CENTER for PRODUCE SAFETY

CPS 2009 RFP
FINAL PROJECT REPORT

Project Title
Mitigation of irrigation water using zero-valent iron treatment

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Principal Investigator
Kalmia E. Kniel
University of Delaware
Email: kniel@udel.edu
Phone: 302-831-6513

Co-Principal Investigators
Dallas G. Hoover, University of Delaware, dgh@udel.edu; 302-831-8772
Pei C. Chiu, University of Delaware, pei@udel.edu; 302-831-3104
Manan Sharma, USDA-ARS, manan.sharma@ars.usda.gov; 301-504-9198
Daniel Shelton, USDA-ARS, dan.shelton@ars.usda.gov; 301-504-5760
Jitu Patel, USDA-ARS, jitu.patel@ars.usda.gov; 301-504-9198

Objectives
1. Design and evaluate ZVI columns to remove bacterial pathogens taking various water conditions into consideration. Bacterial cells that survive ZVI treatment will be assessed for survival and attachment to lettuce as if irrigated with ZVI-treated water.
2. ZVI columns will be scaled-up and built into irrigation systems that are currently used in high tunnels, greenhouses and growth chambers. These systems will be used to water leafy greens and assess for bacterial survival.

Kalmia E. Kniel, University of Delaware
Mitigation of irrigation water using zero-valent iron treatment

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Abstract

Zero-valent iron (ZVI) filters may provide an efficient method to mitigate the contamination of produce crops through irrigation water. Laboratory- and field-scale systems were utilized to evaluate the effectiveness of a biosand filter (S) compared to a biosand filter with ZVI incorporated (ZVI) in decontaminating irrigation water that was inoculated with *Escherichia coli* O157:H12 or O157:H7 or *Salmonella* Newport. *Escherichia coli* O157:H12 was used as an allowable field organism. Filtered waters were analyzed immediately or subsequently used to overhead irrigate 'Tyee' spinach plants. This type of spinach was selected for the ability to sustain growth during warmer temperatures in Maryland. Pulse tests were conducted on laboratory-scale columns. The incorporation of iron into the columns increased removal by 1-2 logs consistently over a ten-hour pulse. The ZVI column maintained effectiveness following reconditioning by a chlorine wash after several pulse tests containing approximately 12 logs of bacteria. Batch tests indicated that bacteria absorbed to the iron significantly more ($P < 0.05$) as compared to sand. In the field, water, spinach plant and soil samples were obtained on days 0, 1, 4, 6, 8, 10, 13 and 15 and analyzed for *E. coli* O157:H12 populations. ZVI filters inactivated 6 log CFU 100 ml^{-1} *E. coli* O157:H12 during filtration on day 0, significantly ($P < 0.05$) more than S filter (0.49 CFU 100 ml^{-1}). On day 0, spinach plants irrigated with ZVI-filtered water had significantly lower *E. coli* O157 counts (0.13 log CFU g^{-1}) than spinach irrigated with either S-filtered (4.37 log CFU g^{-1}) or control (5.23 log CFU g^{-1}) water. Soils irrigated with ZVI-filtered water contained *E. coli* O157:H12 populations below the detection limit (2 log CFU g^{-1}). ZVI biosand filters were more effective in reducing microbial populations in irrigation water than sand filters. Zero-valent ion treatment may be a cost-effective mitigation step to help small farmers reduce risk of foodborne *E. coli* infections to consumers.

Background

Over the past decade, significant problems have occurred in the U.S. with regard to the contamination of produce by the enteric bacterial pathogens *E. coli* O157:H7 and *Salmonella*. Minimally processed produce lacks a "kill" step to aid in reduction or elimination of the occasional and incidental contamination that can lead to widespread outbreaks and national recalls. Consequently, greater emphasis has been placed on pre-harvest Good Agricultural Practices and post-harvest Good Manufacturing Practices to ensure safety, but the American food production and distribution system is vast, complex and global. Environmental fecal contamination is not uncommon in these foods; transmission of human pathogens to plants through contaminated irrigation water has been documented under both laboratory and field conditions.

