

CA Lettuce Research Board 2007-08 Interim Research Report Summary

Project Title: Concentration and Deposition of Viable *E. coli* in Airborne Particulates from Composting and Livestock Operations

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Immediate Objectives:

1. Verify and improve practical bioaerosol detection and recovery platforms for viable *E. coli*/fecal indicators and pathogens and detection and molecular confirmation of viable but non-cultureable cells of target pathogens in aerosols.
2. Determine the recoverable concentrations of viable *E. coli*/fecal indicator and pathogens (**EFIP**) in ambient air, and in airborne source particulates, in bidirectional gradient modes (prevailing upwind and downwind) within and beyond current industry standards for source separation from composting and livestock/animal operations.

Summary of Preliminary Findings:

- Methods to be used in this project to detect, recover, and culture-confirm *E. coli* O157:H7, non-O157 EHEC, shigatoxin gene carrying enteric coliforms, and other presumptive indicators of fecal matter transfer were verified on cattle feedlot and environmental samples.
- Media to recover environmental and potentially environmentally-stressed cells of *E. coli* were verified using available aerosol capture equipment.
- Aerosol capture of high populations of viable *E. coli* within 30 feet and diffuse concentrations at 100 feet of a feedlot point source was demonstrated. Under the same conditions detection was not observed at 200 feet or greater.
- Direct detection of pathogenic *E. coli* DNA from aerosol capture events using Button Samplers was demonstrated at 30 feet of a feedlot point source.
- Aerosol capture of EFIP was not demonstrated in multiple attempts at an experimental leafy green trial (AC3) adjacent to a dairy facility within or beyond the key 'metrics' described in Lettuce and Leafy Greens Commodity Specific Guidance documents.
- Detection and recovery of EFIP was infrequently demonstrated at various times and locations on the AC3 plants and immediately adjacent environmental samples. No gradient or pattern of dispersal of these positive events from the dairy was observed.
- Culture confirmed *E. coli* O157:H7 and presumptive non-O157 EHEC were detected on plant samples during preharvest growth.

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Project Status: Due to delays in securing acceptable field sampling locations, this project has continued into 2008 per standard no-cost extension provisions of the CLRB contract. Unexpended funds from 2007 will cover these research activities. The report will serve as an Interim Final Report on research outcomes to date.

Introduction: Animal and compost production facilities have long been known to be associated with the transfer of microorganisms via airborne particulates and bioaerosols. Lettuce and leafy greens metrics require that growers keep a 30-400 feet distance between their fields and any adjacent livestock, compost production facility, or other significant source of fecal or compostable organic matter likely to contain or be subject to food-borne pathogen contamination matter. The foundation assumptions and appropriateness of these distances have not been adequately tested in environments pertinent to production of these commodities in California. This project will conduct an analysis of the concentration of viable nonpathogenic *E. coli* and related bacterial fecal indicators and pathogens in airborne particulates transferred from livestock operations or composting production facilities to adjacent crop fields. Depending on the level of cooperation, at compost sites, bioaerosol assessments will be done during mixing and turning of piles, while at livestock operation facilities, assessment will be done during manure scraping/collection, vehicle and animal movements and loading and unloading of vehicles with animals and manure.

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1. Verify and improve practical bioaerosol detection and recovery platforms for viable *E. coli*/fecal indicators and pathogens and detection and molecular confirmation of viable but non-cultureable cells of target pathogens in aerosols.
2. Determine the recoverable concentrations of viable *E. coli*/fecal indicator and pathogens (**EFIP**) in ambient air, and in airborne source particulates, in bidirectional gradient modes (prevailing upwind and downwind) within and beyond current industry standards for source separation from composting and livestock/animal operations.

Methods: Three different types of air samplers are being tested.



SKC Button samplers: They are small in size compared to the other two samplers. Button samplers are fitted with cellulose acetate membrane filter or with gelatin filters during the sampling procedure. The filters can then either be plated on appropriate recovery media or dissolved in buffer and then subjected to membrane filtration capture to increase sensitivity. They are used to determine the **EFIP** content of the samples taken by culturing or detection of target DNA.

Andersen Samplers: For our purposes, these are being used to measure the concentration and particle or aggregate-size association of EFIP in air. The sampler is a multi-orifice, cascade impactor designed to size-sort particles aerodynamically before they are collected on the surface of an agar medium. By understanding particle-size distribution from the types of sources mentioned above, it will be possible to predict their aerodynamics, and therefore distances to deposition, from well established predictive models. These model generated curves will allow us to test the current assumptions of food safety standards and metrics related to adjacent land use and proximity.



MAS Air samplers: These are portable, light weight air samplers that are able to sample at 100L/min. We have used the six units provided to us by the Western Institute of Food Safety and Security at UCD (R. Atwill, WIFSS) to recovery viable EFIP particles from several environmental locations. Though particle-sizing data is not possible, these are easy to use and program with reliable, reproducible results and capable of collecting between 0 to 2000 liters. Internally generated suction draws air into the sampler onto agar plates placed inside the machine.

Samplers are mounted on tripod stands downwind at various distances from 30 to 400 ft from the composting or livestock facility. Depending on the site-specific objective, samplers are arranged in a linear or vector array. All samplers are run simultaneously during the sampling period. Meteorological data documenting the conditions at the time of collection will be acquired using a Hobo on-site unit or, as necessary, will rely on the nearest CIMIS station. Preliminary tests have been done at several collection sites to standardize the best media for the experiment as well as the best protocol for analysis of the samples. In addition to air samples, various other environmental samples will be also be collected from the compost and livestock operation facilities, if permission can be arranged, as well as from the adjacent crop fields upwind and downwind of these facilities at the time that the bioaerosols will be collected. An experimental site near an adjacent source of concern, in a commercial production region, was secured that allowed us to conduct crop sampling over time. Sampling at different distances from the source from emergence to maturity was undertaken to attempt to determine, more broadly than aerosol capture studies alone could reflect, the survival of bacteria on airborne particles following deposition.

Preliminary Trials: Visits to several compost and livestock facilities that were adjacent to grower operations for potential testing locations in Central Coast region were done prior to any experimentation. Several facilities that were offered for testing were located too far from the contamination source or were located upwind of the contamination source with assurance of downwind sampling and therefore were not ideal for running

bioaerosol transfer studies. Some sites were used for preliminary tests to get familiar with the equipment and to standardize the procedure and media for the experiment.

