppers, beets, and apples), to evaluate their performance in comparison to the traditional soil grab method of sampling in a realistic, produce field setting.

Research Methods and Results

Methods for Objective 1

Field set-up. A longitudinal study was performed on research fields at the University of Illinois at Urbana Champaign. Two fields designated for row crop production were selected for sampling. As per standard

practice by the farm, one field had untreated swine manure applied by liquid injection below the ground surface on March 3rd, 2024. The other field had untreated dairy manure applied by dry spreading on the ground surface on April 25th, 2024. Applying untreated animal manure was necessary to ensure recoverable levels of typically rare fecal indicator organisms. A total of 11 sampling visits were performed for this study based on the weather conditions and feasibility of sampling (**Table 1-1**), with 4 visits to the field with untreated swine manure on days 6, 10, 17, and 31 after manure application, and 7 visits to the field with untreated dairy manure on days 0, 2, 4, 9, 14, 24, and 34 after manure application. Since there was no crop present during sampling visits, a unique 100-meter path was randomly selected within each field as the sampling path.

Swab preparation. Drags were prepared as described by Kingston (1981) and Uesugi et al. (2007). The assembly of drags was performed inside of a biosafety cabinet. Each drag was made by tying an autoclaved 1.2-meter cotton string to a 10 cm by 10 cm sterile gauze sponge (DUKAL™ Corporation, Ronkonkoma, NY). The drag was then transferred in its own 710-mL Whirl-Pak bags (Nasco Sampling, Chicago, IL) and UV-disinfected for 30 minutes. Booties, each containing a dry sterile boot swab packaged in a 700-mL bag, were purchased from Romer labs (Newark, DE). The wetting agents used to hydrate drags and booties included SKM (Sigma-Aldrich, St. Louis, MO), TSB (Sigma-Aldrich), BPW (Sigma-Aldrich), PBS (Sigma-Aldrich), and DI. The volumes of wetting agent used to hydrate each drag and bootie were 12 and 18 mL, respectively. Drags and booties were hydrated the night before sampling, refrigerated overnight, and transported to the fields using insulated box with cold packs.

Sample collection. Samples were collected following previously detailed procedures (Micallef et al., 2023; Strawn et al., 2013; Wu et al., 2023). Latex gloves were worn and disinfected using 70% ethanol, changing before collecting each sample. To take soil samples using a drag, one end of cotton string was attached to footwear. The drag was able to swab on the ground while walking along the sampling path. After completing the path, the drag was deposited to its original Whirl-Pak bag. To take soil samples using a bootie, a plastic boot cover was worn and then a hydrated bootie was worn on top of it. The bootie picked up soil each step while walking along the sampling path. After completing the path, the bootie with attached soil was deposited to its original bag. To take composite soil grabs, an aluminum shovel was disinfected using 70% ethanol. Six soil subsamples scooping to a depth of 3 to 5 cm were deposited into a Whirl-Pak bag as one composite grab sample.

To evaluate the wetting agents and validate sampling methods, a group of samples included all drags and booties hydrated with SKM, TSB, BPW, PBS, or DI, and soil grabs taken along the same path. During each visit, 4 replicates of a group of samples were taken, totaling to 44 samples per visit. All samples were transported using the insulated box with cold packs. Upon arrival to the lab, samples were kept refrigerated at 4.2 ± 0.9 °C, and processed within 24 hours (Diekman et al., 2024).

Sample preprocessing. Samples were processed as described by Wu et al. (2023). Upon receiving, the mass of drags, booties, and grabs were recorded. The mass of soil collected using drags was calculated by subtracting the mass of gauze, cotton string, and Whirl-Pak bag (7.2 g) from the mass of drags after sampling, ranging from 5.2 to 17.0 g. The mass of soil collected using bootie was calculated by subtracting the mass of bootie and bag (9.4 g) from the mass of bootie after sampling, ranging from 3.2 to 40.6 g. The mass of soil collected using grabs was calculated by subtracting the mass of Whirl-Pak bag from the grab sample, ranging from 121.2 to 398.2 g. After recording the sample mass, initial dilution was performed for each sample. For drags, 20 mL of PBS was added to each sample which resulted in initial dilution from 1:2.2 to 1:4.8 based on actual soil mass. For booties, 50 mL of PBS was added to each sample which resulted in initial dilution from 1:2.2 to 1:16.6 based on actual soil mass. For grabs, 25 g of soil was weighed into a new Whirl-Pak bag and 100 ml of PBS was added to obtain the initial dilution (1:5).

Enumeration of indicator organisms. The obtained initial diluent was serially diluted in PBS. Standard methods agar (Hardy Diagnostics, Santa Maria, CA) was used to enumerate aerobic bacteria (aerobic plate counts (APCs)). Serial dilutions (100 µL) were spread onto the standard methods agar and incubated at 35 ± 2 °C for 48 h. All colonies were counted. CHROMagar ECC (CHROMagar, Saint-Denis, France) was used to enumerate total coliforms and generic *E. coli*. One mL of initial diluent was plated on ECC to increase the limit of detection (LOD) of the method (Topalcengiz et al., 2023). Serial dilutions (100 µL) were spread onto ECC and incubated 30 ± 2 °C for 20 h. Blue colonies were counted as generic *E. coli* and mauve (purple) colonies were counted as other coliforms per the manufacturer's instructions. Results from colony-forming units (CFU) at given dilutions were used to calculate CFU/g and then log transformed for further analysis.

Data analysis. All samples generated countable APCs and total coliforms results. In the field with untreated swine manure, 152 out of 176 (86%) samples had generic *E. coli* counts below LODs. In the field with untreated dairy manure, 17 out of 308 (5.5%) samples had generic *E. coli* counts below LODs. For the samples below the LODs, the counts were recorded as LODs/2 after log transformation. All counts data were plotted using R (version 4.4.0).

Statistical analyses were performed separately for each field using JMP Pro 17. To compare the wetting agents, the recoveries of APCs, total coliforms, or generic *E. coli* were considered as dependent variables. Sampling days, sample types, and wetting agents were considered as effects to explain the dependent variables. Three statistical models, including main effect model, 2-way interaction model, and 3-way interaction model, were built. The goodness of fit for these models were compared and the main effect model was selected. To compare the performance of sampling methods, data from different wetting agents were pooled for each sample type and indicator organism since no biologically meaningful differences (>1 log(CFU/g) differences) were observed between wetting agents. The pooled data were used to build a main effects model, with the recovery of indicator organisms as the dependent variable and sampling days and sample types as effects. The statistical analysis on the recovery of generic *E. coli* in the field with untreated swine manure was not discussed because 86% samples were below LODs.

Results for Objective 1

The use of different wetting agents for hydrating drags or booties did not result in biologically meaningfully differences in the recovery of APCs, total coliforms, or generic *E. coli*, although small statistically significant differences were observed. In the field with untreated swine manure applied (**Figure 1-1**), die off of APCs, total coliforms, and generic *E. coli* was observed from day 6 to 17. From day 17 to 31, APCs showed minimal changes, total coliforms increased slightly, and generic *E. coli* were below LODs. Drags and booties showed similar overall trends on the recovery of APCs, total coliforms, and generic *E. coli*. The use of various wetting agents for drags or booties did not result in biologically meaningful differences (>1 log(CFU/g) differences) in recovery of indicator organisms.

Statistical analysis for the field with untreated swine manure is shown in **Table 1-2**. Sampling days showed a statistically significant effect on the recovery of APCs ($p < 0.001$) and total coliforms ($p < 0.001$). The mean value of recovery of APCs decreased from 7.2 log(CFU/g) on day 6 to 6.1 log(CFU/g) on day 31. The mean value of recovery of total coliforms decreased from 6.1 log(CFU/g) on day 6 to 5.2 log(CFU/g) on day 31. Sample type also showed a statistically significant effect on the recovery of APCs ($p < 0.001$) and total coliforms ($p < 0.001$). On average, booties recovered 0.1 log(CFU/g) and 0.2 log(CFU/g) more APCs and total coliforms, respectively, compared to drags. Wetting agents showed a statistically significant effect on the recovery of APCs ($p < 0.001$) and total coliforms ($p < 0.001$). For APCs, using BPW as a wetting agent resulted in the highest recovery, with a mean value of 6.6 log(CFU/g), followed closely by TSB (6.5 log(CFU/g)), DI (6.4 log(CFU/g)), PBS (6.4 log(CFU/g)), and SKM (6.4 log(CFU/g)). Drags and booties

hydrated with BPW or TSB showed significantly higher APC recovery compared to those hydrated with DI, PBS, or SKM. For total coliforms, using TSB as a wetting agent resulted in the highest recovery, with a mean value of 5.6 log(CFU/g), followed by BPW (5.5 log(CFU/g)), SKM (5.5 log(CFU/g)), PBS (5.1 log(CFU/g)), and DI (5.0 log(CFU/g)). Drags and booties hydrated with TSB, BPW, or SKM resulted in significantly higher recovery of total coliforms compared to PBS and DI.

In the field with untreated dairy manure (**Figure 1-2**), die off in APCs was observed from day 0 to 34. For total coliforms and generic *E. coli*, although some increases were noted during certain periods, the overall trend showed a decrease over the 34-day period. Both drags and booties exhibited similar patterns in recovering APCs, total coliforms, and generic *E. coli*. The use of various wetting agents for recovery of indicator organisms did not result in biologically meaningful differences between drags and booties results.

Statistical analysis for the field with untreated dairy manure is shown in **Table 1-3**. Sampling days showed a statistically significant effect on the recovery of APCs ($p < 0.001$), total coliforms ($p < 0.001$), and generic *E. coli* ($p < 0.001$). The mean value of recovery of APCs, total coliforms, and generic *E. coli* decreased from 8.6 log(CFU/g), 6.4 log(CFU/g), and 5.8 log(CFU/g) on day 0 to 6.2 log(CFU/g), 4.5 log(CFU/g), and 1.5 log(CFU/g) on day 34, respectively. Sample type also showed a statistically significant effect on the recovery of APCs ($p < 0.001$), total coliforms ($p < 0.001$), and generic *E. coli* ($p < 0.001$). On average, booties recovered 0.1 log(CFU/g), 0.6 log(CFU/g), and 0.6 log(CFU/g) more APCs, total coliforms and generic *E. coli*, respectively, compared to drags. Wetting agents showed a statistically significant effect on the recovery of APCs ($p < 0.001$), total coliforms ($p < 0.001$), and generic *E. coli* ($p < 0.001$). For APCs, using SKM as a wetting agent resulted in the highest recovery, with a mean value of 7.6 log(CFU/g), followed closely by BPW (7.6 log(CFU/g)), TSB (7.6 log(CFU/g)), DI (7.4 log(CFU/g)), and PBS (7.4 log(CFU/g)). Drags and booties hydrated with SKM, BPW, and TSB showed statistically higher recovery of APCs compared to those hydrated with DI and PBS. For total coliforms, TSB performed the best as a wetting agent, with a mean value of 5.8 log(CFU/g), followed by SKM (5.7 log(CFU/g)), BPW (5.7 log(CFU/g)), PBS (5.4 log(CFU/g)), and DI (5.4 log(CFU/g)). Drags and booties hydrated with TSB, SKM, and BPW showed statistically higher recovery of total coliforms compared to those hydrated with PBS and DI. For generic *E. coli*, SKM performed the best as a wetting agent, with a mean value of 3.7 log(CFU/g), followed by BPW (3.6 log(CFU/g)), TSB (3.6 log(CFU/g), DI (3.3 log(CFU/g), and PBS (3.3 log(CFU/g). Drags and booties hydrated with TSB, SKM, and BPW showed statistically higher recovery of generic *E. coli* compared to those hydrated with PBS and DI.