This project proposed to develop and evaluate treatment for irrigation water utilizing filtration through columns of mixtures of zero-valent iron (ZVI) and sand. ZVI has been successfully used for over ten years in commercial water treatment operations to remove chemical contaminants. Evidence has described the adherence and inactivation of viruses and coliphage by ZVI during water treatment. ZVI treatment has been used in permeable reactive barriers to remove a broad range of chemical contaminants in groundwater (Meggyes and Simon 2000). ZVI oxidizes continuously in water through reactions with dissolved oxygen and protons to form amorphous iron hydroxides which are subsequently converted into more stable oxides and oxyhydroxides, such as magnetite, goethite, and lepidocrocite (Odziemkowski *et al.* 1998; Phillips *et al.* 2000). Iron hydroxides, oxides, and oxyhydroxides have a relatively high pH_{pzc} (point of zero charge) and can strongly adsorb viruses and other negatively-charged microorganisms via electrostatic interactions. ZVI does not generate

potentially harmful byproducts like other chemical treatments. ZVI-based technology has achieved greater than 5-log removal of two model viruses (bacteriophages MS2 and ØX174) in synthetic groundwater in minutes (You *et al*. 2005). These results strongly suggest that ZVI can remove viruses quickly and effectively from water without using a chemical treatment; however, ZVI has not been evaluated to remove and inactivate potential bacterial pathogens in irrigation water. As water becomes a more critical resource in agriculture, ZVI treatment could provide an agriculturally sustainable approach to providing irrigation water of sufficient quality to produce growers, and potentially allow more sources of water to become eligible to irrigate produce.

The main objectives of this project included the determination of a way to optimize the effectiveness of ZVI/sand water treatment columns by challenging with inoculum from two serotypes of *E. coli* (O157:H7 and O157:H12) and *Salmonella* Newport. The potential benefits are that ZVI is inexpensive and readily available, has a very high surface area and a long service life. Additionally, the ZVI process is not based on a chemical oxidant such as chlorine and therefore does not generate disinfectant by-products. Microbial inactivation is not based on physical trapping and therefore does not require small pore or particle size or incur significant pressure fluctuations; however, potential pitfalls include the fact that experimental variables are numerous due to the changing environment within a ZVI-containing filtration unit. The level of oxidation changes with exposure to air and water. We do not yet have a good understanding of the mechanisms for absorption or removal, which likely change over time and the life of the filtration material.

Research Methods and Results

Objective 1. Preparation and Pulse Test Analysis using Laboratory-scale Columns

Column Design: Virus removal experiments were conducted using pairs of acrylic columns (3.8-cm internal diameter, 10-cm length, bed volume = 113 cm^3) that were fabricated at the University of Delaware in the Engineering Machine Shop. Each column was wet-packed with sand only (S) or both sand and ZVI (SI). The ZVI used for all experiments was commercial iron granules ETI850/50 from Peerless Metal Powders and Abrasive (Detroit, MI). The iron was sieved, and the size fraction 0.25-0.5 mm was used for column packing. Accusand (Unimin, Le Sueur, MN) was used with a size distribution of 0.1-1.0 mm with ~70% 0.25–0.5 mm. The suspending solution for all studies was artificial ground water (AGW), composed of $CaCl_2$, $MgCl_2$, KCl, $NaHCO_3$ at an ionic strength of 0.002 M and pH 7.5. Two combinations of iron were assessed. Two formulations of columns containing 20% iron either in one layer or two was compared to a column containing 40% iron in one layer.

Microbial Cultures: *E. coli* O157:H7 strain 4407 (2006 Spinach outbreak strain) and *E. coli* O157:H12 (a non-pathogenic strain isolated from a Baltimore County, MD watershed) were grown overnight in Tryptic soy broth supplemented with 50 µg/ml nalidixic acid. *Salmonella* Newport (tomato outbreak isolate from Virginia) was grown in tryptic soy broth. After filtration through the columns *E.coli s*amples were collected on Tryptic soy agar supplemented with 50 µg/ml nalidixic acid and *Salmonella* on XLT-4 agar. Control solutions were compared to serial dilutions of the collected fractions.

