Examination of overhead and drip irrigation and chlorine dioxide treatment of irrigation water within an organic farming system

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INTRODUCTION

- Contaminated open surface irrigation water has contributed to produce outbreaks (Ackers et al., 1998; Barton et al., 2011), and growers must take steps to minimize the risk of crop contamination with agricultural water.
- The currently proposed Standards for the Growing, Harvesting, Packing and Holding of Produce for Human Consumption (Proposed Produce Rule) associated with the Food Safety Modernization Act states that "all agricultural water must be safe and of adequate sanitary quality for its intended use" and that agricultural water "used during growing activities for covered produce…using a direct water application method you must test the quality of the water …if you find that there is more than 235 CFU/MPN generic *E. coli* per 100 ml for any single sample or a rolling geometric mean (n=5) of more than 126 CFU/MPN per 100 ml of water, you must immediately discontinue use of that source of agricultural water and/or its distribution system" (FDA, 2013).
- Chlorine dioxide is an antimicrobial with 2.5 times the oxidizing potential compared to chlorine gas (Suslow, 2000), however it is primarily used in post-harvest treatment of fruits and vegetables.

MATERIALS AND METHODS

- The study involved vegetable plots amended with broiler litter. Four plots per treatment were randomly assigned to: 1) untreated overhead irrigation 2) untreated drip irrigation 3) overhead irrigation treated with chlorine dioxide and 4) drip irrigation treated with chlorine dioxide.
- Irrigation water was pumped from a creek. Treated water involved filtration and application of chlorine dioxide (CP33 injector; Chemilizer, Largo, FL).
- Irrigation water was sampled during four irrigation events. The creek was sampled in two ways, collecting water from a free flowing area near the center (two sampling sites) and collecting water near the bank including sediment (two sampling sites). Samples were collected after the drip filter and after chlorine dioxide treatment, as well as at the point of application in the vegetable plots (4 plots per treatment).
- Soil was sampled on three dates (16 total samples/date). For each 20 x 50 ft plot, 12 soil samples (2.5 cm cores, 0 to 10 cm depth) were collected and composited.
- Lettuce heads (24 per plot) were cut in half and three half-heads were composited for microbial testing (8 composite samples per plot). Outer leaves removed from lettuce heads in the field were collected and composited into 1-2 samples per plot. Samples were massaged with 0.1% peptone water at a 1:1 ratio for 2 min. Collected liquid was analyzed for pathogen presence for all samples, and half were selected for MPN analysis.
- Indicator organisms (fecal coliforms and generic *E. coli*) were quantified using a 5-tube most probable number technique (FDA-BAM, 2002).
- The presence of *E. coli* O157 was examined using immunomagnetic separation and standard plating techniques for isolation and latex agglutination for confirmation (LeJeune et al., 2001). Conventional PCR examined the presence of virulence genes: Shiga toxins (stx1 and stx2), haemolysin (hlyA), and intimin (eaeA).
- The presence of *Salmonella* spp. was examined by standard plating techniques and latex agglutination for confirmation (FDA-BAM, 2007).
- Data were analyzed using mixed model procedure of SAS®. Water samples collected prior to application were analyzed separately. For irrigation water at the point of application and soil data, a randomized complete block design with fixed effects of irrigation system and treatment was used. For lettuce data, harvest was considered a fixed effect. Individual plots were designated as blocks (random factor).

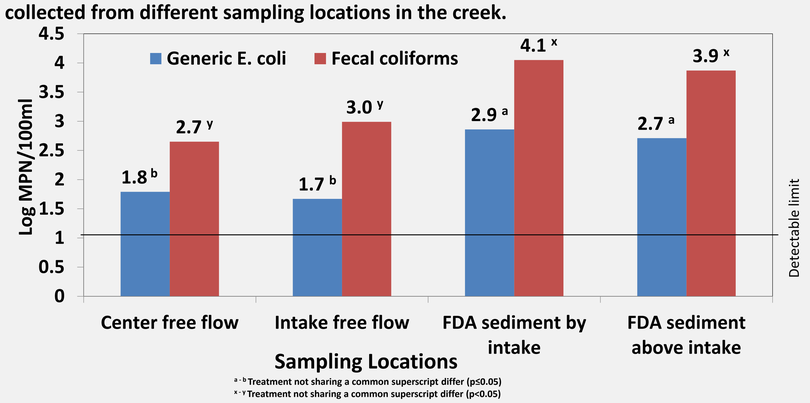
Drip irrigation lines

Cleaning and sanitizing soil sampling tool

OBJECTIVE

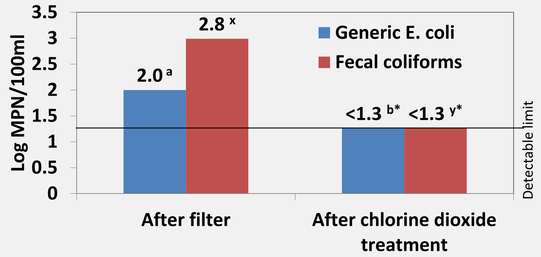
- To evaluate the effect of irrigation practices (overhead and drip) and water treatment (with or without chlorine dioxide) on pathogen presence (*E. coli* O157 and *Salmonella*) and indicator organisms levels (fecal coliforms and generic *E. coli*).