More complex statistical models confirmed that sample types and wetting agents had statistically significant impact on mean recovery of indicator organisms, but likely not biologically meaningful. Similar trends were observed across the main effects model, the 2-way interaction model, and the 3-way interaction model in both fields (**Tables 1-4** & **1-5**). Sampling days, sample types, and wetting agents showed statistically significant effects ($p < 0.001$) on the recovery of indicator organisms. In general, sampling days showed the most significant effect, with p values way smaller than that of sample types and wetting agents. The p values for sample types and wetting agents were similar.

Although statistically significant differences were observed in the 2-way interaction terms and 3-way interaction terms, these models did not substantially improve model fit compared to the main effects model, as indicated by R2 values. In the field with untreated swine manure (**Table 1-4**), the 2-way interaction model explained 1% and 11% more variation in the recovery of APCs and total coliforms, respectively, while the 3-way interaction model explained 2% and 12% more variation in APCs and total coliforms, respectively, compared to main effect model. Similarly, in the field with untreated dairy manure (**Table 1-5**), the 2-way interaction model explained 2%, 8%, and 1% more variation in the recovery of APCs, total coliforms, and generic *E. coli*, respectively, while the 3-way interaction model explained 3%, 8%, and

2% more variation in the recovery of APCs, total coliforms, and generic *E. coli*, respectively, compared to main effect model. Overall, the effects of sampling days, sample types, and wetting agents alone appear sufficient to explain the recovery of indicator organisms, as the inclusion of 2-way and 3-way interaction terms did not provide meaningful improvements in model fit. The statistical analysis for the main effects model is presented in the following sections.

In general, drags and booties performed similar to or better than grabs in recovering indicator organisms, although some discrepancies were observed on certain sampling days. In both fields (**Figure 1-3**), results of drags and booties did not show consistent differences from those of grabs for recovery of APCs. For total coliforms, drags and booties performed similarly to grabs in the field with untreated dairy manure but slightly outperformed grabs in the field with untreated swine manure. For generic *E. coli*, drags and booties performed similarly to grabs in the field with untreated swine manure. However, in the field with untreated dairy manure, while drags and booties were comparable to grabs on most sampling days, grabs performed slightly better on days 9 and 24.

In both fields (**Table 1-6**), sampling days and sample types had statistically significant effects ($p < 0.001$) on the recovery of indicator organisms. Sampling days showed more significant effect compared to sample types, with p values much smaller than that of sample types. In the field with untreated swine manure, grabs recovered 0.3 log(CFU/g) more APCs than drags and 0.1 log(CFU/g) more than booties. For total coliforms, grabs recovered 0.9 log(CFU/g) less than drags and 1.1 log(CFU/g) less than booties. In the field with untreated dairy manure, grabs recovered 0.3 log(CFU/g) more APCs than drags and 0.4 log(CFU/g) more than booties. For total coliforms, grabs recovered 0.5 log(CFU/g) more than drags but 0.1 log(CFU/g) less than booties. For generic *E. coli*, grabs recovered 0.9 log(CFU/g) more than drags and 0.3 log(CFU/g) more than booties.

Methods for Objective 2

Preparation of swabs and processing of samples followed the same protocol for all sampling visits.

Sample collection methods. Three sample collection methods were evaluated during this study, which include cotton boot covers, drag swabs, and aggregate soil grabs. Cotton boot covers were sourced from Romer Labs (Union, MO). Drag swabs were constructed from a 4x4 inch sterile gauze pad and 1.2 m of cotton kitchen twine, which is autoclaved individually, then sterilized under UV light for 15 minutes after assembly. Soil grabs were collected by scooping about 50 g of surface soil, no deeper than 5 cm, 6 times throughout one walk of a path using a handheld spade that is cleaned with 70% ethanol between each repetition. Swabs were stored in individual Whirl-Pak bags to be transported to and from each sampling site.

Each repetition (n=48 repetitions) consisted of paired 1 boot swab and 1 drag hydrated with sterile phosphate buffered saline (PBS) (Sigma-Aldrich, St. Louis, MO) worn by the sampler on their right leg, 1 boot and 1 drag swab hydrated with sterile buffered peptone water (BPW) (Millipore, Burlington, MA) on their left leg, and a composite soil sample where 6 soil grabs were taken with a clean, disinfected spade repetition, as pictured in **Figure 2-1**. Each drag and bootie was pre-hydrated with 12 and 18 mL of the designated wetting agent, respectively, prior to arriving at the sampling field. Nitrile gloves were worn and disinfected with 70% ethanol before collecting each repetition of samples and exchanged with each repetition (Strawn et al., 2013), and plastic boot covers (Romer Labs, Union, MO) worn under the cotton bootie swabs were changed after each repetition to prevent additional contamination from the bottom of the samplers' shoes. After samples were collected, they were placed in a cooler with ice packs and kept at refrigeration temperatures (~4°C) until processing.

Sample collection walking patterns. For melons, a total of n=20 repetitions were collected over a 2-day sampling trip to a commercial melon farm in the United States (n= 10 repetitions per day). The sampling field was approximately 750 m wide and 300 m long (**Figure 2-2**). Every 4th bed was used as the sample collection path. Each repetition was a 300-m walk in a straight line down the field, or the entire length of the field. At the end of each 300-m walk, the sampler then used the adjacent empty bed to change direction and collect the next repetition of samples, walking back across the length of the field. The walking route creates a serpentine pattern across the field, walking down and back across the field. Samples were overnight shipped back to Illinois and processed within 36 h of sampling. A brief study was conducted to evaluate the stability of the microorganisms after sampling but before processing, as previous methodology states that samples must be processed in 24 h (Stasiewicz, 2024). N=12 repetitions were processed following the basic enumeration protocol to understand the stability of APC, TC, and generic *E. coli* over time in storage post sampling. Samples were kept at 4°C for 7 days, and were tested at days 0, 1, 2, and 7, and the samples were stable for ACP, TC, and generic *E. coli* up to day 2.

The next n=18 repetitions of samples were collected across two sampling dates in a small-scale mixed horticultural field, measuring approximately 75 m long by 100 m wide. Samples collected on this field were divided into 3 different zones based on commodity type, where zone 1 was beets, zone 2 was mixed leafy greens (herbs, lettuce, and cabbage), and zone 3 was various types of sweet peppers grown above black paper. Each zone consisted of sample paths totaling approximately 75 m per repetition (**Figure 2-3**).

The final n=10 reps were collected over two trips to the apple orchard in southern Illinois (n=5 per day). The walking path is further specified on **Figure 2-4;** however, in summary, a walking path under the canopy of 10 consecutive trees (where 10 m was the total circumference of walking under each tree, calculated by circumference = 2 πr and r = approximately 1.6 m from the trunk of the tree), to total approximately 100 m of walking with each repetition. Rows were approximately 7 m apart. The ground under each of the canopies was during peak harvesting, and the facility harvests the fruit by hand.

Sample processing, plating, and enumeration. Samples were preprocessed to obtain the initial dilution for enumerating indicator organisms including total coliforms (TC), aerobic plate counts (APC), and generic *E. coli*. For each bootie swab sample, the initial weight of the swab and bag that contained it was recorded. The mass of the bootie swab after sampling was also recorded. Drag sample weights were recorded before and after sampling using the same process of the booties. The mass of soil collected was measured by subtracting the initial mass from the final weight of the sample post-collection. Soil mass collected by the drag swabs averaged 9.5 ± 3.8 g, and bootie swabs averaged 22.0 ± 15.0 g across all 48 repetitions. Within 18-36 h of collection, samples were then diluted by fixed volumes of 20, 50, and 100 mL of sterile PBS to the drag, boot cover, and 25-g subsamples of the composite grabs, respectively. Dilution range for drag swabs was from 1:2.0 to 1:4.6 dilution based on the actual collected soil mass, where boot drags dilution ranged from 1:1.8 to 1:26.2, which is used in the data analysis in the following steps.

Soil grabs were thoroughly mixed, and 25 g of the subsample was removed and diluted with 100 mL of PBS to create 1:5 dilution for further enumeration. Samples were mixed by massaging by hand for approximately 2 minutes to fully disperse the soil collected into the PBS as a homogenous solution while dilutant aliquots were removed for enumeration. One mL of the initial dilutant was extracted and used for serial dilutions using PBS on CHROMagar ECC (CHROMagar) and Standard Methods Agar (SMA) (Criterion, New York, NY).

A 1-mL undiluted sample was removed and plated on CHROMagar to raise the limit of detection (LOD) for the TC and generic *E. coli*. 100 µL of the diluent was reserved for serial dilutions. All plates were spiral plated with 100 µL of sample. SMA plates were spiral plated and incubated at 35 ± 2 °C for approximately 48 h, and CHROMagar was incubated at 30 ± 2 °C for 24 h. The number of colony forming units (CFU) were

collected, and calculated to find the concentration based on the mass of soil for the serial dilutions (CFU/g), then log transformed (Jarvis, 2016).

Plates were manually counted, as the CHROMagar required differentiation between the blue colonies (generic *E. coli*) and purple colonies (TC). APCs were recorded from the SMA agar, where <1 CFU were considered too few to count. One mL of the initial dilutant was extracted and poured on a CHROMagar plate to lower the LOD.

Enrichment of samples for the detection of generic *E. coli*. 2x EC broth (Sigma-Aldrich, St. Louis, MO) was added in equal volume as the remaining PBS in the sample bag containing the swab or soil sample and the bag was incubated at 45°C for 24 h. After incubation, enriched sample bags were massaged by hand for 30 s. Samples were then streaked to isolation on CHROMagar and incubated at 37°C for 24 h. Plates with any blue colonies, indicating the presence of generic *E. coli*, were counted as positive.

DNA extraction, amplification, sequencing, and analysis from soil samples. A total of 150 samples (n=30 samples from each commodity type) were selected for DNA extraction. All bootie, drag, and soil grabs for the beets, peppers, and leafy greens were selected (n=90 total, 30 for each commodity type). From the melon farm, n=30 (10 booties, 10 drags, 10 soil grabs) out of 100 total samples were randomly selected using a random number generator. Similarly, a total of n=30 (10 booties, 10 drags, 10 soil grabs) out of 50 total samples were also randomly selected from the apple orchard. Of the 150 samples where DNA was extracted, only 144 were selected for sequencing, as 6 of the samples did not contain high enough DNA for sequencing.

DNeasy PowerSoil Pro kit (QIAGEN, Maryland) was used to extract the soil microbiota DNA following the manufacturer's directions. The purity and preliminary concentration of the extracted samples were measured by NanoDrop 2000c Spectrophotometer (Thermo Scientific, California), the base pair length was analyzed using gel imaging, and lastly the concentration was measuring using Quant-iT PicoGreen dsDNA reagent and kit (Invitrogen, California). Extracted samples were diluted to 15–49 ng/µL and stored at -20°C before submitting to the Roy J. Carver Biotechnology Center DNA Services Laboratory at the University of Illinois at Urbana-Champaign. DNA amplifications were done via Fluidigm with 16S V3-V4 targets. Sequencing was conducted using Illumina MiSeq V2 (Illumina Inc., USA).