Trial 1: Done at a compost facility using all three kinds of samplers. Samplers were placed downwind, approximately 100ft, of the compost windrows (new-wet and finished-dry) and the windrows were turned at the start of each sampling period. The samplers were able to capture large amounts of environmental background (Gram-positive bacteria and molds); however most plates were overcrowded due to the high density of growth and therefore did not give any patterns. Direct sample collection from finished compost had no detectable generic *E. coli* and very low coliform counts. Direct sample collection from new compost windrows (high moisture/low particulate release on turning), containing dairy manure, were strongly positive for coliforms and generic *E. coli* but these viable colonies were not detected in aerosol capture equipment on the same media used to assess viability of the windrow source material. All samples were negative (1 < 100gm; n=5) for pathogenic *E. coli* using rapid test kits.

Interval Trial: Detection and Recovery of *E. coli* O157:H7 from Feedlot Bedding

Tests were conducted to confirm operator ability to detect and recover *E. coli* O157:H7 from naturally contaminated environmental samples that would be typical of aerosolized particulates targeted in this project. Two feedlot materials were collected by Co-PI Millner and provided to Suslow Lab. Ninety ml of pre-warmed mEHEC enrichment media was added to 10g of the feedlot bedding material. In addition, 100 g of feedlot bedding material was suspended in 200 ml of a sodium phosphate plus Tween extraction buffer, thoroughly manipulated to break up all large aggregates and allowed to settle for 30 min. Ninety ml of mEHEC was added to 10ml of the partially clarified upper phase of the extraction suspension. After enrichment, these samples were tested using GDS *E. coli* O157:H7 and one sample was found to be positive. The immunomagnetic capture beads from the positive GDS O157 enrichment were diluted and plated out on Chromagar O157:H7. Dark pink colonies (atypical of the expected mauve/lighter pink colonies presumptive for *E. coli* O157:H7 on this media) were among the various recovered possible positive colonies that appeared on the plates and these single colonies were picked and restreaked to purify. DNA was extracted from these colonies and various characterization tests were conducted. Multiplex PCR diagnostic for O157:H7 was run on the single colony DNA extracts. They were positive for *stx2*, *eae* and *hlyA*. GDS O157 was run on some of the single colonies and they were positive for *E. coli* O157:H7 and with GDS Shigatoxin they were positive for SLT2 (equivalent to *stx 2*) and negative for SLT1. The single colonies were tested using an immunodiagnostic test (RapidCheck – SDI) and were all positive. Presumptive colonies were also tested using the FDLB genetic markers (CADHS RIMS Method FM-613 for O157:H7) and were positive for *stx2* and *uidA*, but negative for *stx1*. Collective evidence from the various identification schemes supported the detection and recovery of culture-confirmed isolates of *E. coli* O157:H7 from feedlot bedding material by the Suslow lab.

Trial 2: MAS samplers were used at a UC livestock research facility in very close proximity to animals from a herd known to persistently shed *E. coli* O157:H7. Elevated samplers were positioned at a standard height of 1.5 meter from ground level and a distance of 3 to 10ft from the corral fencing and chute barrier at the animal inspection shed. Successful capture of very low numbers of environmental coliforms was

demonstrated. Twenty-five to thirty animals were manually stimulated by sound to move frequently, as a group, in a holding pen with a dense layer of dry bedding and new fecal matter, or prodded with a plastic cow-paddle to encourage rapid movement through the inspection chute. The bedding and recently deposited cow pats were subsequently shown by sampling conducted by UCD host-faculty from the Dept. of Animal Sciences to be positive for *E. coli* O157:H7 on the day of aerosol trapping. In addition, groups of heifers were made to pass through a narrow dry-manure impacted chute for pregnancy testing, not connected with this experiment, and therefore kicked up dust during herding that was captured by the air samplers. As before, most plates were overcrowded with environmental background on non-selective plates and selective media for EFIP had only a few coliform colonies on one sampling period. Impression samples of corral surfaces using the same EFIP media were positive for generic *E. coli* and coliforms. Samples were also taken from several water troughs as well as bedding material. One water sample from a trough was found to be positive for *E. coli* O157:H7 using rapid test kit, however, viable isolation was not successful. In independent sources of feedlot bedding material from a known colonized herd, the same techniques were successful in our lab for detection and viable colony recovery of multiple *E. coli* O157:H7 isolates in two separate screenings.

Trial 3: A grower cooperator was identified and a site to establish a non-harvested, simulated commercial leafy green experimental site upwind of a large livestock facility (Fig. 9). For reporting, this site-code is referred to as AC3. For this trial the MAS air samplers and SKC button samplers were used, as equipment problems with Andersen Samplers were encountered. The trial sub-objective was to finalize a recovery media during sampling as well as to assess the capture of background bacteria during normal pre-harvest field operations such as bed shaping, planting, and equipment movement that may impact an adjacent existing lettuce or leafy greens crop.

In addition to air sampling, due to the upwind position, failure to secure cooperation from the dairy for downwind and direct-source sampling, and limitations on duration of aerosol trapping, plant samples were taken at varying distances from the dairy side to evaluate the potential for detection of EFIP within and beyond the boundaries of LGMA compliance criteria. Source samples from the immediately adjacent environment, under the control of the grower cooperator, were taken to establish the presence of a trackable EFIP that would potentially be deposited on the experimental crop as it emerges and grows to harvest maturity.

1st sample collection (02/13/08)

Eighteen bioaerosol samplings using MAS were taken by collecting 100L/min during 10 minutes to assess our sampling methodology for EFIP prior to ground-work and seedbed preparation activity and seeding dates. Wind direction during this period positioned the dairy and solids-spreading areas in a downwind position from AC3. In addition, 10 environmental samples (water, soil and mud) from the tailwater ditch and the storm runoff diversion ditch adjacent to the farm access road between the dairy and AC3 were taken to conduct an initial screen and characterization of EFIP populations at the test area.

During transportation, samples were kept in a cooler containing a layer of frozen gel-ice and upon arrival in the Mann Lab (typically 6-8h) stored overnight at 2.5C prior to

microbiological analyses the following day. Thermotolerant coliform and generic *E. coli* were enumerated using appropriate media by direct plating or by membrane filtration. Samples were also subjected to standard enrichment prior to screening for *E. coli* O157:H7 or other pathogenic *E. coli*.