Pulse Tests: The first pulse of 300 ml AGW contained ~10^6 cfu/ml and was followed by a flush of 300 ml uninoculated AGW. Fractions were collected continuously, 120 x 5 ml at a flow rate of 1 ml/min. For analysis fractions were plated as stated above. Breakthrough curves were assessed as reduction over time per volume and final log reduction values determined using the calculation of the negative of N/N_0. To assess bacterial interaction with the ZVI, a pulse test using an elution buffer (100 mM Tris, 50 mM glycine, 3% beef extract, 50 mM $MgCl_2$, pH 9.6) to detach the bacteria from the column, followed by

300-ml AGW. Additionally after that a pulse test of 200-ppm chlorine was used to remove any residual bacteria, followed by 300-ml AGW.

Figure 1. Breakthrough curves showing bacterial removal by sand (yellow diamonds) and SI (brown squares) filtration columns over time. For *E. coli* O157:H7 removal by ZVI ranged from 2.5-3.0 logs. For *E. coli* O157:H12 removal by ZVI ranged from 2.9-4.4 logs. For *S.* Newport removal by ZVI ranged from 3.8-4.6 logs.

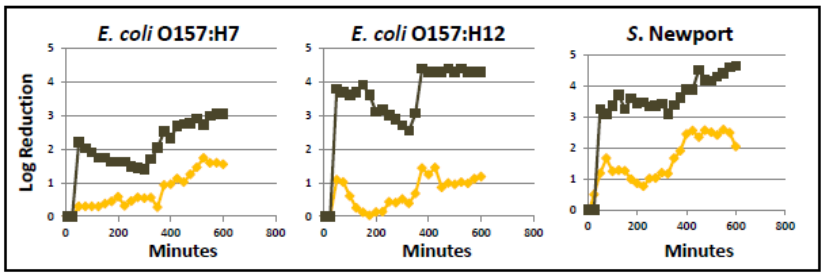


Figure 2. To assess reversible and irreversible interactions bacteria were eluted from S (yellow diamonds) and SI (brown squares) columns by 3% beef extract buffer, pH 9.6 were enumerated on selective media. The bacteria eluted from the columns may indicate reversible binding. Eluted bacteria are all after the initial pulse test (0-300 min).

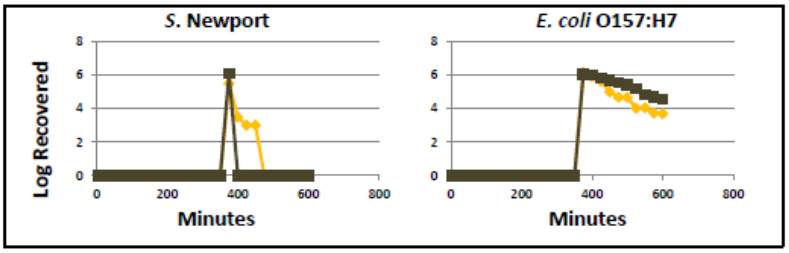
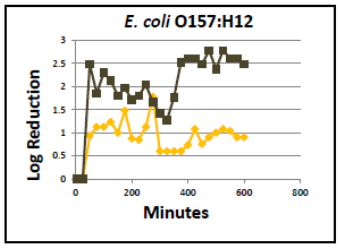


Figure 3. The S and SI columns were cleared of residual bacteria using 200-ppm chlorine. Bacteria were removed by S (yellow diamonds) and SI (brown squares) columns over time in reconditioned columns in a pulse test using inoculated AGW, as performed previously.



Zeta Potential: The zeta potential was measured using a zetasizer (ZEN 3600, Malvern Instruments Ltd.) with a background solution of AGW (ionic strength 0.002 M, pH 7.5).

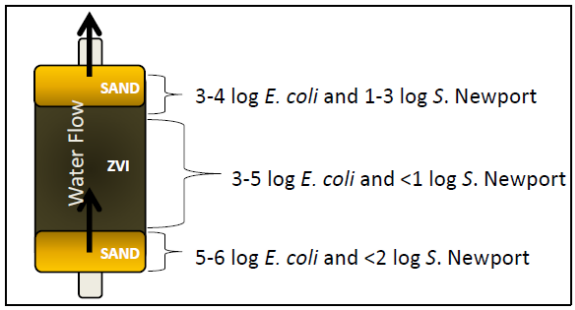
Table 1. shows zeta potential measured for each organism.