Microbial community analysis for alpha and beta diversity was performed by the High-Performance Computing in Biology (HPCBio) group at Roy J. Carver Biotechnology Center at the University of Illinois. Data was imported and processed in R version 4.4.1, with quality control checks using DADA2 (Callahan et al., 2016). Data was passed through filters to remove artifacts, prune out low-count taxa, and remove any possible bad or uninformative samples, using phyloseq (version 1.48.0) and vegan (version 2.6-8). No full samples were filtered out. 26,854 taxa were available for further analysis after filtering. Alpha diversity metrics were: Observed, Chao1, ACE, Shannon, Simpson, Fisher, and Faith's PD. Histograms and Koenker's test checks for normality ruled out the use of parametric tests. Therefore, nonparametric Kuskal-Wallis, Wilcoxon rank sum, and Wilcoxon signed rank tests were used to evaluate differences in diversity metrics between the wetting agents and sample types. Principal coordinate analysis (PCoA) showing Bray-Curtis distance visualizing the beta diversity were created and statistical significance was tested using permutational multivariate analysis of variance (PERMANOVA, (Anderson, 2017)) for wetting agent and sample type.

Data analysis. Plate counts were log transformed as is standard microbiology practice, as raw counts in CFU/g typically are more normally distributed once log transformed (Granato et al., 2014; Jarvis, 2016). The limit of detection (LOD) was calculated using the known weight of soil collected by each sample type and aliquot of PBS of its initial dilution. Less than 1 colony were considered too few to count. On 1-mL pour plate, <1 CFU = 0.7 log CFU/g of soil. After the log transformation, ½ LOD was used for statistical

analysis, which is consistent with the EPA (United States Environmental Protection Agency (U.S. EPA), 1992) and the approach in previous aggregate sampling work in beef trim (Arthur & Wheeler, 2021). As the mass of soil collected on the drags and boot swabs, the LOD varies slightly with each individual sample.

All statistical analyses and graphs were made using JMP Pro 18 or R version 4.3.3. Scatterplots were used to visualize whether there was a difference in each wetting agent's ability to detect APC and TC. Next, a matched pairs comparison was run between paired sample types (bootie and drag swabs) by microbial test (TC and APC), to understand that if the wetting agents (PBS and BPW) performed differently in each microbial test by sample type. The same quantitative statistical analysis was not conducted on the generic *E. coli* data, as most individual samples across all sampling trips were <LOD, and therefore the data is highly censored. Instead, a Fisher's exact test was used to analyze post-enrichment analysis to understand the ratio of positives for generic *E. coli* collected for each collection method and wetting agent.

Paired t-tests to evaluate the mean differences for APC and TC to compare mean difference between bootie and drag swab types and soil grabs for each commodity type. The differences between each paired sample of drag or bootie swab to soil grabs were normally distributed when evaluated with histogram and QQ Plots so parametric tests were appropriate.

Results for Objective 2

Overall, results show that bootie and drag swabs were able to detect higher concentrations of microorganisms in comparison to the aggregate soil grabs. In addition, generic *E. coli* enumeration results are much lower than total coliforms (TC) and aerobic plate counts (APC) (**Figure 2-5**).

Wetting agents performed similarly for detecting aerobic plate counts, total coliforms, and generic *E. coli*. **Figure 2-6** demonstrates the linear relationship between the log CFU/g measured between paired samples, separated by test type (TC or APC). The APC R^2 of 0.37, and the correlation coefficient of 0.61 means there is a weak, positive correlation for APC between paired BPW and PBS samples. The TC R^2 of 0.72, and correlation coefficient of 0.85, means there is a positive, stronger, linear relationship between paired samples.

A paired comparison analysis checked for an overall difference in performance between PBS or BPW (**Table 2-1**). Generally, there was no statistically significant difference ($p > 0.05$) in means for APC or TC between the wetting agents for drag or bootie swabs when comparing paired sample types (i.e., comparing PBS drags to BPW drags for APC), with the exception of TC results for drag swabs, where BPW collected, on average, 0.26 log(CFU/g) ($p < 0.05$) more TC than the swabs hydrated with PBS. However, even the largest mean difference of 0.26 log(CFU/g) is not likely biologically meaningfully (<1 log(CFU/g)) different.

Considering microbial diversity within and between samples, there is no statistically significant alpha diversity difference in samples using PBS or BPW ($p > 0.05$) in 3 of the 5 metrics assessed (**Figure 2-7**, top), while Chao1 and Shannon do show a significant difference in alpha diversity ($p < 0.05$). However, there is still substantial overlap in community richness across samples collected with the PBS and BPW wetting agents. In **Figure 2-7** (bottom), a PCoA Bray-Curtis distance shows that where there is a statistically significant compositional difference ($p = 0.004$), there is still >50% overlap in individual data points, again showing a high amount of compositional overlap between the samples collected using PBS or BPW.

Drag and bootie swabs are more effective than soil grabs at detecting aerobic plate counts, total coliforms, and generic *E. coli.* As there was no major difference between the wetting agents, these data were then combined to evaluate the overall effectiveness of sample type (booties, drags, soil grabs) for each microbiological test (APC, TC, generic *E. coli*) and commodity type. All drag and bootie swabs detected significantly higher ($p < 0.05$) levels of APC and TC, except for booties swabs detecting no significant

differences APC in peppers (0.13±0.39 log(CFU/g), p=0.24) in comparison to soil grabs (**Table 2-2**). APC differences between booties and soil ranged from 0.13±0.39 log(CFU/g) to 1.83±0.24 log(CFU/g), and the difference between drags and soil ranged from 0.42±0.52 log(CFU/g) to 1.63±0.74 log(CFU/g). TC differences between booties and soil range from 1.76±1.05 log(CFU/g) to 5.05±1.06 log(CFU/g), and the differences between drags and soil ranged from 1.32±0.96 log(CFU/g) to 5.32±1.03 log(CFU/g). In summary, for each of the commodity types, bootie and drag swabs recovered similar or greater levels of APC and TC, compared to the soil grabs, when controlling for wetting agent.

Considering microbial differences between sample types, differences in alpha-diversity (**Figure 2-8**, top) show that there is variation in the difference between the amount of diversity recovered from samples drag, bootie, and soil grab collection methods, with bootie swabs showing the greatest within-sample diversity of the three methods. In **Figure 2-8** (bottom), a PCoA Bray-Curtis distance shows that there is compositional difference ($p < 0.05$) between the separate collection methods. As >50% of the points for bootie and drag swabs are overlapping, as seen as clustering on the PCoA plot, there is more compositional similarity between these sample collection methods, in comparison to the cluster seen in the soil grab.

Enrichment results show that bootie and drag swabs test positive for generic *E. coli* significantly more frequently than soil grabs. There was a statistically significant difference (Fisher's exact $p = 3.5 \times 10^{-11}$) in generic *E. coli* positives between the combined drag and bootie samples (173 positive out of 192 samples) compared to soil grabs (21 positives out of 48 samples), meaning that for these data, it was more likely for soil grab samples to test negative for generic *E. coli* than hydrated swab methods.

Outcomes and Accomplishments

Objective 1

The team compared skim milk, tryptic soy broth, buffered peptone water, phosphate buffered saline, and deionized water as wetting agents for drag and bootie swabs for the recovery of indicator organisms from fields with untreated animal manure applied to soil for fallow row-crop fields (to ensure sufficient levels of indictors for the screening analysis). Aerobic plate counts, total coliforms, and generic *E. coli* were enumerated for the drag and bootie swabs and composite soil grab samples to assess microbial quality and safety. Skim milk was the reference to evaluate alternative wetting agents against. Composite soil grabs were the gold standard to evaluate swab performance against. Data was collected in a series of experiments consisting of on-site soil sampling at cooperating farms, and laboratory analysis of collected samples.

The major outcomes include:

- Wetting agents used for hydrating drags and booties showed only small (<1 log(CFU/g)) statistically significant effects on recovering indicator organisms, and these differences are likely not biologically important.
- Drag and bootie sampling methods have similar or better recovery of indicator organisms than composite soil grabs, showing potentially as an easier-to-perform improvement over grabs.
- All wetting agents for drag and bootie swabs performed similarly, suggesting the choice of wetting agents can be determined by which are most practical to use.

Objective 2

Phosphate buffered saline and buffered peptone water were selected as the optimal wetting agents from Objective 1, to represent a non-nutritive wetting agent and a nutritive wetting agent, respectively. The team brought bootie and drag swabs hydrated in these wetting agents to three different commercial produce fields, to evaluate their effectiveness and compare them to traditional soil grabs in a realistic, field setting for various commodities (melons, leafy greens, beets, peppers, and apples). Aerobic plate counts, total coliforms, and generic *E. coli* were enumerated. Samples were also enriched to increase the detection power of generic *E. coli.* Microbial community analysis was also conducted to further understand and compare the performances of both the wetting agents and sample collection methods.

The major outcomes include:

- There is no biologically meaningful difference in the performance of phosphate buffered saline or buffered peptone water for detecting indicator microorganisms in produce soils. Because phosphate buffered saline is non-nutritive, non-allergenic, and does not require refrigeration it may be the preferred wetting agent for soil sampling.
- Bootie and drag swabs performed similar to each other in detecting indicator microorganisms from produce soils, and in some cases better than aggregate soil grabs. These results suggest the aggregative sampling methods could be determined by practicality, noting that in our work booties were easier to use than drag swabs.
- Bacterial community analysis shows there are no major differences within or between sample diversity when comparing samples collected with different wetting agents or sample collection methods.

APPENDICES

Publications and Presentations

Publications

1. CPS has reviewed the following manuscript. The team is currently working on revisions with the goal of submitting it to the *Journal of Food Protection*.
   Wu, J., Pinto, G., Kealey, E., Barnett-Neefs, C., Stasiewicz, M.J. (202X). Aggregative soil sampling using drags and booties hydrated with various wetting agents showed similar recovery of indicator organisms compared to soil grabs from fields with untreated manure applied.
2. CPS is reviewing the following manuscript, before submission to the *Journal of Food Protection:*
   Kealey, E., Wu, J., Elementi, R., Frankowski, Z., Barnett-Neefs, C., Valizadegan, N., Margenot, A., Banerjee, P., Stasiewicz, M. J. (202X). Testing Wetting Agents for Drag and Bootie Swabs Against Aggregative Soil Grabs for Preharvest Soil Sampling for Varied Produce Commodities.

Presentations

1. Submitted to IAFP annual meeting 2025 as a technical presentation:
   Wu, J., Kealey, E., Stasiewicz, M.J. (2025). Aggregative soil sampling using drags and booties hydrated with alternative wetting agents shows promising recovery of indicator organisms across diverse soils.

Budget Summary

During the primary performance period of the grant, the 2024 calendar year, the project spent approximately $117,000 of the $142,000 budgeted for the project. During the final 6 months of the project, we anticipate about $10,000 in additional expenses, for two open-access publication fees for two manuscripts (about $5,000) and PI Stasiewicz and lead graduate student travel to the 2025 CPS Research Symposium to share results of the project (about $5,000). Therefore, we anticipate leaving about $15,000 of the project budget unspent.

Tables and Figures (see below)

Objective 1 tables

Table 1-1. Timeline for soil sampling in the field with untreated swine manure and the field with untreated dairy manure.