AC3 Results from Sample Date 1:

All bioaerosol samples were negative for generic *E. coli* and thermotolerant coliform. Given these outcomes, we decided to reassess our recovery media to assure it would allow for recovery of desiccation-stressed cells in dairy bedding particulates. It is recognized that aerosol sampling can create desiccation-stress to cells during collection of 1000L volumes, though this is more typical for aerosolized water droplets. Our concern was that we would underestimate the viable, culturable population as a consequence of using selective and differential media to detect *E. coli*. Nonpathogenic *E. coli* were not detected in any environmental sample, at the detection limit used, and thermotolerant coliform populations were variable by source (Fig. 1). In addition, all samples were negative for *E. coli* O157 using Biocontrol Assurance GDS® detection technology.

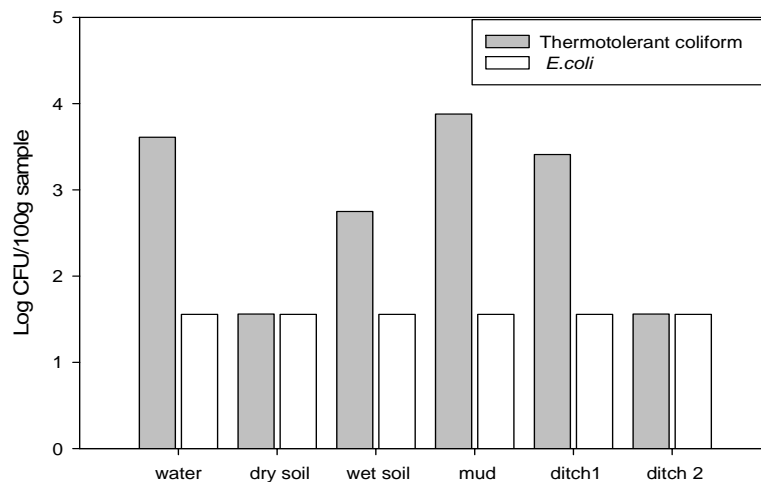


Fig. 1 – Presence of thermotolerant coliforms and non-pathogenic *E. coli* in environmental samples surrounding the experimental planting. The Limit of Detection was log 1.47 CFU/sample.

Interval Trial: UCD Feedlot

In response to the results observed in pre-emergence sampling at AC3, additional developmental experiments were done at the UC Davis Feedlot to verify or improve the bioaerosol sample recovery methodology. Three collection time points of 10 dry bedding samples were taken to determine the EFIP (mostly thermotolerant coliform and *E. coli* populations) at corresponding bioaerosol sampling dates. Paired to this source-sampling, 30 bioaerosol samples were collected using MAS to compare the efficacy of general media, selective media amended with sodium pyruvate (incorporated into our standard method for stress-recovery) and selective media plus pyruvate plus a resuscitation membrane. Background samples, when visual evidence of dust or particulates could not

be detected, were taken to compare with manual activity within the animal pens to generate a suspended particulate source from the feedlot bedding. Bioaerosol samples were collected at downwind distances of 30-400 feet from the feedlot point-source. The results indicated that the best media to recover viable coliform and generic *E. coli* is the standard selective media plus pyruvate (ECCP) without the membrane. The average *E. coli* population in the bedding material was essentially log 7.0/100g of bedding material. Aerosol capture at 30 feet with ECCP consistently recovered 200 *E. coli* /1000L of air in comparison to 50 CFU/1000L air at 100 ft and non-detectable levels at greater distances. From these results we concluded that ECCP was adequately sensitive to allow the recovery of environmental sources of *E. coli* and thermotolerant coliforms associated with dry, aerosolized particulates. Non-detection at AC3 was indicative of an absence of significant population densities at the specific collection point and time. Without manual or front-end loader activities to promote aerosolization of bedding particulates, no recovery of viable *E. coli* or thermotolerant coliform was detected, even at 30 feet from the source.

Additional capture tests were conducted at the UC Feedlot using button samplers to assess direct detection of pathogenic *E. coli* using real time PCR. Samples collected at 30 feet for 30 min and processed, as described above, were positive for virulence associated markers.

2st sample collection (02/27/08)

From a downwind position, 18 bioaerosol samples were collected using MAS throughout planting activities. In addition, 18 bioaerosol samples were collected during simulated bed-shaping that created air-borne particulates and dust: linear array samples were taken from distances of 10 to 100 feet from this activity and compared to samples from 250 to 400 feet. Each bioaerosol sample was collected for 10 minutes with 100L/min. At this time a comparison between the two best media, previously tested in the lab during this project period to enhance recovery of *E. coli* and thermotolerant coliform, was made to continue the process of optimizing the recovery methodology for viable bacteria in bioaerosols. During the same period of preharvest agricultural activities, 6 button samplers with gelatin filter inserts were run for 30 minutes each with a flow of 15L/min to collect bioaerosol particulates for DNA-based screening methods assessments.

Fifteen environmental samples from sites surrounding the plot close to the dairy (soil, mud, run-off, ditch water, mud and algae) and 30 soil samples from the field used for this study were taken. Field soil samples taken from pre-existing beds that had not been pre-irrigated were very dry at the time of collection. All samples were transported in coolers and processed the following day after been stored overnight at 2.5 C. Soil samples were enriched and composited into 10 samples for analysis according to the distances from the dairy that they had been collected. Background coliforms and generic *E. coli* were also enumerated from composites ranging from 30 to 400ft from the dairy.

Results (02/27/08):

During the extended bed shaping activity very low numbers, 25 CFU or fewer/1000L of thermotolerant coliforms were detected from samples taken at 10 to 100 feet from the upwind dust/particulate source. In comparison, 10 CFU or fewer/1000L of thermotolerant

coliform were detected at sampling distances 250 to 400 feet from the source activity. No viable *E. coli* or thermotolerant coliform were detected in 1000L of captured-air during the planting activity which generated very little visible particulates.

Nonpathogenic *E. coli* were not detected in soil samples (Fig. 2), were found in very low numbers in irrigation overflow ditch water, but were not detected in irrigation runoff water collected directly at the ends of planted furrows (Fig. 3).

All samples were negative for *E. coli* O157:H7 and shigatoxin-genetic markers using Biocontrol Assurance GDS® detection with the exception of one environmental sample that was positive for shigatoxin genes (Table 1). Viable colonies were recovered and determined not to be consistent with *E. coli* O157:H7 but appear to be nonO157 STEC (Shiga-toxin producing *E. coli*); final characterization is in progress.