Figure 1: Indicator organism levels (generic *E. coli* and fecal coliforms) in irrigation water collected from different sampling locations in the creek.



a-b Treatment not sharing a common superscript differ (p≤0.05)
x-y Treatment not sharing a common superscript differ (p<0.05)

Figure 2: Indicator organisms levels (generic *E. coli* and fecal coliforms) in irrigation water collected after filter and after chlorine dioxide treatment.



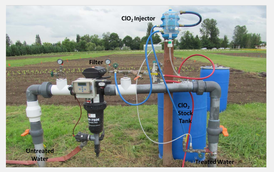
a-b Treatment not sharing a common superscript differ (p≤0.05)
x-y Treatment not sharing a common superscript differ (p<0.05)
* Estimated count. Most observations were below the detectable limit.

Sample processing

Table 1: Indicator organism levels (generic *E. coli* and fecal coliforms) of soil* and lettuce* irrigated with drip and overhead irrigation

Sample source	Irrigation system	Generic *E. coli*	Fecal Coliforms
Soil	Overhead	2.0[a]	4.4[a]
	Drip	1.2[b]	3.7[b]
Lettuce	Overhead	1.8[a]	5.7[a]
	Drip	<1.3[b†]	5.4[a]

*Soil samples are calculated as log MPN/gdw, and lettuce samples are calculated as log MPN/100ml.
[a-b]Means within a sample source and indicator organism followed by a different superscript are significantly different (P <0.05).
† Estimated count. Most observations were below the detectable limit



Chlorine dioxide treatment system

Sampling lettuce heads

Water sample collection at creek

RESULTS AND DISCUSSION

Results represent the first season of a two-year study.

Irrigation water

- Free-flowing surface water samples were significantly lower in generic *E. coli* and fecal coliforms compared to samples collected near the bank that included sediment (Figure 1).
- Chlorine dioxide treatment significantly ($p<0.05$) reduced generic *E. coli* and fecal coliform levels by at least 0.7 and 1.5 log MPN/100ml, respectively (Figure 2).
- Generic *E. coli* and fecal coliform levels at the point of application were influenced by irrigation water treatment but not irrigation method.
  - Generic *E. coli* levels of the treated irrigation samples at the point of application were significantly lower (below the detectable limit) than untreated samples (1.4 log MPN/100 ml).
  - Fecal coliforms levels at the point of application were also significantly lower (below the detectable limit) compared to untreated plots (2.6 log MPN/100ml).
- *E. coli* O157:H7 was detected prior to chlorine dioxide treatment at the filter (1/13); however, no pathogens were detected from the creek samples collected on the same day. One untreated overhead irrigation sample at the point of application was positive (1/72) for *E. coli* O157:H7.

Soil

- Irrigation system significantly affected the indicator organism levels in soil (Table 1). Treatment of irrigation water was not significant ($p>0.05$).

Lettuce

- Type of irrigation system significantly affected generic *E. coli* levels on lettuce (Table 1). Treatment of irrigation water did not show a significant ($p>0.05$) effect on indicator organism levels.
- Drip irrigation samples had significantly lower generic *E. coli* levels on lettuce by approximately 1.0 log MPN/100ml. Fecal coliform levels on lettuce were higher than 5 log MPN/100ml for all treatments (Table 1).
- For the first harvest, lettuce outer leaves (7/10) yielded *E. coli* O157:H7 in treated and untreated drip and untreated overhead plots; no lettuce heads were positive (0/64).
- For the second harvest, *E. coli* O157:H7 was detected from lettuce head samples (2/64) associated with untreated overhead and untreated drip irrigation plots.

SUMMARY

- Use of a chlorine dioxide water treatment system appeared to reduce generic *E. coli* levels in irrigation water. The influence of irrigation water treatment on generic *E. coli* levels in the soil and on lettuce was less clear. The role of irrigation water treatment for pathogen risk reduction on lettuce was not clear.
- Drip irrigation appeared to reduce generic *E. coli* levels on lettuce. Changing irrigation methods may be an effective strategy for risk reduction when using open surface water bodies with poor water quality for irrigation.

ACKNOWLEDGEMENTS

We would like to thank the Washington State Department of Agriculture Specialty Crop Block Program for project funding and support. A special thank you to Liz Myhre, technicians, staff, graduate and undergraduate students who assisted with this project.

REFERENCES

Ackers et al., 1998. J Infect Dis. 177:1588–1593.
Barton, et al., 2011. N Engl J Med. 364:918-927.
FDA. 2013. Proposed Produce Safety Rule. *Federal Register* 78 : 3504-3646.
FDA-BAM. 2002. Enumeration of *Escherichia coli* and the Coliform Bacteria.
FDA-BAM. 2007. *Salmonella*.
LeJeune et al., 2001. American Society of Microbiology. 67:3053-3057.
Suslow, T.V. 2000. Fruit and Vegetable Processing. 3-14.

Strategizing Sampling Methods