Manure types	Events	Dates	Notes
Swine	Manure application	Mar 3, 2024	Day 0 (The field applied swine manure by liquid injection)
	Sampling	Mar 9, 2024	Day 6 (The earliest time we got access to the fields)
	Sampling	Mar 13, 2024	Day 10
	Sampling	Mar 20, 2024	Day 17
	Sampling	Apr 3, 2024	Day 31
Dairy	Manure application	Apr 25, 2024	Day 0 (The field applied dairy manure by dry spreading)
	sampling	Apr 25, 2024	Day 0
	sampling	Apr 27, 2024	Day 2
	sampling	Apr 29, 2024	Day 4
	sampling	May 4, 2024	Day 9
	sampling	May 9, 2024	Day 14
	sampling	May 19, 2024	Day 24
	sampling	May 29, 2024	Day 34

Table 1-2. Main effects of sampling days, sample types, and wetting agents on the recovery of APCs, total coliforms, and generic *E. coli* in the field with untreated swine manure.

Count types	Sources	P value	Variable	Mean (log(CFU/g))	Level	Parameter estimates	P value
APCs	Sampling days	$2.9 \cdot 10^{-42}$	Day 6	7.2	A	0.68	<.0001
			Day 10	6.4	B	-0.09	0.0110
			Day 17	6.2	C	-0.26	<.0001
			Day 31	6.1	C	reference	reference
	Sample types	$2.9 \cdot 10^{-4}$	booties	6.5	A	0.07	0.0003
			drags	6.4	B	reference	reference
	Wetting agents	$4.4 \cdot 10^{-4}$	BPW	6.6	A	0.17	<.0001
			TSB	6.5	A B	reference	reference
			DI	6.4	B	-0.04	0.28
			PBS	6.4	B	-0.05	0.22
			SKM	6.4	B	-0.10	0.01
Total coliforms	Sampling days	$1.4 \cdot 10^{-32}$	Day 6	6.1	A	0.77	<.0001
			Day 10	5.4	B	0.04	0.50
			Day 17	4.6	B	-0.69	<.0001
			Day 31	5.2	C	reference	reference
	Sample types	$1.2 \cdot 10^{-3}$	booties	5.4	A	0.11	0.001
			drags	5.2	B	reference	reference
	Wetting agents	$7.6 \cdot 10^{-8}$	TSB	5.6	A	reference	reference
			BPW	5.5	A	0.12	0.07
			SKM	5.5	A	0.12	0.08
			PBS	5.1	B	-0.22	0.0008
			DI	5.0	B	-0.28	<.0001
Generic *E. coli*	Sampling days	$1.2 \cdot 10^{-8}$	Day 6	0.6	A	0.20	<.0001
			Day 10	0.3	B	-0.04	0.21
			Day 17	0.4	B	-0.02	0.46
			Day 31	0.2	B	reference	reference
	Sample types	$3.6 \cdot 10^{-4}$	booties	0.5	A	0.07	0.0004
			drags	0.3	B	reference	reference
	Wetting agents	0.2	BPW	0.4	A	0.03	0.48
			TSB	0.4	A	reference	reference
			DI	0.4	A	-0.03	0.50
			PBS	0.4	A	-0.0003	0.99
			SKM	0.3	A	-0.07	0.07

Table 1-3. Main effects of sampling days, sample types, and wetting agents on the recovery of APCs, total coliforms, and generic *E. coli* in the field with untreated dairy manure.

Count types	Sources	P value	Variable	Mean	Level	Parameter estimates	P value
APCs	Sampling days	$2.1 \cdot 10^{-135}$	Day 0	8.6	A	1.10	<.0001
			Day 2	8.5	A	0.97	<.0001
			Day 4	8.2	B	0.70	<.0001
			Day 9	7.7	C	0.12	0.0096
			Day 14	7.3	D	-0.22	<.0001
			Day 24	6.3	E	-1.26	<.0001
			Day 34	6.2	E	reference	reference
	Sample types	$1.0 \cdot 10^{-5}$	Booties	7.6	A	0.08	<.0001
			Drags	7.5	B	reference	reference
	Wetting agents	$1.8 \cdot 10^{-5}$	SKM	7.6	A	0.08	0.03
			BPW	7.6	A	reference	reference
			TSB	7.6	A	-0.12	0.002
			DI	7.4	B	-0.12	0.0009
			PBS	7.4	B	0.09	0.02
Total coliforms	Sampling days	$2.7 \cdot 10^{-85}$	Day 0	6.2	A	0.64	<.0001
			Day 2	6.4	A	0.85	<.0001
			Day 4	5.7	BC	0.08	0.13
			Day 9	5.6	C	0.007	0.89
			Day 14	5.9	B	0.31	<.0001
			Day 24	4.8	D	-0.81	<.0001
			Day 34	4.5	E	reference	reference
	Sample types	$1.2 \cdot 10^{-33}$	Booties	5.9	A	0.30	<.0001
			Drags	5.3	B	reference	reference
	Wetting agents	$1.0 \cdot 10^{-18}$	TSB	5.8	A	reference	reference
			SKM	5.7	A	0.16	0.0003
			BPW	5.7	A	0.11	0.0085
			PBS	5.4	B	-0.21	<.0001
			DI	5.4	B	-0.31	<.0001
Generic *E. coli*	Sampling days	$7.0 \cdot 10^{-149}$	Day 0	5.8	A	2.28	<.0001
			Day 2	5.7	A	2.17	<.0001
			Day 4	4.1	B	0.67	<.0001
			Day 9	2.5	C	-0.97	<.0001
			Day 14	3.8	D	0.32	<.0001
			Day 24	1.1	E	-2.43	<.0001
			Day 34	1.5	F	reference	reference
	Sample types	$6.7 \cdot 10^{-19}$	Booties	3.8	A	0.29	<.0001
			Drags	3.2	B	reference	reference
	Wetting agents	$7.0 \cdot 10^{-6}$	SKM	3.7	A	0.20	0.0012
			BPW	3.6	A	0.11	0.08
			TSB	3.6	A	Reference	Reference
			DI	3.3	B	-0.18	0.004
			PBS	3.3	B	-0.22	0.0003

Table 1-4. Comparison of main effects, two-way interaction, and three-way interaction models for evaluating the effects of sampling days, sample types, and wetting agents on the recovery of indicator organisms in fields with untreated swine manure.

Count types	Sources	Main effects model			2-way model			3-way model		
		P value	R^2	R^2 adjusted	P value	R^2	R^2 adjusted	P value	R^2	R^2 adjusted
APCs	Sampling days	$2.9 \cdot 10^{-42}$	0.74	0.73	$2.4 \cdot 10^{-40}$	0.78	0.74	$1.2 \cdot 10^{-39}$	0.81	0.75
	Sample types	$2.9 \cdot 10^{-4}$			$2.6 \cdot 10^{-4}$			$1.9 \cdot 10^{-4}$		
	Wetting agents	$4.4 \cdot 10^{-4}$			$3.8 \cdot 10^{-4}$			$2.6 \cdot 10^{-4}$		
	Wetting agents*Sample types	N.A.			0.8			0.8		
	Wetting agents*Sampling days	N.A.			0.5			0.4		
	Sample types*Sampling days	N.A.			0.03			0.03		
	Wetting agents*Sample types* Sampling days	N.A.			N.A.			0.1		
Total coliforms	Sampling days	$1.4 \cdot 10^{-32}$	0.68	0.66	$7.3 \cdot 10^{-39}$	0.81	0.77	$7.9 \cdot 10^{-38}$	0.83	0.78
	Sample types	$1.2 \cdot 10^{-3}$			$9.4 \cdot 10^{-5}$			$7.7 \cdot 10^{-5}$		
	Wetting agents	$7.6 \cdot 10^{-8}$			$1.5 \cdot 10^{-11}$			$5.9 \cdot 10^{-11}$		
	Wetting agents*Sample types	N.A.			$4.6 \cdot 10^{-7}$			$3.5 \cdot 10^{-7}$		
	Wetting agents*Sampling days	N.A.			$2.0 \cdot 10^{-3}$			$1.5 \cdot 10^{-3}$		
	Sample types*Sampling days	N.A.			$7.2 \cdot 10^{-4}$			$5.9 \cdot 10^{-4}$		
	Wetting agents*Sample types* Sampling days	N.A.			N.A.			0.2		
Generic *E. coli*	Sampling days	$1.2 \cdot 10^{-8}$	0.30	0.26	$1.9 \cdot 10^{-8}$	0.38	0.26	$3.6 \cdot 10^{-8}$	0.42	0.24
	Sample types	$3.6 \cdot 10^{-4}$			$4.0 \cdot 10^{-4}$			$5.0 \cdot 10^{-4}$		
	Wetting agents	0.2			0.2			0.2		
	Wetting agents*Sample types	N.A.			0.7			0.7		
	Wetting agents*Sampling days	N.A.			0.5			0.5		
	Sample types*Sampling days	N.A.			0.3			0.3		
	Wetting agents Sample types* Sampling days	N.A.			N.A.			0.7		

Table 1-5. Comparison of main effects, two-way interaction, and three-way interaction models for evaluating the effects of sampling days, sample types, and wetting agents on the recovery of indicator organisms in fields with untreated dairy manure.

Count types	Sources	Main effects model			2-way model			3-way model		
		P value	R^2	R^2 adjusted	P value	R^2	R^2 adjusted	P value	R2	R^2 adjusted
APCs	Sampling days	$2.1 \cdot 10^{-135}$	0.91	0.91	$4.0 \cdot 10^{-140}$	0.94	0.93	$1.1 \cdot 10^{-134}$	0.95	0.94
	Sample types	$1.0 \cdot 10^{-5}$			$2.4 \cdot 10^{-7}$			$6.5 \cdot 10^{-8}$		
	Wetting agents	$1.8 \cdot 10^{-5}$			$1.7 \cdot 10^{-7}$			$3.5 \cdot 10^{-8}$		
	Wetting agents*Sample types	N.A.			0.2			0.2		
	Wetting agents*Sampling days	N.A.			$3.9 \cdot 10^{-4}$			$8.6 \cdot 10^{-5}$		
	Sample types*Sampling days	N.A.			$1.4 \cdot 10^{-12}$			$1.2 \cdot 10^{-13}$		
	Wetting agents*Sample types* Sampling days	N.A.			N.A.			$3.5 \cdot 10^{-3}$		
Total coliforms	Sampling days	$2.7 \cdot 10^{-85}$	0.82	0.82	$2.3 \cdot 10^{-106}$	0.92	0.90	$8.6 \cdot 10^{-100}$	0.93	0.90
	Sample types	$1.2 \cdot 10^{-33}$			$1.5 \cdot 10^{-49}$			$7.8 \cdot 10^{-48}$		
	Wetting agents	$1.0 \cdot 10^{-18}$			$2.2 \cdot 10^{-30}$			$1.3 \cdot 10^{-29}$		
	Wetting agents*Sample types	N.A.			$6.5 \cdot 10^{-17}$			$1.1 \cdot 10^{-16}$		
	Wetting agents*Sampling days	N.A.			$9.8 \cdot 10^{-12}$			$1.6 \cdot 10^{-11}$		
	Sample types*Sampling days	N.A.			$1.7 \cdot 10^{-7}$			$1.8 \cdot 10^{-7}$		
	Wetting agents*Sample types* Sampling days	N.A.			N.A.			0.4		
Generic *E. coli*	Sampling days	$7.0 \cdot 10^{-149}$	0.93	0.93	$4.0 \cdot 10^{-148}$	0.95	0.94	$8.2 \cdot 10^{-140}$	0.96	0.95
	Sample types	$6.7 \cdot 10^{-19}$			$1.6 \cdot 10^{-22}$			$4.8 \cdot 10^{-23}$		
	Wetting agents	$7.0 \cdot 10^{-6}$			$2.0 \cdot 10^{-7}$			$9.6 \cdot 10^{-8}$		
	Wetting agents*Sample types	N.A.			0.01			0.01		
	Wetting agents*Sampling days	N.A.			$3.6 \cdot 10^{-5}$			$1.6 \cdot 10^{-5}$		
	Sample types*Sampling days	N.A.			$6.5 \cdot 10^{-5}$			$3.6 \cdot 10^{-5}$		
	Wetting agents*Sample types* Sampling days	N.A.			N.A.			0.05		

Table 1-6. Main effects of sampling days and sample types on the recovery of APCs, total coliforms, and generic *E. coli* in the field with untreated swine manure and the field with untreated dairy manure.