Organism	zeta potential
E. coli O157:H7	-27.7 ± 1.0
E. coli O157:H12	-12.2 ± 4.9
Salmonella Newport	-12.2 ± 4.8

Column analysis: Columns were broken down following 3-4 months of use and bacterial contents analyzed in 2-cm sections of the entire 10-cm long column. Sections were mixed with buffer

and samples plated as stated above or subjected to enrichment solution of modified Tryptic soy broth for *E. coli* and lactose broth and RV broth for *Salmonella*.

Figure 4. SI columns were dissembled to determine reversible interactions of the pathogens.



Batch experiments: To better assess the apparent difference between *E. coli* and *Salmonella* in terms of interacting with the colloids, sand and ZVI samples were inoculated in 1.5-ml centrifuge tubes and samples removed after 0, 0.5, 1, and 4 hours.

Table 2. Viable *S.* Newport was detected in both supernatant and pellet samples of the sand and iron mixture and in the supernatant of the ZVI alone, indicating a weaker interaction or absorption compared to both types of *E. coli*.

Organism	time (hours)	ZVI:sand (1:1)		ZVI alone	
		supernatant	pellet	supernatant	pellet
***E. coli* O157:H7**	**0.5**	**1.5**	**0**	**0**	**0**
	4	**0**	**0**	**0**	**0**
***E. coli* O157:H12**	**0.5**	**2.7**	**0**	**0**	**0**
	4	**1.5**	**0**	**0**	**0**
***Salmonella* Newport**	**0.5**	**2.6**	**1.9**	**2.3**	**0**
	4	**2.8**	**2.6**	**2.1**	**0**

Objective 2. Preparation and Overhead Irrigation using Field-scale Columns

Preparation of biosand ZVI columns. Commercial HydrAid Biosand Filters (Cascade Engineering, Grand Rapids, MI) were built as recommended by the manufacturer, each containing under-drain gravel, filtration gravel and 45.4 kg (100 lbs) filtration sand into a 20-L water column. Dimensions of the filters (referred to as columns) were 0.77-m (2.5-ft) high with a diameter of 0.14 ;m (0.38 ft). Columns were modified in the filtration layer to contain either a filtration sand layer only (S), or a combination of zero-valent iron (ZVI) and filtration sand at a 1:1 ratio by weight (SI). ZVI (Peerless Metal Powder and Abrasives, Detroit, MI) were incorporated into columns without modification. In SI columns, 23 kg (45 lbs) of filtration sand and an equivalent amount of ZVI were added to columns. Functional columns were prepared by gravity-feeding 20 L of uninoculated ground water through each column every day for 10 days to allow a biological layer to develop in the filtration sand layer. Groundwater originating from the USDA-ARS Beltsville Area Research Center (BARC) farm (Beltsville, MD), contained no viable *E. coli* and was collected at a groundwater pump station prior to transport in water-tanker trucks to raised-bed planters.

Raised-bed plot construction and seeding. Located on the north farm of BARC, three raised-bed planters were constructed using Jersey barriers, standard-sized poured-concrete highway dividers, for perimeter support. A 50% sandy-loam soil/compost mixture was poured into each planter and leveled at approximately 15 cm (6 in) from the top of each barrier. Only organically-approved products were used on these plots during the past five years. Organically-certified spinach seed (*Spinacia oleracea*, c.v.

'Tyee' F1, Johnny's Selected Seeds, Winslow, ME) was sprinkled manually into each row at approximately 300 seeds per square foot. 'Tyee' was chosen for its heat tolerance, slow-bolting, savoyed leaf properties, and its commercial use in Maryland. Seed was covered to a depth of ¼ inch with soil from the plot. Each planter was oriented east/west as were the spinach rows. Prior to each irrigation event, plastic barriers were raised in each plot to prevent unintended impacts on adjacent plots from wind drift of water droplets during overhead irrigation.