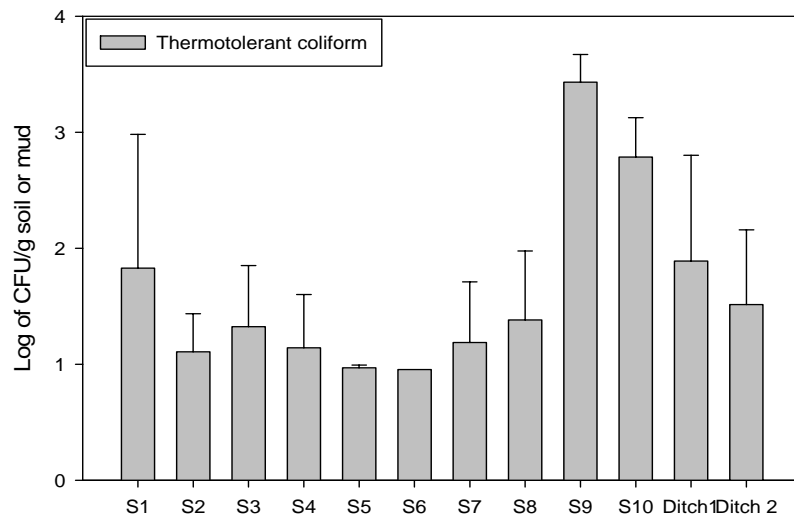


Fig. 2 – Background thermotolerant coliform populations in soil composites from pre-plant field samples and an adjacent irrigation overflow collection ditch at AC3. No nonpathogenic *E. coli* were detected among these samples at the detection limit of the assay (log 0.9CFU/g).

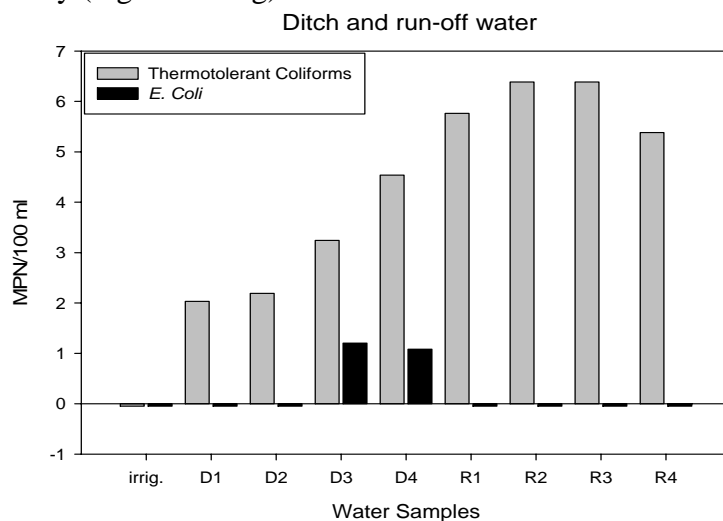


Fig. 3 - Background thermotolerant coliform populations in irrigation source water, an adjacent irrigation overflow collection ditch, and irrigation run-off collected during the post-seeding irrigation at AC3. Nonpathogenic *E. coli* were detected among these samples at the detection limit of the assay (<1 MPN/100ml).

AC3 3rd sample collection (03/24/08)

Spinach plants at baby leaf stage, 26 days since the planting wet-date (PWD), were collected immediately prior to an overhead irrigation event. Plant samples were processed for general microbial background and EFIP to establish whether a spatial deposition gradient had established relative to the dairy. The hypothesis was that a higher EFIP populations on plants located closer to the dairy would be potential evidence for aerosol transfer or a risk-indicator of adjacent land use activity consistent with LGMA metrics. Plants were collected at 6 spatially distinct sampling line perpendicular to the approximate prevailing upwind and sporadic down wind direction relative to the dairy and dairy access road frequented by solids-hauling vehicles. Alleys of sampling, 25cm wide from the entire bed, were created at every bed ($n = 9$). Sampling position distances were 5, 46, 61, 91, 122, 396 m (16, 150, 200, 298, 400 and 1300 feet) from the dairy border, and each sample had approximately 400g for each of the 54 baby spinach samples.

An additional 7 environmental samples (ditch water) were taken on this date.

Aerosol sampling was conducted for about 2 hours, from 5:30 to 7:30pm at the time of day when animals are most active, before sunset. This temporal focus was selected to correspond to the times when the creation of volumes of suspended 'heifer dust' were present. Forty-two bioaerosol samples using MAS were taken during this time interval. During this and surrounding dates the hourly wind direction has been shifting to put the AC3 site in a down and cross-wind direction from the source dairy. However, these periods were brief and inconsistent. Unfortunately, at the specific sampling time the AC3 site was upwind.

All samples were transported in coolers and kept at 2.5C overnight until the processing time the following day. In the lab, plant samples from each collection-distance point were combined as three groups (beds 1, 2 & 3; 4, 5 & 6; 7, 8, & 9). Twenty five grams of leaves were randomly selected from each composite sample and were washed with 75ml BPW and plated directly on non-selective or semi-selective/differential agar for the general microbial background and EFIP enumeration. Also, 150g of leaves from each composite sample were combined with 400 ml of mEHEC enrichment broth and held at 42C for 18h; cultures were then processed for presence/absence (P/A) screening for *E. coli* O157:H7 and Shigatoxin markers. In addition, another 150g composite sample was added to 400 ml Buffered Peptone Water (BPW) and held at 37C for 18h for P/A screening for *Salmonella*. Any residual plant samples were kept at 2.5C.

AC3 4th sample collection (03/25/08)

Plants samples were collected immediately subsequent to the AC3 3rd sampling following an overhead irrigation event. Samples were collected at the nearest proximate distances on beds 1 and 9 to the previous day samples. Plants were processed following the same protocol described above to determine general microbial background and EFIP. An additional 13 environmental samples were drawn from run-off water, ditch water, and algae, as well as another 12 bioaerosol samples using MAS.

AC3 Results 3rd and 4th sampling:

No viable *E. coli* or thermotolerant coliform was detected among any of the 54 aerosol samples, giving a result of less than 1 CFU/1000L of air under the prevailing conditions.