Manure	Count types	Sources	P value	Variable	Mean	Level	Parameter estimates	P value
Swine	APCs	Sampling days	$2.1 \cdot 10^{-43}$	Day 6	7.2	A	0.67	<.0001
				Day 10	6.4	B	-0.07	0.04
				Day 17	6.2	C	-0.28	<.0001
				Day 31	6.2	C	reference	reference
		Sample types	$6.7 \cdot 10^{-6}$	booties	6.6	A	-0.01	0.7
				drags	6.4	B	-0.16	<.0001
				grabs	6.7	C	reference	reference
	Total coliforms	Sampling days	$1.3 \cdot 10^{-32}$	Day 6	6.1	A	0.81	<.0001
				Day 10	5.3	B	0.02	0.7
				Day 17	4.5	B	-0.70	<.0001
				Day 31	5.1	C	reference	reference
		Sample types	$2.8 \cdot 10^{-16}$	booties	5.4	A	0.47	<.0001
				drags	5.2	B	0.25	<.0001
				grabs	4.3	C	reference	reference
	Generic *E. coli*	Sampling days	$1.4 \cdot 10^{-10}$	Day 6	0.6	A	0.24	<.0001
				Day 10	0.3	B	-0.06	0.1
				Day 17	0.4	B	-0.04	0.2
				Day 31	0.3	B	reference	reference
		Sample types	$1.5 \cdot 10^{-4}$	booties	0.5	A	0.006	0.9
				drags	0.3	A	-0.13	<.0001
				grabs	0.6	B	reference	reference
Dairy	APCs	Sampling days	$1.7 \cdot 10^{-143}$	Day 0	8.7	A	1.12	<.0001
				Day 2	8.5	A	0.97	<.0001
				Day 4	8.3	B	0.72	<.0001
				Day 9	7.6	C	0.06	0.2
				Day 14	7.4	D	-0.20	<.0001
				Day 24	6.3	E	-1.27	<.0001
				Day 34	6.2	E	reference	reference
		Sample types	$9.0 \cdot 10^{-12}$	Booties	7.6	A	-0.05	0.09
				Drags	7.5	B	-0.22	<.0001
				grabs	7.9	C	reference	reference
	Total coliforms	Sampling days	$1.3 \cdot 10^{-81}$	Day 0	6.3	A	0.65	<.0001
				Day 2	6.4	A	0.83	<.0001
				Day 4	5.7	B	0.09	0.1
				Day 9	5.7	BC	0.06	0.3
				Day 14	5.9	C	0.32	<.0001
				Day 24	4.8	D	-0.85	<.0001
				Day 34	4.5	D	reference	reference
		Sample types	$1.8 \cdot 10^{-27}$	Booties	5.9	A	0.23	<.0001
				Drags	5.3	A	-0.37	<.0001
				grabs	5.8	B	reference	reference
	Generic *E. coli*	Sampling days	$1.4 \cdot 10^{-164}$	Day 0	5.8	A	2.25	<.0001
				Day 2	5.7	A	2.14	<.0001
				Day 4	4.2	B	0.64	<.0001
				Day 9	2.7	C	-0.87	<.0001
				Day 14	3.8	D	0.29	0.0002
				Day 24	1.2	E	-2.39	<.0001
				Day 34	1.5	E	reference	reference
		Sample types	$7.5 \cdot 10^{-22}$	Booties	3.8	A	0.07	0.1
				Drags	3.2	B	-0.50	<.0001
				grabs	4.1	C	reference	reference

Objective 1 figures

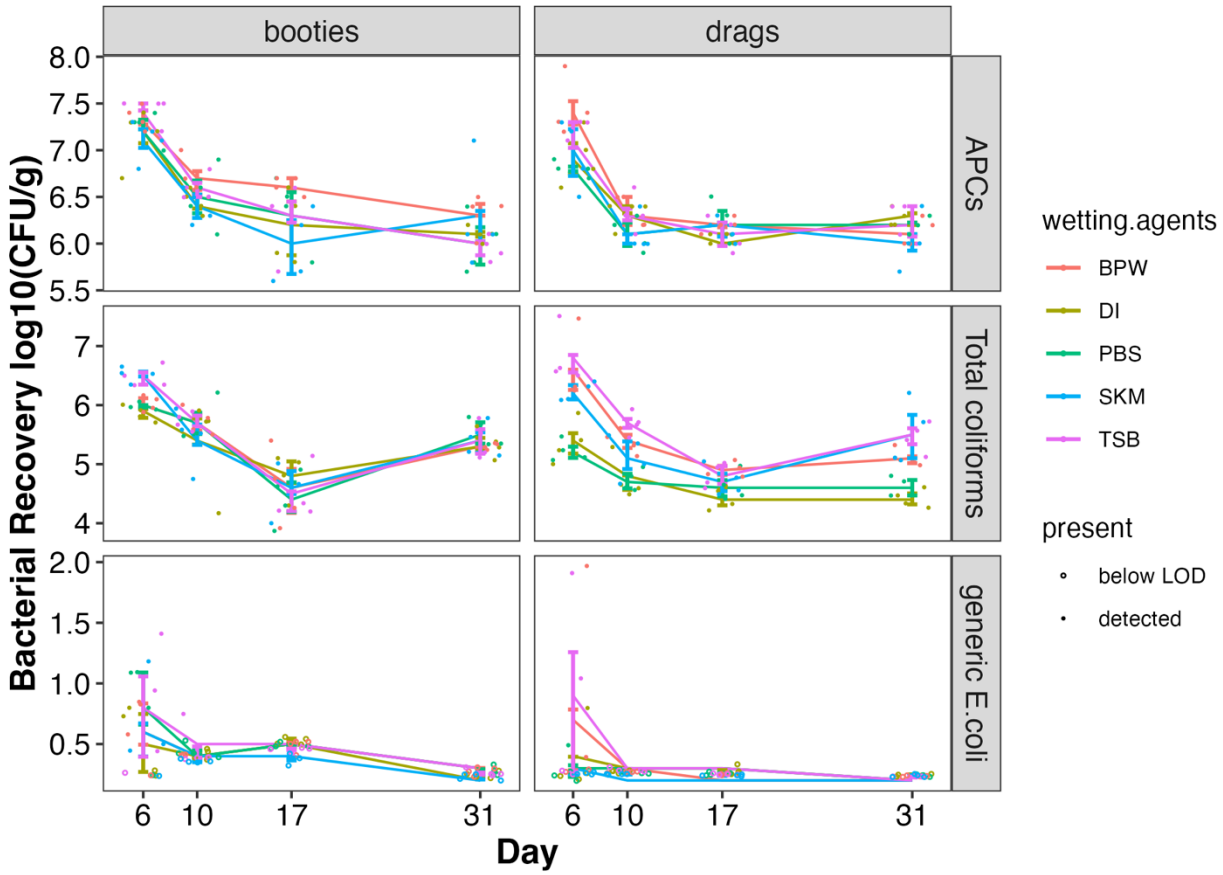

Figure 1-1. Bacterial counts of aerobic plate counts, total coliforms, and generic *E. coli* for soil collected using drags and booties hydrated by different wetting agents in the field with untreated swine manure. The whiskers represent the interquartile range. Solid jittered points represent the counts of detected samples. Open jittered points represent the LODs/2 of the samples under LODs. Different wetting agents are represented using different colors.

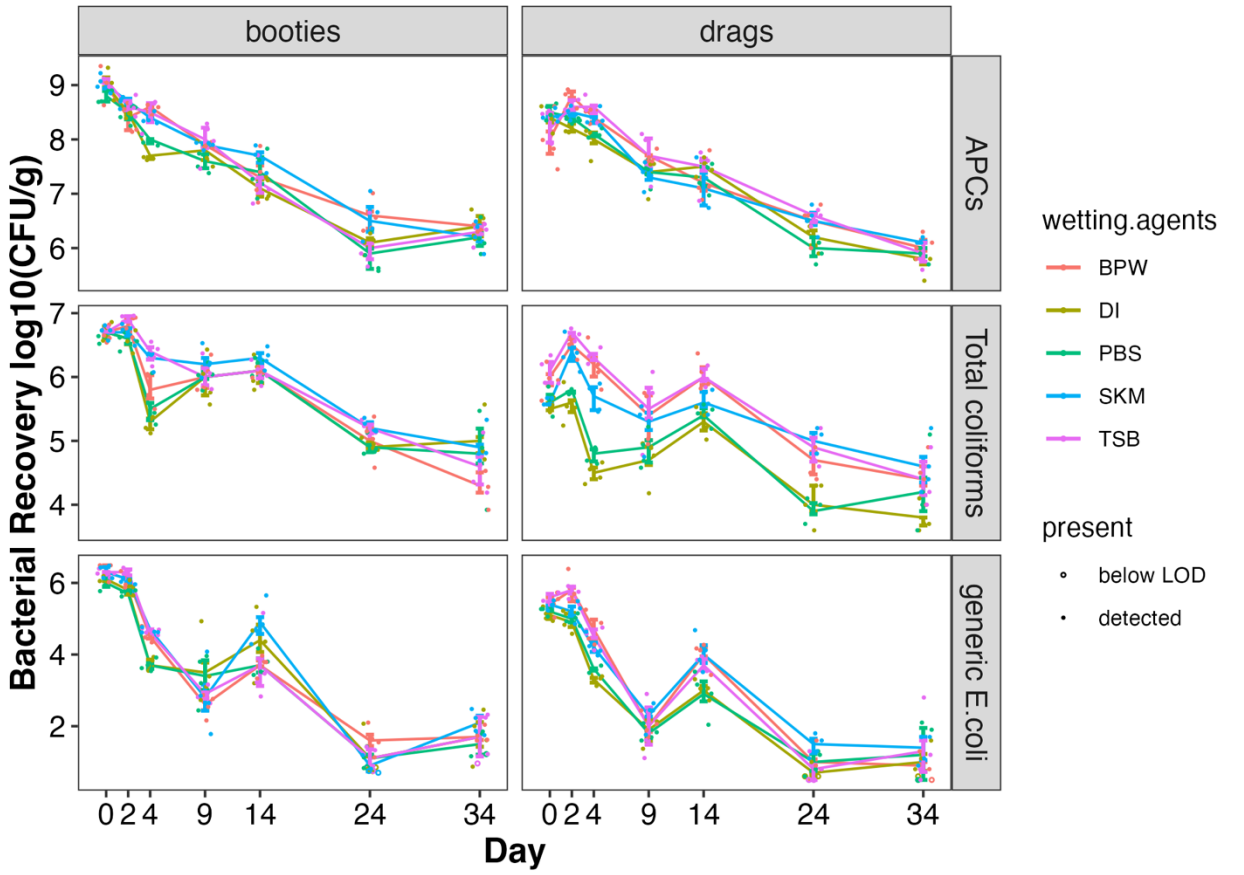

Figure 1-2. Bacterial counts of aerobic plate counts, total coliforms, and generic *E. coli* for soil collected using drags and booties hydrated by different wetting agents in the field with untreated dairy manure. The whiskers represent the interquartile range. Solid jittered points represent the counts of detected samples. Open jittered points represent the LODs/2 of the samples under LODs. Different wetting agents are represented using different colors.