Construction of irrigation system. Each treatment plot contained an independent, overhead irrigation system. Each plot contained two industrial stainless steel spray nozzles that delivered a uniform distribution of each irrigation treatment onto the surface of each plot. When pressurized to 40 pounds per square inch (psi), each spray nozzle delivered an evenly distributed mist at a 60-degree angle in a square 3 ft x 3 ft pattern at 3 L min^{-1}. During each irrigation event, approximately 10 L of irrigation treatment water was applied to each plot (pump run for 100 s).

Strains used. The same strains that were used in Objective 1 were used in Objective 2.

Microbial analysis of water. Appropriate volumes (1, 10, 100 or 1000 ml) from 1-L fractions were collected and then filtered through 0.45-mm MicroCheck II beverage monitors (Pall Corp., Ann Arbor, MI) placed on a vacuum manifold (Pall Corp). After filtration, monitors containing *E. coli* O157:H12 cells were aseptically removed with forceps and transferred to MACN. Water was analyzed after each of the following actions: 1) Inoculation of dairy manure (day 0); 2) Filtration through control, S, or SI treatments and collection in sterile carboys (days 0, 1, 4, 8, 13, and 15); and 3) Irrigation through emitter sprinkler head and collection at sprinkler head before irrigation of spinach plants (days 0, 1, 4, 8, 13 and 15).

Spinach tissue harvest and analysis. 'Tyee' spinach was planted in late May 2011 and grown for four weeks in raised beds until late June 2011 before irrigation, harvest, and microbial analysis. Spinach was irrigated and harvested on days 0, 1, 4, 8, 13 and 15 from June 23 to July 8, 2011. On each day after irrigation, spinach was harvested after water droplets had dried on spinach leaves (within 60 – 90 min). From each plot, five 10-g samples of foliar tissue were collected by cutting leaves with sterile shears, and homogenized. Enumeration by direct plating or using a three-tube most probable number (MPN) was performed on 10, 1 and 0.1 ml of the homogenate added to each of three tubes containing 0, 9, and 9.9 ml mEHECN, respectively. MPN values were used when *E. coli* O157:H12 populations were not detected by plate counts.

Microbial analysis of soil. On each day that spinach was harvested and analyzed for *E. coli* O157:H12 populations, soil samples were also collected. Composite soil samples from each plot were collected from 0-3 cm depth using a sterile, gloved hand and deposited into a sterile whirl-pack bag, which was sealed and stored at 4^{o}C until analysis. Microbial contents of samples were analyzed by bacterial enumeration or enrichment methods.

The following **Tables 3-5** are adapted from Ingram *et al.* (2012).

Table 3. Populations of *E. coli* O157:H12 in inoculated water filtered by either no treatment (control), sand, or sand-zerovalent iron (ZVI) and collected in a carboy before irrigation of spinach plants.

	Population (log CFU 100 ml^{-1}) of *E. coli* O157:H12 in irrigation water collected in carboys after filtration		
	Treatment		
Day	Control	Sand	ZVI
0	8.29a [1]x [2]	7.80ax	2.34ay
1	0.11by	7.56abx	1.75aby
4	0.46by	5.85bx	1.25abcy
6	0.85by	3.91cx	−0.46bcy
8	0.85by	3.34cx	−0.66cy
13	< 1 by	2.84cx	--

[1]Within Treatment, means of day in the same column followed by a different(a,b,c) letters are significantly (P < 0.05) different.

[2]Within Day, means of treatment in the same row followed by a different letter (x,y) are significantly (P < 0.05) different.
[3] -- indicate no *E. coli* O157:H12 populations were recovered.

Table 4. Populations of *E. coli* O157:H12 in inoculated water filtered by either no treatment (control), sand, or sand-zerovalent iron (ZVI) and collected from emitter before irrigation of spinach plants.

	Population (log CFU 100 ml^{-1}) of *E. coli* O157:H12 in irrigation water collected from emitters		
	Treatment		
Day	Control	Sand	ZVI
0	8.33a [1]x [2]	5.74aby	2.82az
1	6.02ax	7.67ax	1.71aby
4	1.94by	5.96abx	' 0.69aby
6	1.05by	4.32bcx	<0.10by
8	1.47by	3.51bcx	−0.23by
13	0.55bxy	2.21cx	<0.10by
15	0.76bx	2.00cx	0.07bx

[1]Within Treatment, means of day in the same column followed by a different(a,b,c) letters are significantly (P < 0.05) different.