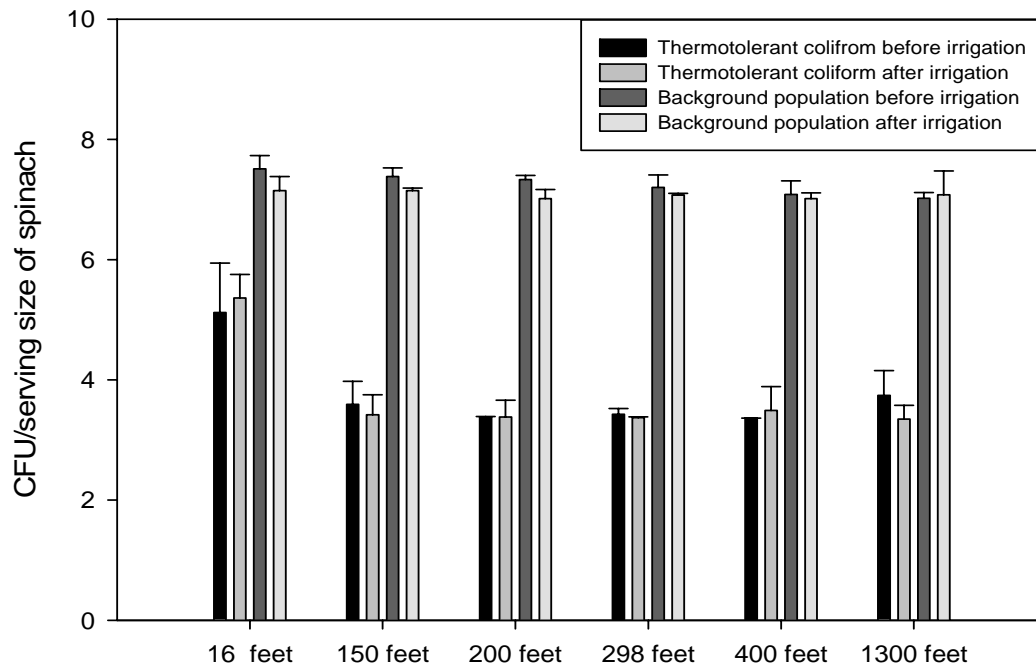


Fig. 4 - Influence of overhead irrigation on recovered total aerobic mesophilic bacteria (background), thermotolerant coliforms, and nonpathogenic *E. coli* from spinach leaves at AC3. Only two leaf samples had detectable *E. coli* above the non-normalized detection limit of log 1.47 CFU/g or log 3.4 CFU/prewash serving size (85g) (data not included on figure).

Moderate evidence for a gradient effect (Fig. 4 and Fig 7), relative to adjacent land use, was observed based on the bacterial populations evaluated on plant samples. No evidence for an influence of overhead irrigation on comparable populations within 16h of the irrigation event was observed.

All plant samples were negative in screening for *E. coli* O157:H7, non-O157 EHEC, STEC, EPEC, and *Salmonella* with the exception of one composite sample at 61m (200 ft) in beds 1-3, closest to the farm west-perimeter road.

Analysis of Positive Composite Leaf Samples (from 03/24/08)

An additional analysis of the positive composite spinach leaves was done by exposing the retained samples to elevated temperature to determine whether the harvested leaves could serve as an initial enrichment of low levels of survivors. Leaves harvested from the positive section at 200 feet from the road/dairy and already composited as 200-1 (beds 1,2 &3), 200-2 (beds 4, 5&6) and 200-3 (beds 7, 8 &9) were washed with tap water treated with sodium thiosulfate to neutralize residual chlorine, drained of excess free water and sealed in micro-perforated film appropriate for spinach. Two replicate thermally sealed bags were filled with 60g of washed leaves per sampled area and stored at 7.5, 10 and 15C for 96 hours. For detection and recovery processing, 160 ml of mEHEC was added into each 60g sample and analyzed by GDS-PCR for *E. coli* O157 and Shigatoxin run after 8 and 18 hours enrichment.

All results for *E. coli* O157:H7 and Shigatoxin were negative.

AC3 5th sample collection (04/15/08)

Spinach leaves at 48 PWD were collected at the same 6 proximate distances described above for sample date 3/24/08, from each of the 9 beds for a total of 54 samples of about 400g each. Additional environmental samples were taken (7 water samples and 5 algae samples). All samples were transported and processed in the same way as described above.

Results:

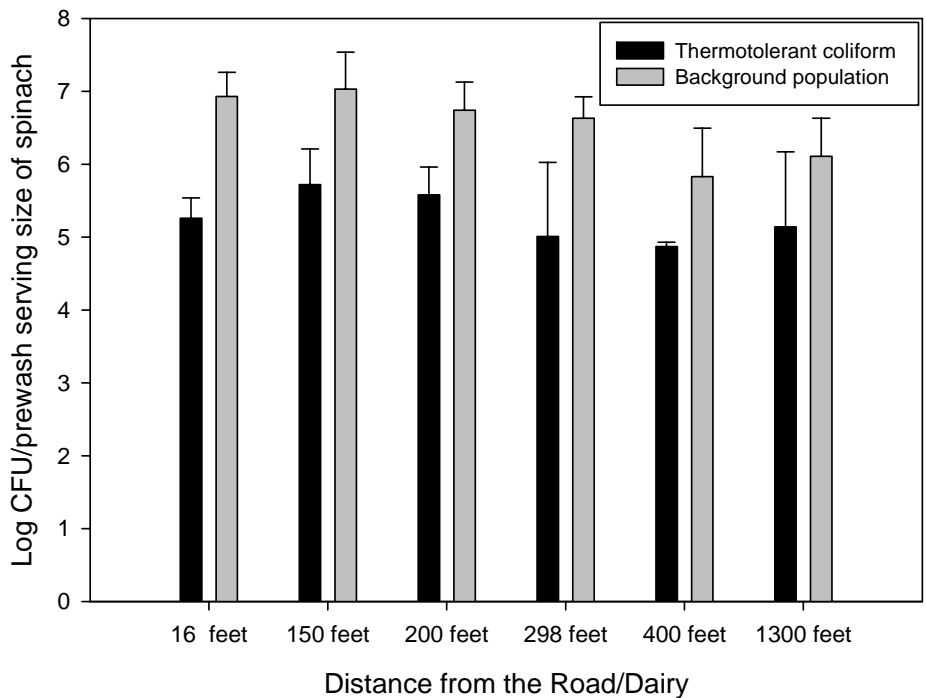


Fig. 5 – Evaluation of total aerobic mesophilic bacteria (background), thermotolerant coliforms, and nonpathogenic *E. coli* from spinach leaves at AC3

as a function of proximity to the adjacent dairy and associated activities. Only two leaf samples had detectable *E. coli* at the non-normalized detection limit of log 1.47 CFU/g or log 3.4 CFU/prewash serving size (85g).

All plant samples were negative in screening for *E. coli* O157:H7, non-O157 EHEC, STEC, EPEC, and *Salmonella* with the exception of 1 environmental sample and 2 plants samples (composite beds 4,5,6 at 16 feet and composite beds 1,2,3, at 200 feet) which were all Shigatoxin positive (Table 1; Fig. 10).

No evidence for a gradient effect relative to adjacent land use was observed based on the bacterial populations evaluated on plant samples.