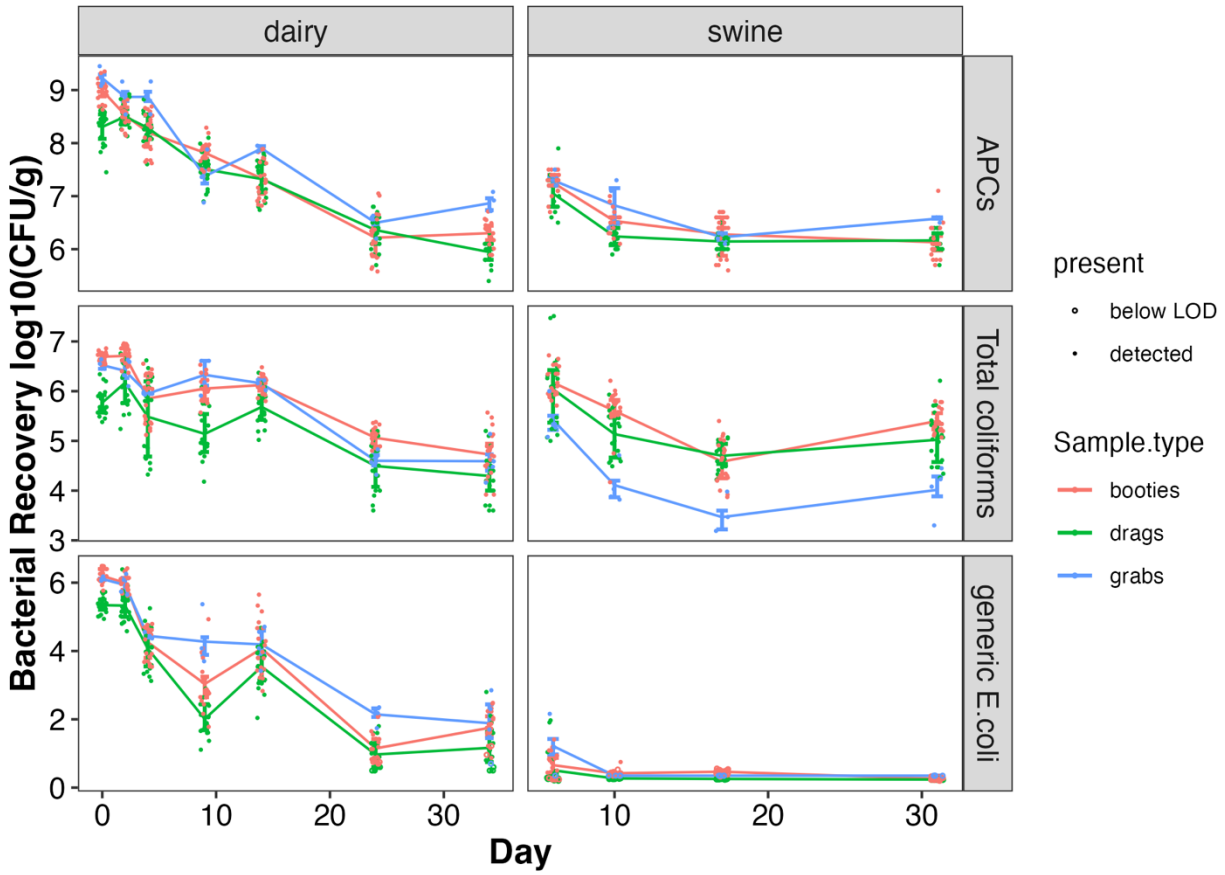

Figure 1-3. Bacterial counts of aerobic plate counts, total coliforms, and generic *E. coli* for soil collected using drags, booties, and grabs in the field with untreated swine manure and the field with untreated dairy manure. The whiskers represent the interquartile range. Solid jittered points represent the counts of detected samples. Open jittered points represent the LODs/2 of the samples under LODs. Different sample types are represented using different colors.

Objective 2 tables

Table 2-1. Comparing the mean difference of paired samples (BPW samples – PBS samples) log CFU/g[a].

Test Type	Sample Collection Type	Mean Difference of Paired samples, (BPW – PBS) log CFU/g ±St. Error	Prob > |t|	FDR Correction
APC	Drag	0.14 ± 0.11	0.197	0.369
	Bootie	-0.02 ± 0.09	0.812	0.818
TC	Drag	0.26 ± 0.09	0.005	0.020
	Bootie	-0.02 ± 0.07	0.818	0.818

[a] This is a summary of the matched pairs that were compared in the top and bottom scatterplots of Figure 5.

Table 2-2. Table comparing the mean and standard deviations of detected microorganisms by collection method.

	Aerobic Plate Count (APC), log CFU/g					Total Coliforms (TC), log CFU/g				
Soil from Given Commodity	Bootie	Drag	Grab	Bootie – Grab[a]	Drag – Grab[a]	Bootie	Drag	Grab	Bootie – Grab[a]	Drag – Grab[a]
Melons	8.15±0.11	8.04±0.11	6.41±0.16	1.74±0.94	1.63±0.74	7.27±0.12	7.54±0.12	2.21±0.17	5.05±1.06	5.32±1.03
Beets	7.13±0.10	6.93±0.10	6.51±0.14	0.62±0.38	0.42±0.52	6.09±0.14	5.62±0.14	3.59±0.20	2.50±0.88	2.03±0.91
Leafy Greens	7.36±0.12	7.21±0.12	6.53±0.17	0.83±0.37	0.68±0.48	6.60±0.16	6.21±0.16	3.97±0.22	2.80±0.93	2.42±1.03
Peppers	6.52±0.07	6.97±0.07	6.38±0.10	0.13±0.39[a]	0.59±0.17	5.38±0.23	4.94±0.23	3.61±0.33	1.76±1.05	1.32±0.96
Apples	8.07±0.07	7.40±0.07	6.24±0.10	1.83±0.2	1.16±0.44	6.94±0.13	6.26±0.13	3.64±0.19	3.30±0.65	2.62±0.93

[a] Paired t-tests were conducted on the mean differences between bootie and soil grabs or drag swabs and soil grabs to show if the differences between the drag or booties swab and the soil grab were equal to 0, meaning there was no difference between the swab collection method enumeration results and the corresponding mean of soil grabs. APC of peppers showed that there was no significant difference ($p = 0.24$) between bootie swabs and soil grabs. All other mean differences were significantly ($p < 0.05$) different from 0.

Table 2-3. Contingency table comparing the counts of positive and negative results of the presence (indicated on CHROMagar) of generic *E. coli* (gEC) by wetting agent and collection type.

Wetting Agent	Collection Type	Positive gEC	Negative gEC	Total
PBS	Drag	45	3	48
	Boot	42	6	48
BPW	Drag	42	6	48
	Boot	44	4	48
Swabs total		**173** [a]	**19** [a]	**192**
Soil Only	**Grabs**	**21** [a]	**27** [a]	**48**
Total		**194**	**46**	**240**

[a] Fisher's exact test on the total samples for each collection method significantly different ($p = 3.5 \times 10^{-11}$) between swabs and soil grabs.

Objective 2 figures

Figure 2-1. Sample collection set up for 1 repetition. Left foot: 1 BPW Bootie, 1 BPW Drag. Right Foot: 1 PBS Bootie, 1 PBS Drag. Not pictured but collected: composite soil grab using a small, handheld shovel and a sterile bag.

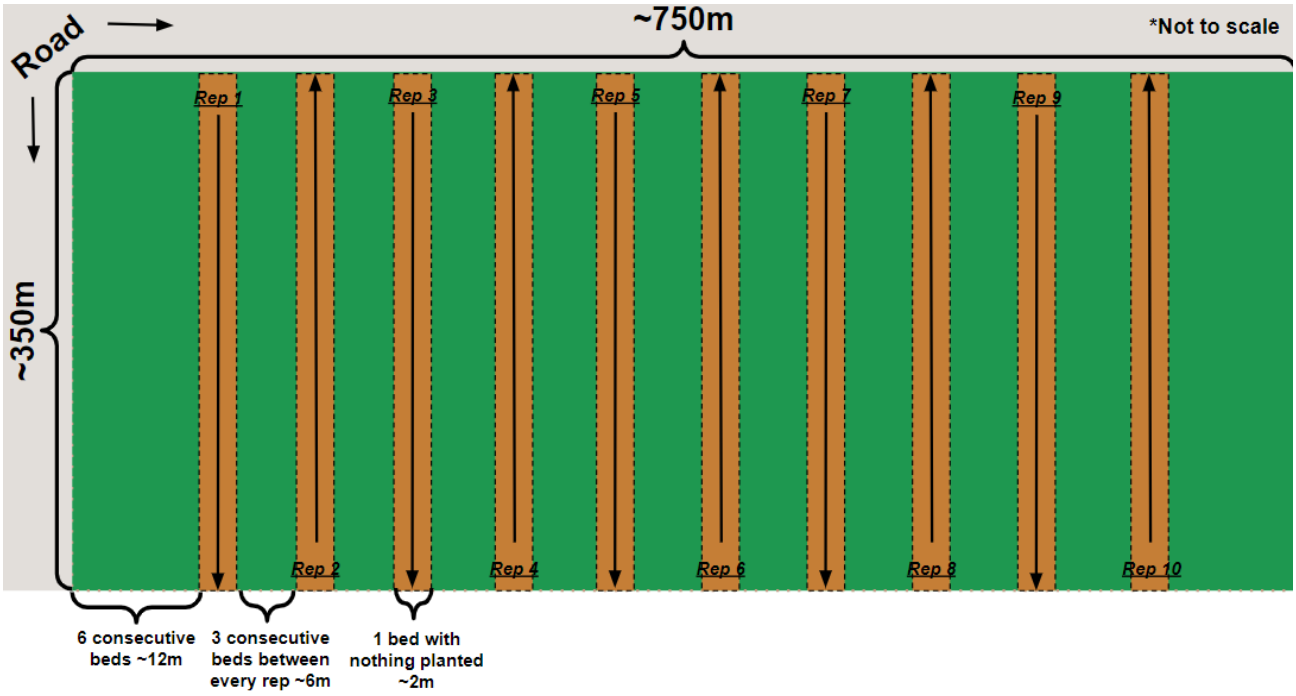

Figure 2-2. Dimensions and walking path of melon field. A commercial melon field in the continental United States. The designated sampling area was ~750m wide by ~350 long. Each sampler walked down 1 bed (~2m wide) in one direction, and collected the following as 1 repetition; 1 boot swab hydrated in PBS, 1 boot swab hydrated in BPW, 1 drag swab hydrated in PBS, 1 drag swab hydrated in BPW, and 1 aggregate soil grab, that consisted of the sampler stopping 6 times in one repetition to take a small scoop of soil, approximately 50g each time. The sampler then reset and moved onto the next empty bed and collected the next repetition on the way back. This process was completed until 10 repetitions were completed and was repeated 24 hours later following the same paths, equaling a total on n=20 repetitions on the melon field.