[2]Within Day, means of treatment in the same row followed by a different letter (x,y) are significantly (P < 0.05) different.

Table 5. Populations of *E. coli* O157:H12 on spinach irrigated with water filtered through control (no treatment), sand, or sand-zerovalent iron (ZVI) and collected from emitter before irrigation of spinach plants.

	Population (log CFU g^{-1}) of *E. coli* O157:H12 on spinach		
	Treatment		
Day	Control	Sand	ZVI
0	5.23a [1]x [2]	4.37ax	0.13ay
1	2.78bx	4.28ax	−0.18ay
4	2.07bcx	3.29abx	−0.29ay
6	0.64cx	0.84bx	1.05ax
8	−0.37dx	0.56bcdx	--
13	−1.15dx	−1.24dx	--
15	−0.95dx	−0.72cdx	--

[1]Within Treatment, means of day in the same column followed by a different(a,b,c) letters are significantly (P < 0.05) different.

[2]Within Day, means of treatment in the same row followed by a different letter (x,y) are significantly (P < 0.05) different.
[3] -- indicate no *E. coli* O157:H12 populations were recovered.

Outcomes and Accomplishments

- Zero-valent iron (ZVI) is a useful addition to a sand filtration system to reduce bacterial contamination. Efficiency of removal was >2 log over 3 months, and ranged from 2-4 log removal.
- Removal and inactivation of bacteria varies with microorganism type and ZVI oxidation. The bacterial surface charge may affect the interaction with the ZVI, but even this will be altered by water quality and water type.
- Based on its presence and elution from the ZVI, *E. coli* appears to survive better in the ZVI compared to *S.* Newport. This was supported by column reconditioning steps, disassembly, and batch-testing over a 4-hour period.
- The ZVI column can be "cleaned" using chlorine and reconditioned for removal of bacteria.
- ZVI filtration is a simple and effective way to use water that may not have previously been acceptable for irrigation, including surface water.

Summary of Findings and Recommendations

These studies indicate that even though sand filtration reduced microbial populations in irrigation water, the lack of an immediate reduction in *E. coli* and *Salmonella* populations by sand filtration may make it a less effective mitigation treatment compared sand-ZVI filtration.
These results indicate the sand-ZVI filter may provide an important, cost-effective mitigation step in decontamination of irrigation water for small growers of leafy greens. Future work should evaluate the effectiveness of ZVI in treating surface waters used for irrigation. With optimization and further investigation, ZVI-treatment of irrigation water may prove to be an inexpensive and simple method for maintaining compliance with LGMA standards while simultaneously allowing for more diverse sources of irrigation water to be used when irrigating leafy green commodities. Due to constraints of flow that would be necessary for irrigation of large fields and contact time with ZVI that is likely necessary for efficient removal of microbial pathogens, ZVI may be more useful for smaller farms compared to larger ones; however, this has not yet been assessed scientifically and engineering likely plays an essential role in design.

APPENDICES

Publications and Presentations (required)

One manuscript is still in preparation (Kniel *et al*.) and one is *in press*.

1. Ingram, D., Callahan, M., Ferguson, S., Hoover, D., Shelton, D., Millner, P., Camp, M., Patel, J., Kniel, K., and Sharma, M. 2012. The use of zero-valent iron biosand filters to reduce *E. coli* O157:H12 in irrigation water applied to spinach plants in a field setting. J. Appl. Micro. *In press.*

Presentations

1. Shortlidge, K., Johnson, C., Hernandez, C., Wei, J., Hoover, D., and Kniel, K. Removal of pathogens from irrigation water using zero-valent iron. CANR Fourth International Symposium on Global Issues in Nutrient Management: Science, Technology and Policy, Newark, DE, August 2011.

2. Mudd, C., Callahan, M.T., Ferguson, S., Ingram, D.T., Shelton, D., Patel, J., Hoover, D.G., Wei, J., Kniel, K.E., and Sharma, M. The use of zero-valent iron and biosand filtration to inactivate *E. coli* O157:H7 in irrigation water. IAFP Annual Meeting, Milwaukee, WI, August 2011. P2-62.