Analysis of Positive Composite Leaf Samples (from 04/15/08)

From each of the 2 composite plant locations that were positive for shigatoxin genetic markers, 25 leaves were randomly selected. Each leaf was placed into a bag with 2 ml of buffer, then manually macerated with a heavy instrument for 10 seconds and incubated in enrichment media at 42C for 18h. The DNA of each leaf-enrichment was extracted and real time PCR was done to screen for the presence of EHEC, STEC, or EPEC pathogens. All 50 samples were negative for genetic markers associated with pathogenic *E. coli* or shigatoxins.

AC3 6th sample collection (04/30/08)

Spinach leaves at 63 PWD were harvested immediately prior to an overhead irrigation event at the same 6 distances from the road described above from each of the 9 beds for a total of 54 samples of about 400g each. Eighteen additional bioaerosol samples were taken using MAS. No water or algae were present in the ditch at this time. All samples were processed as described above.

AC3 7th sample collection (05/01/08)

Spinach leaves were collected the morning following an overhead irrigation event at the same locations as the previous day, from each one of the 9 beds in a total of 54 samples with 400g each. Additional 12 bioaerosol samples with MAS were taken. All samples were processed as described above.

Results from Sample Dates 6 & 7:

No viable *E. coli* were detected in any aerosol samples. Very low levels of thermotolerant coliform, 5 CFU/1000L of air was detected in aerosol samples taken adjacent to the field.

No strong evidence for a gradient effect (Fig. 6), relative to adjacent land use, was observed based on the bacterial populations evaluated on plant samples. A moderate elevation of populations was observed within 16h of an overhead irrigation event on comparable populations.

All plant samples were negative for *E. coli* O157:H7 and shigatoxin-genetic markers using Biocontrol Assurance GDS® detection methodologies. All plant samples screened by real time PCR for the presence of virulence factors associated with EHEC, STEC, or EPEC pathogens were negative.

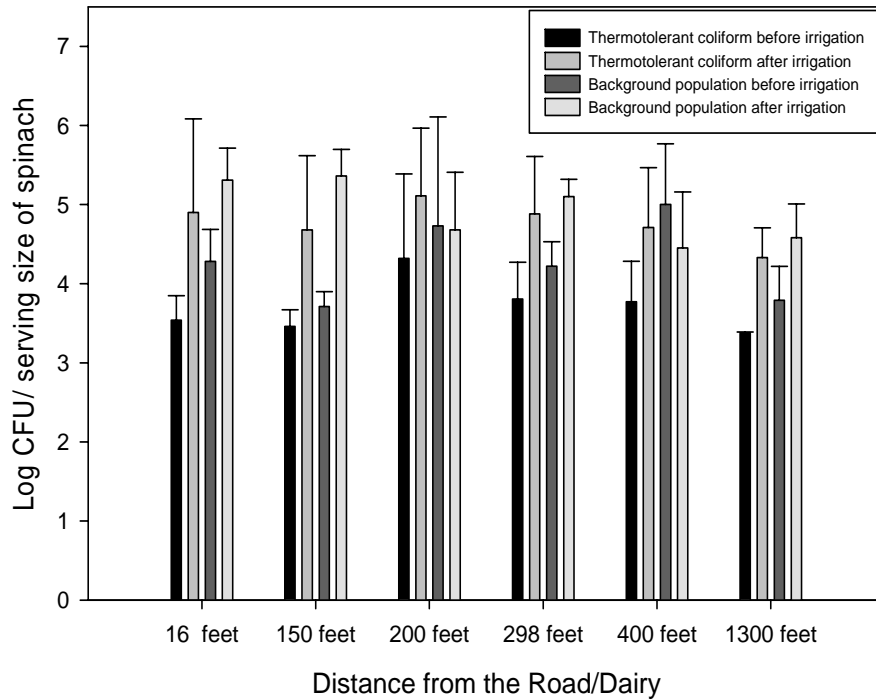


Fig. 6 - Influence of overhead irrigation on recovered total aerobic mesophilic bacteria (background), thermotolerant coliforms, and nonpathogenic *E. coli* from spinach leaves at AC3. No leaf samples had detectable *E. coli* at the non-normalized detection limit of log 1.47 CFU/g or log 3.4 CFU/prewash serving size (85g).

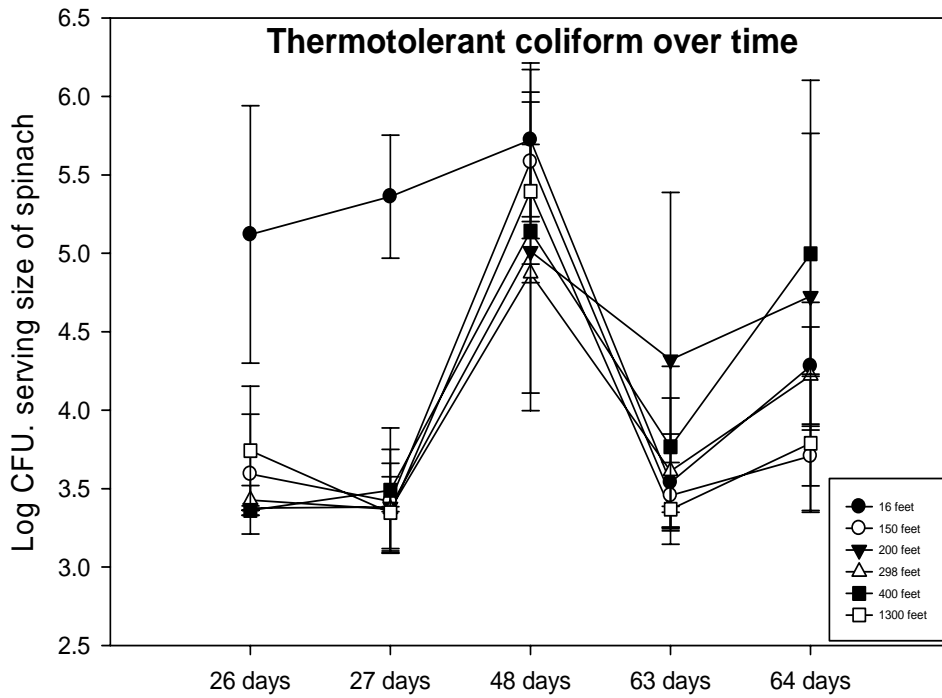


Fig. 7 – Variation in thermotolerant coliform on spinach leaves as a function of time post-seeding and distance from a dairy as a potential source of enteric microflora. Nonpathogenic *E. coli* were essentially below the limit of detection of log 1.47 CFU/g or log 3.4 CFU/prewash serving size (85g).

