

SOIL FILTERING REDUCES ONION BULB EXPOSURE TO *E. COLI* FROM IRRIGATION WATER

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Summary

Surface irrigation systems that reuse water may deliver bacteria to produce destined for fresh consumption. During 2015 four irrigation systems delivered 1) well water free of *Escherichia coli* via subsurface drip irrigation, 2) canal water with moderate levels of *E. coli* via subsurface drip irrigation, 3) canal water with moderate levels of *E. coli* via furrow irrigation, and 4) canal water with enhanced levels of *E. coli* via furrow irrigation. The four irrigation systems (replicated five times) applied water to onion on silt loam repeating a similar trial conducted in 2014. Water was sampled hourly for *E. coli* and the lateral movement of *E. coli* in the soil solution was tracked by soil samples following four irrigations. Onion bulbs were sampled for *E. coli* contamination. The most probable numbers of *E. coli* in the irrigation water and in the soil water were determined using the water testing products IDEXX Colilert[®] and Colisure[®], respectively, and Quanti-Tray/2000[®]. Under both furrow and subsurface drip irrigation, a fraction of *E. coli* in the irrigation was delivered to the soil water immediately adjacent to the onion bulbs. The silt loam retained most of the *E. coli* content away from the onion bulbs and close to where the water entered the soil. *E. coli* was detected on the bulb exteriors prior to and immediately after lifting, but there was no relationship between the exterior contamination and the water source, irrigation system, or soil water content sampled prior to sampling. When onions were sampled immediately after irrigation with contaminated water, no *E. coli* was detected inside of the onion bulbs grown with any irrigation treatment. At packout there were no *E. coli* on the outside or inside of the bulbs. Current subsurface drip or furrow (flood) irrigation practices do not appear to pose a significant risk for *E. coli* contamination of dry bulb onion grown on silt loam in the Treasure Valley.

Introduction

The transmission of foodborne pathogens from fresh produce to humans has been documented and is of increasing concern (Bernstein 2011, 2013; De Roeveer 1998; Oliamat and Holley 2012). Since many fresh commodities are consumed without cooking or other treatment, microbes on

fresh produce can be directly consumed. Much of the microbial contamination on produce is of fecal origin and can be introduced into or onto produce directly or indirectly from handling, soil, water, or animals. Once microbes are on produce, their survival or multiplication depends on many factors including the nature of the organism, the suitability of the produce for growth, the place of contact, the physiological status of the produce, and the environmental conditions of handling and storage.

Microbial contamination of produce via irrigation water varies with the commodity and whether the water is in direct or indirect contact with the produce (Oliamat and Holley 2012, Steele and Odumeru 2004). Sprinkler irrigation can directly deposit its microbial load onto fruit and vegetables. Subsurface drip irrigation (SDI) is thought to be less apt to deliver microbes to produce than furrow (flood) and sprinkler irrigation. Okafo et al. (2003) showed that garden vegetables can be readily contaminated by contaminated irrigation water in Nigeria.

Agriculture in the Treasure Valley of eastern Oregon and southwestern Idaho is highly diversified, with numerous crops and many cattle (Shock et al. 2000a, USDA Economic Research Service 2011a,b). Dry bulb onions are the most economically valuable crop grown in the region; they are grown on 9,000 ha in the valley, with an annual farm gate value of \$150 million. Onions are subject to the Food Safety Modernization Act (FSMA) rules (U.S. Food and Drug Administration 2015).

In the Treasure Valley, irrigation canal systems mix relatively clean water with runoff water. This intermixing can result in high counts of *E. coli* in irrigation water throughout large parts of the water distribution systems (Shock et al. 2013b, Shock and Shock 2014). The burdens of the agricultural water standards of the FSMA rules on onion growers in the Treasure Valley will consist of the labor for water sampling and record keeping, the cost of laboratory analysis, and any additional costs for potential water quality remediation procedures. Losses from the proposed rules to the community could extend to lost investment in onion production equipment, onion storage buildings and packing facilities, and potential loss of employment and property values.