3. Kniel, K.E., Wei, J., Shelton, D., Patel, J., Hoover, D.G., and Sharma, M. Optimization for the removal of *Salmonella*, *E. coli* O157:H7 and *E. coli* O157:H12 from water using zero-valent iron. IAFP Annual Meeting, Milwaukee, WI, August 2011. P3-25.

Two additional presentations were given by Casey Johnson (Animal Science undergraduate research student) at University of Delaware Undergraduate Research Symposia in August 2010 and August 2011.

Budget Summary (required)

A research associate was paid from this project to ensure consistency in the project during this time period along with undergraduate researchers conducting undergraduate thesis research projects. Undergraduate research is extremely important at the University of Delaware and several students valued greatly from this funding and this opportunity. Additional funds to supplies included funding for materials to hold and transfer surface water, plastic consumables, and reagents for batch tests to assess bacterial survival in the presence of ZVI.

Breakdown of the grant funds for University of Delaware includes funds of $31,702.99 spent on supplies and materials and $95,034.26 spent on personnel. The USDA-ARS had a subcontract of $100,000 of which $52,087.66 was spent on supplies and other materials and the rest on personnel.

Tables and Figures (optional)

Figures 5-7 are adapted from Ingram *et al.* (2012).

Figure 5. Cross section of a raised-bed spinach plot showing the angle of the square jet spray nozzle and the height above plots.

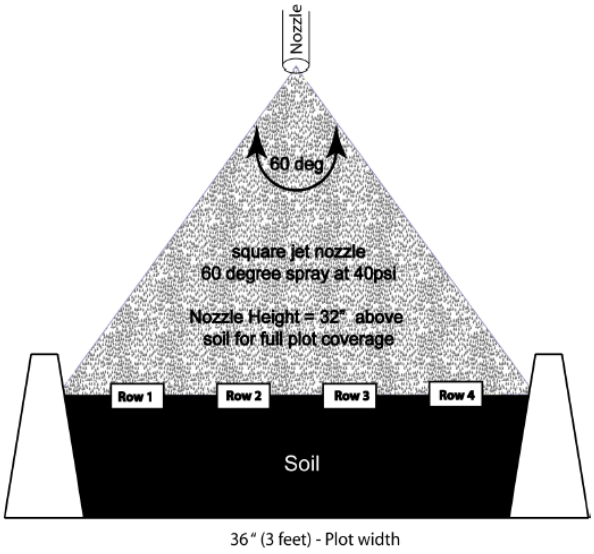


Figure 6. Dimensions of spinach-planted rows and irrigation emitter positions to evenly distribute a single treatment across all spinach tissue.

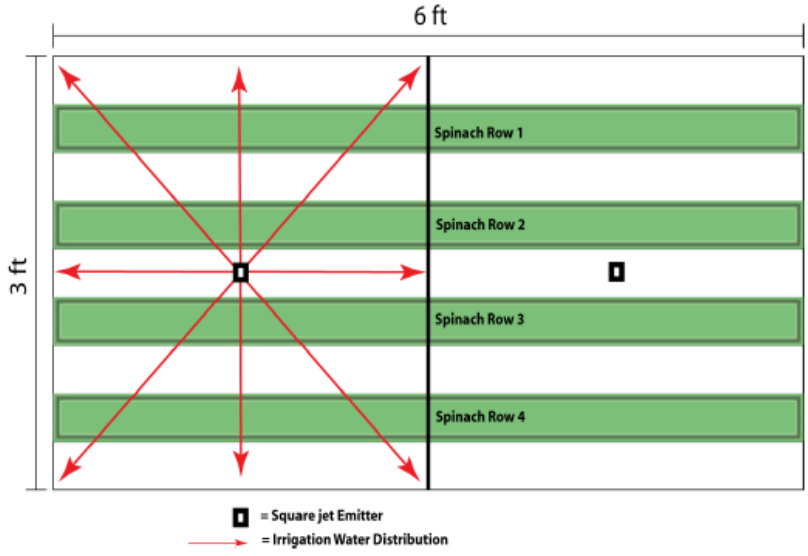


Figure 7. Experimental treatment layout for three plots containing each irrigation water treatment (control, sand or zero-valent iron treatment) within a single raised-bed plot.