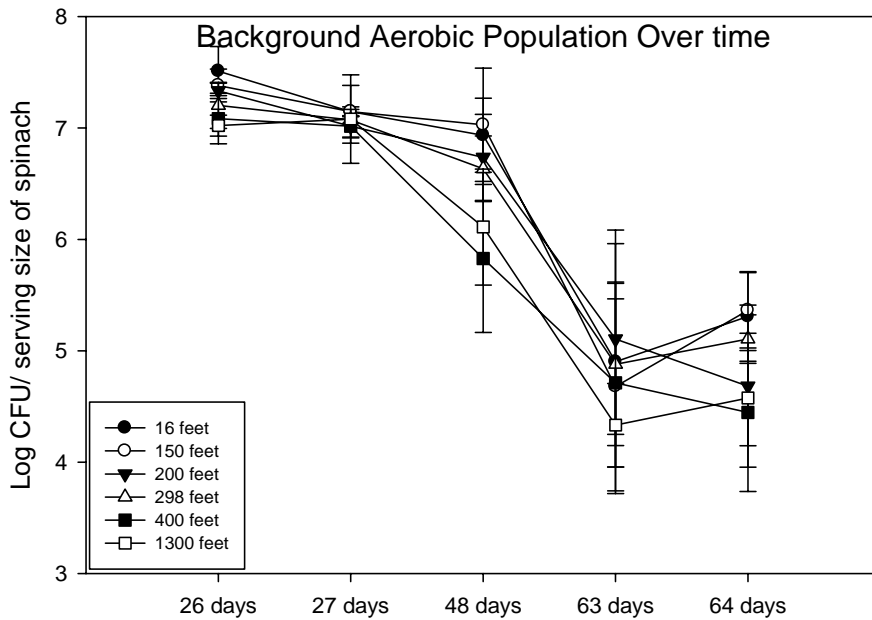


Fig. 8 - Variation in aerobic mesophilic bacteria populations on spinach leaves as a function of time post-seeding across the length of the experimental field planting.

Samples	Primary Screening Tests		Secondary Screening Test				Culture Confirmatory Tests	
	GDS E.coli	GDS Shigatoxin	Pathogenic E.coli	stx1 FDLB	stx2 FDLB	uidA FDLB	rfbE	Multiplex PCR
Algae from irrigation overflow ditch 02/27	negative	positive stx1	Positive				negative	stx1 eae hlyA
Presumptive colonies from algae enrichment 02/27			Positive	positive	negative	negative	negative	stx1 eae hlyA
Plant from 03/24 (1, 2 & 3 at 200 feet)	positive	positive stx1	Positive					
Presumptive colonies from positive plant sample enrichment 3/24			Positive	negative	positive	positive	positive	stx2 eae hlyA
Algae from irrigation overflow ditch 03/25	positive	positive stx1 and stx2						
Presumptive colonies from algae enrichment 3/25			Negative	negative	negative	negative	negative	negative
Plants from 04/15 (beds 1,2,3 at 150 feet)	negative	positive stx1 and stx2	Negative					
Plants from 04/15 (beds 4,5,6 at 16 feet)	negative	positive stx1	Negative					
Water from irrigation overflow ditch 04/15 (7 composited samples)	negative	positive stx1 and stx2						
Plants from 04/30 (beds 4,5,6 at 150 feet)	negative	negative	Positive					
Plants from 05/01 (beds 1,2,3 at 16 feet)	negative	negative	Positive					
Plants from 05/01 (beds 4,5,6 at 1300 feet)	negative	negative	Positive					
DNA isolation from aerosol capture with button samplers			4/4 positive					
Feedlot bedding source material (from 3 different sampling days)	negative	negative	15/20 positive					

Table 1 – Summary of key observations of potential target pathogen association with environmental or plant samples at AC3. Only outcomes from enrichment screenings that were positive for at least one category of Primary or first level Secondary screening are reported. Screening and confirmatory tests included;

- GDS E.coli – Biocontrol Assurance GDS AOAC Official Method 2005.04
- GDS Shigatoxin – Assurance GDS Shigatoxin AOAC Official Method 2005.05
- Pathogenic E.coli – A broad real-time PCR screening for pathogenic *E. coli* including O157:H7, other EHEC, STEC, EPEC (Suslow Lab; not an official method)
- FDLB – stx 1 (shigatoxin 1); stx 2 (shigatoxin 2); uidA (O157:H7) real-time PCR methods from CADHS Method FM-613
- rfbE - gene for O157 from Bertrand and Roiga Water Research. 41 (2007) 1280 – 1286
- Multiplex PCR – conventional PCR for stx1, stx 2, eae, and hlyA considered inclusive of O157:H7 and most EHEC

Total hours during experiment	1536
Total hours field downwind	131
% of time wind field downwind	8.5
When downwind average direction (degrees)	27.2
When down wind average speed (mph)	4.5
When downwind average temperature (F)	57.7

Table 2. Maximum and minimum wind speed when the planted field was at a downwind position from the dairy were 9.5 and 2.2 mph, respectively. During these periods the wind direction was towards a northeastern vector from 45-90 degrees.