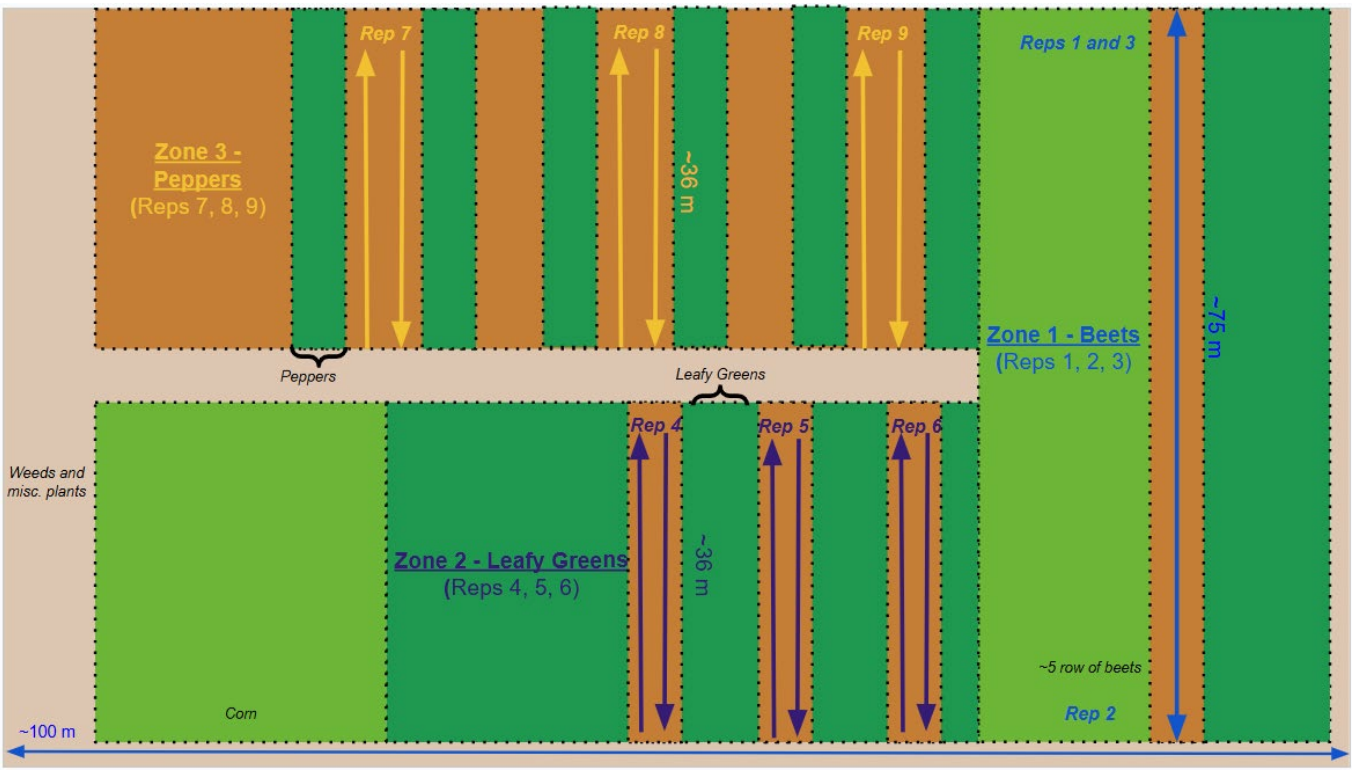

Figure 2-3. Dimensions and paths of beets, peppers, and leafy greens. Beets, peppers, and leafy greens are all grown on the same small, sustainable farm, that is approximately 100m wide and 75m long. As the commodities were grown and irrigated in sections, samplers were able to treat each commodity as a different "zone." Zone 1 consisted of 3 repetitions walking down the same path, approximately 75m long, where each change in direction was 1 repetition, and was done n=3 times. Zone 2 was the location of the leafy greens. Due to the planting pattern of this farm, samplers had to divide Zone 2 into 3 walking paths, where walking down and back on the same path was 1 repetition a total of n=3 times, spacing it equally between 3 rows of leafy greens, totaling approximately 72m for each repetition. Zone 3 was conducted in a similar manner to Zone 2 for the pepper plants. This sampling sequence was repeated 14 days later, totaling n=6 repetitions for each commodity (beets, leafy greens, or peppers).

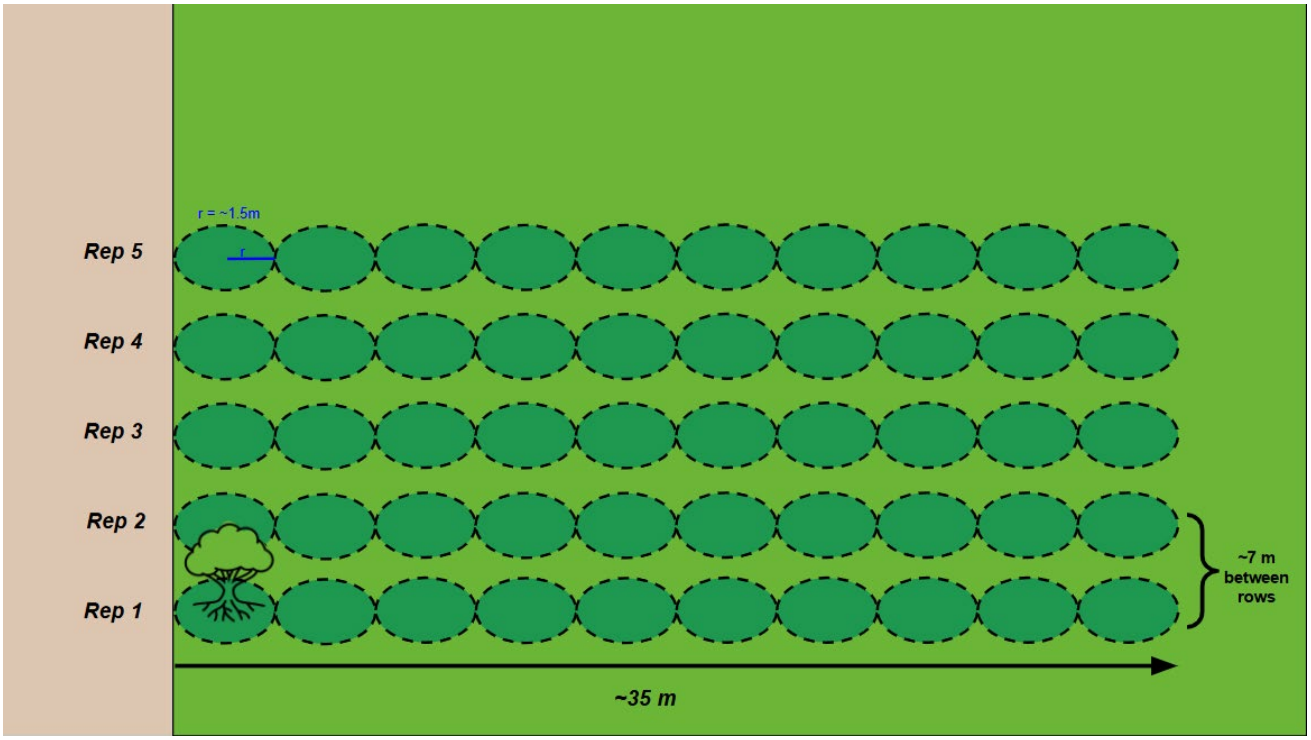

Figure 2-4. Dimension and walking paths of southern IL orchard. The samplers sampled 5 consecutive rows of apple trees in an orchard. Each row of trees was approximately 7m apart, and the length of 10 consecutive trees was approximately 35m, where trunk to trunk each tree was approximately 3m apart.

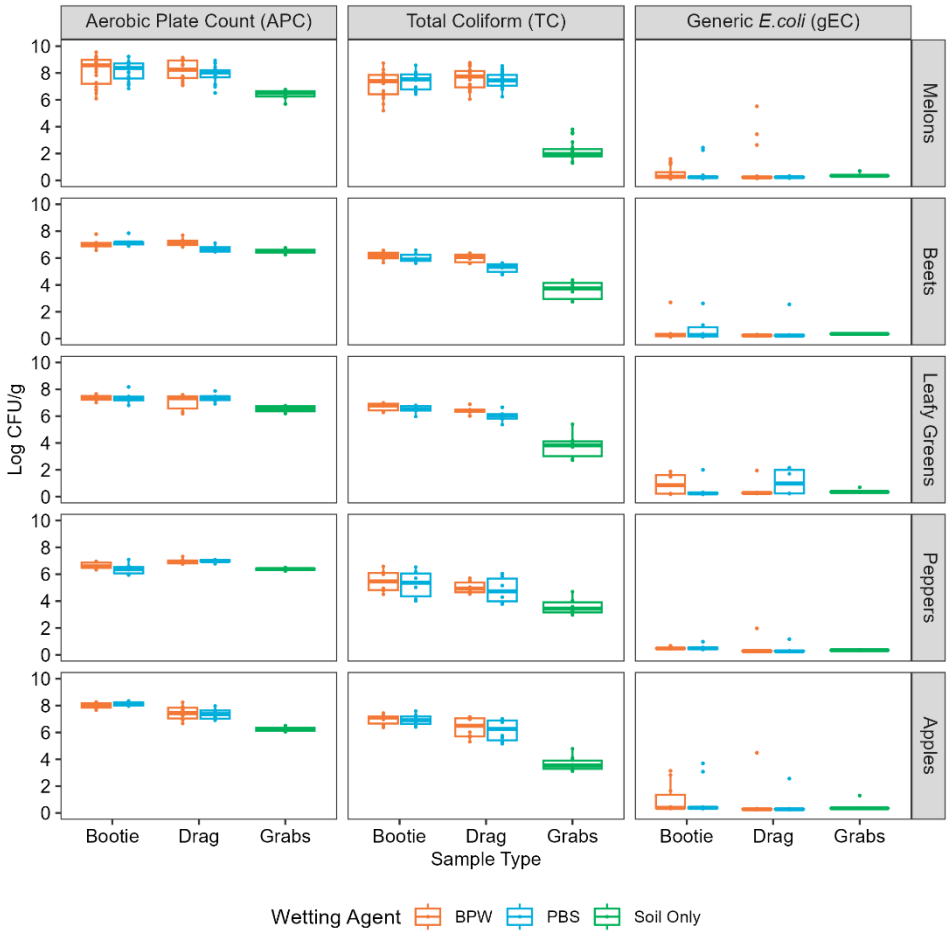

Figure 2-5. Summary of enumeration results for each sample collection method, wetting agent, and commodity. The boxes represents mean, Q1, Q3, and whiskers show 1.5x the interquartile range. gEC data points, if measured below the LOD, ~0.7 log CFU/g, are graphed at ½ LOD, ~0.35 log CFU/g.