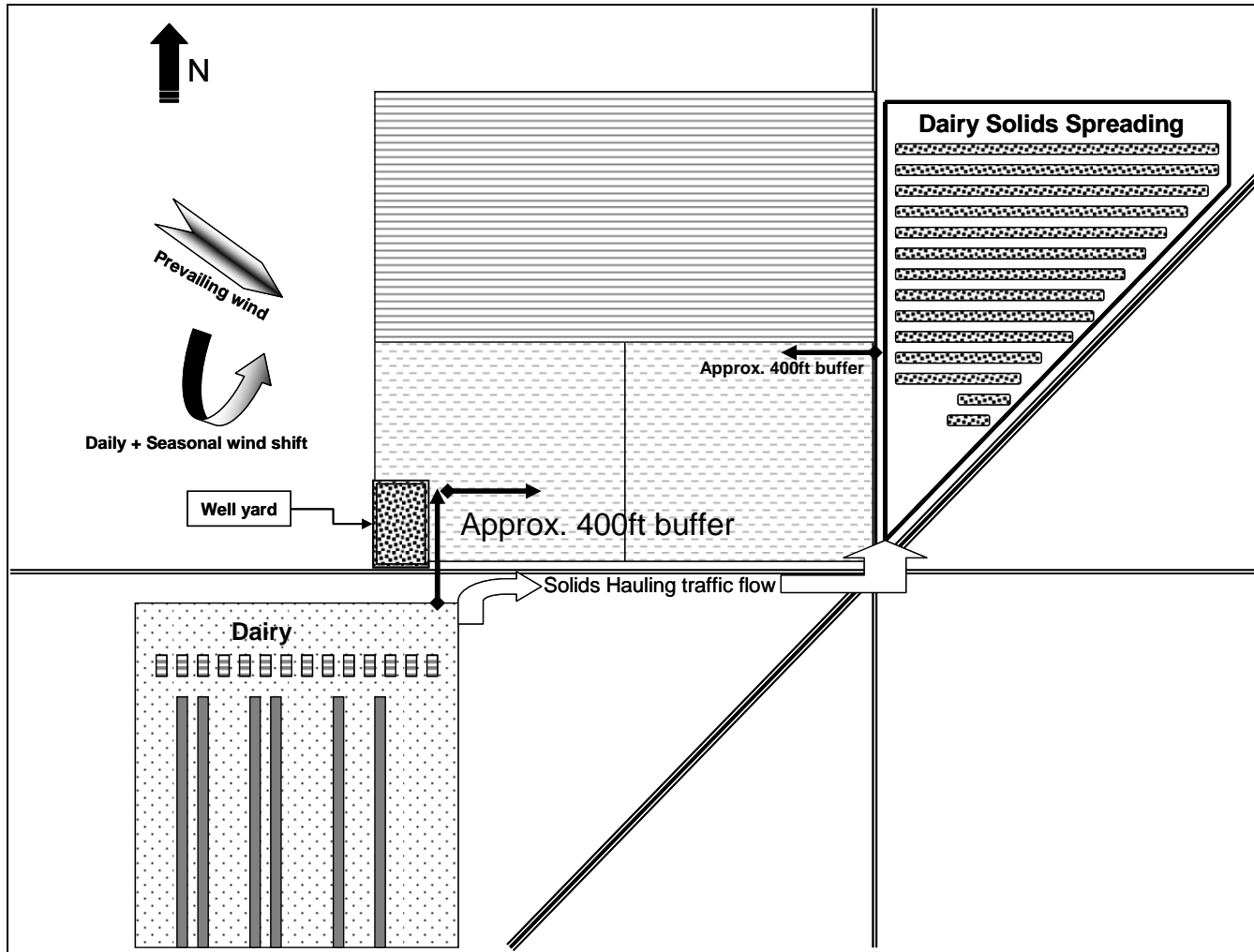


Fig. 9 – Schematic overview of experimental trial AC3

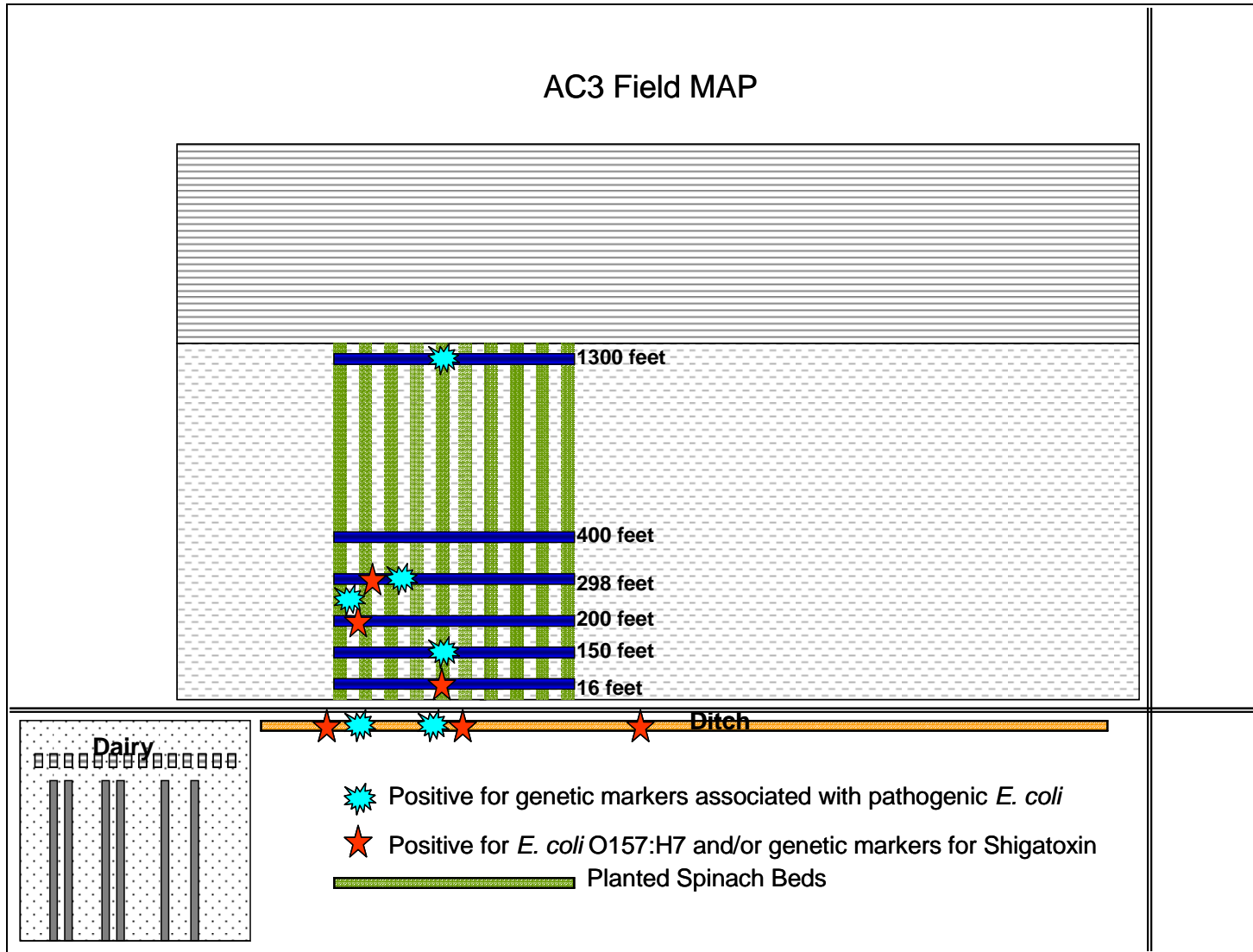


Fig. 10 - Schematic overview of experimental trial AC3 detection locations on composite plant and environmental samples.