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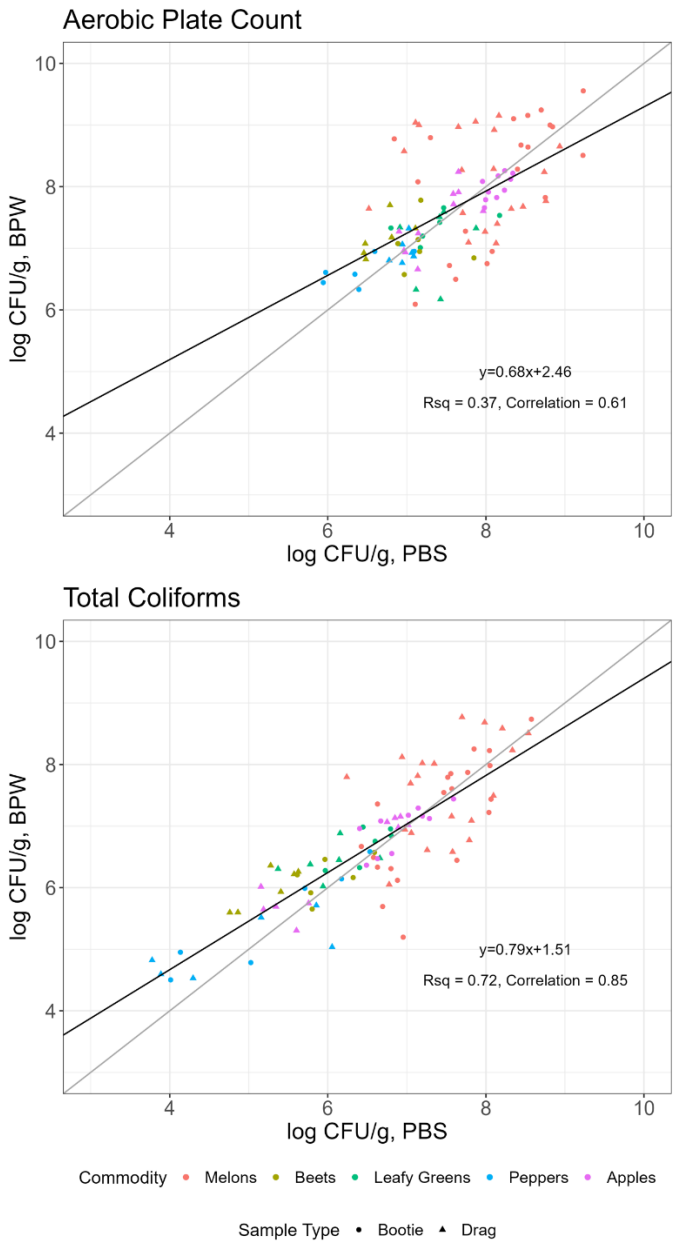

Figure 2-6. Comparing the performance of both wetting agents for APC and TC. The shapes represent different collection types, and colors represent different commodities. The grey line is the reference y = x graph, and the black line represents the linear relationship between the log CFU/g detected of APC or TC by PBS and BPW as wetting agents. Slope, R^2, and correlation coefficients are on the corresponding graphs for the black line. Both graphs have a positive correlation between log CFU/g detected by PBS and BPW as wetting agents. Matched pairs comparison for sample collection method can be seen on Table 2-1.

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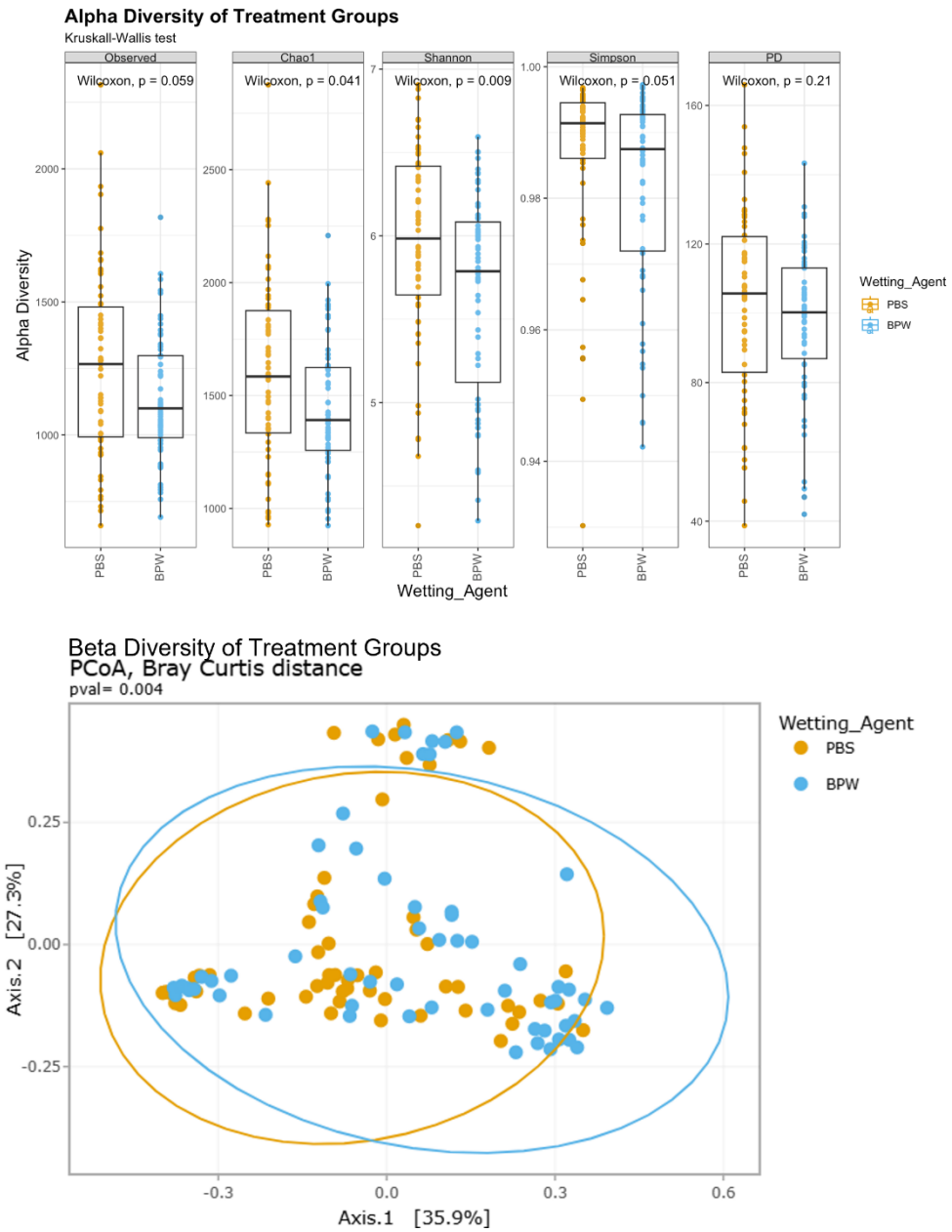

Figure 2-7. Alpha and Beta Diversity of V3V4 targets by Wetting Agent. (Top) Alpha diversity of selected Fluidigm targets in the top figure compares the alpha diversity by wetting agents. Wilcoxon signed-rank comparisons were done to compare individual box and whisker plots, where the box represents mean, Q1, Q3, and whiskers show 1.5x the interquartile range, and each dot represents an individual sample. Each individual dot represents an individual sample. (Bottom) Beta diversity analyzes microbiome compositional differences between the soil collected by different wetting agents, shown on PCoA Bray-Curtis distance.

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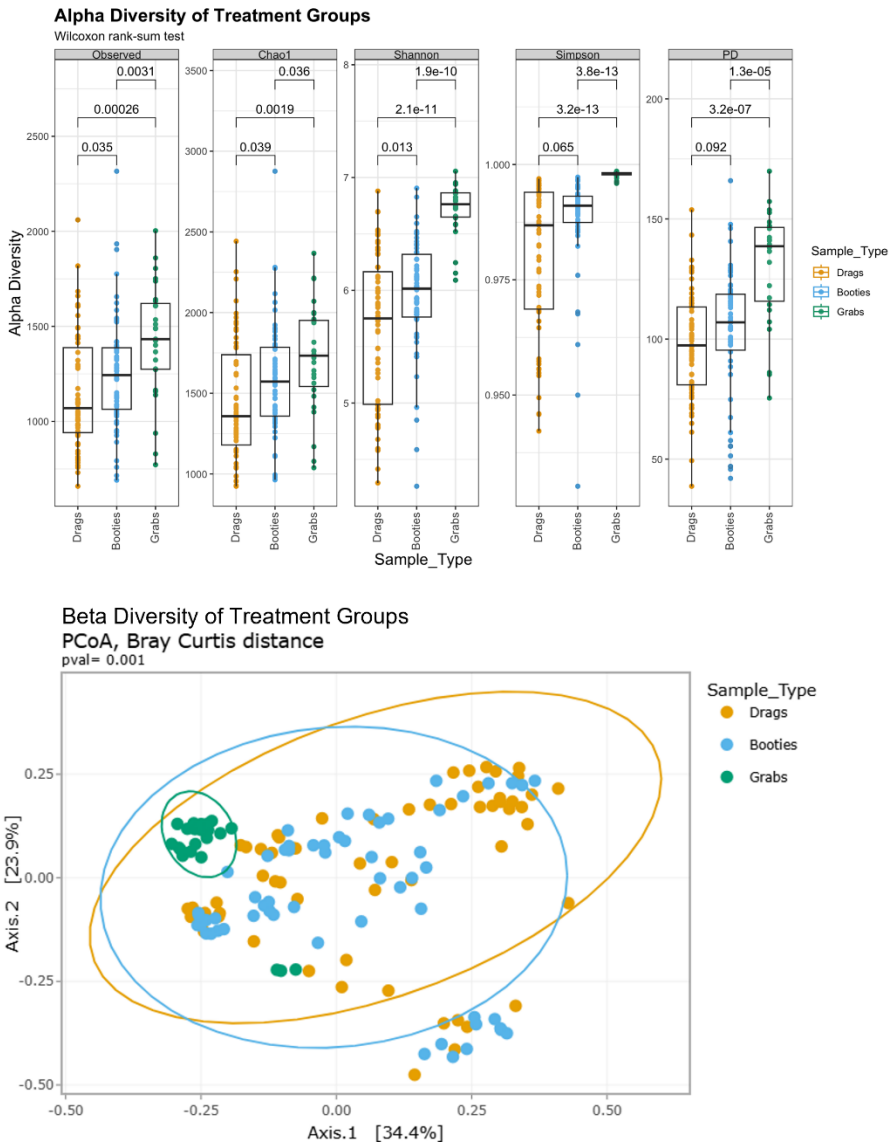

Figure 2-8. Alpha and Beta Diversity of V3V4 targets by Sample Type. (Top) Alpha diversity of selected Fluidigm targets in the top figure compares the alpha diversity by sample collection method. Wilcoxon signed-rank comparisons were done to compare individual box and whisker plots, where the box represents mean, Q1, Q3, and whiskers show 1.5x the interquartile range. and each dot represents an individual sample. Each individual dot represents an individual sample. (Bottom) Beta diversity analyzing microbiome compositional differences between the soil collected by different sample collection method, shown on PCoA Bray-Curtis distance.

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