Historically, onions in the Treasure Valley have been grown under furrow irrigation. Today, approximately half of the onions are grown under SDI (Shock et al. 2013a). However, conversion from furrow to SDI has not been an economic option for all growers. Onion seed is planted in late March and early April and irrigated from April until mid-August or early September. Onion bulbs mature in the field, are lifted in late August through the beginning of October, and transported to storage or sold directly out of the field after curing to packing operations.

The leaves of some plants develop biofilms that harbor human pathogens (Heaton and Jones 2008). *E. coli* applied to onion leaf tissue in the fall in Georgia at 1,000,000 colony-forming units (CFU)/100ml could be recovered up to 74 days after application (Islam et al. 2005), but the population declined logarithmically over time. *Salmonella* and *E. coli* populations that were applied to onions through irrigation water declined logarithmically over time to traces over 28 days following the last irrigation during onion bulb maturation and curing (Emch and Waite-Cusic 2016). Similar to the exteriors of other vegetables, onion bulbs may acquire *E. coli* contamination in the field from various sources. Most of the *E. coli* from irrigation water that gets on onion bulbs when they are growing in the fields may not be internalized and die before marketing (Shock et al. 2013b). Other *E. coli* probably land on the bulbs by chance while they

are growing and curing. Onions receiving no *E. coli* in the irrigation water could be contaminated with *E. coli* by the time they are lifted and cured. By the time that onions are lifted and cured, bulbs grown with contaminated water had just as low external contamination as those grown with zero *E. coli* irrigation water (Shock et al. 2013b). It is unknown to what extent *E. coli* present in irrigation water could reach onion roots, bulbs, and ultimately internally in marketed onion bulbs, and thus be a human health risk.

The work discussed here approaches the possibility that the soil might filter out *E. coli* before it reaches the onion bulbs. If soil can be used to filter out bacteria, water with high bacteria counts might have a much lower count as it soaks through the soil toward the onion bulb. This experiment sought to determine whether water containing *E. coli* would be filtered by the soil from its point of application, greatly reducing the *E. coli* in the soil water that actually reaches onion bulbs. The experiment also sought to compare the potential filtration of *E. coli* in the soil water that occurs under furrow irrigation with the filtration that occurs under subsurface drip irrigation. Harvested onion bulbs grown with water free of *E. coli* were compared with bulbs grown with water containing substantial *E. coli* for the internal presence of *E. coli*.

Materials and Methods

Onions were grown in 2015 on an Owyhee silt loam at the Oregon State University Malheur Experiment Station, Ontario, Oregon. The field had been planted to wheat in 2014. In the fall of 2014, the wheat stubble was shredded and the field was irrigated. The field was then disked, moldboard plowed, and groundhogged. A soil sample was taken in the fall of 2014 and analysis showed that the top foot of soil had a pH of 7.1, 3.1-5.5% lime, 1.66% organic matter, 101% base saturation, 20 ppm nitrate, 7 ppm ammonium, 34 ppm phosphorus (P), 252 ppm potassium (K), 3709 ppm calcium (Ca), 293 ppm Magnesium (Mg), 146 ppm sodium (Na), 4.8 ppm zinc (Zn), 1.2 ppm copper (Cu), 7 ppm Manganese (Mn), 9 ppm iron (Fe), and 0.8 ppm boron (B). Based on the soil analysis, 75 lb/acre of P, 200 lb/acre of K, 23 lb/acre of S, 20 lb/acre of Mg, 7 lb/acre of Mn, and 1 lb of B/acre were broadcast before plowing. After plowing, the field was fumigated with K-Pam[®] at 15 gal/acre and bedded at 22 inches.

Seed of 'Vaquero' onion (Nunhems, Parma, ID) was planted on March 13 in double rows spaced 3 inches apart at 150,000 seeds/acre. Each double row was planted on beds spaced 22 inches apart. Planting was done with customized John Deere Flexi Planter units equipped with disc openers. Immediately after planting, the onions received a narrow band of Lorsban[®] 15G at 3.7 oz/1000 ft of row (0.82 lb ai/acre) over the planted rows, and the soil surface was rolled. Onion emergence started on March 30.

The field had drip tape laid at 4-inch depth between 2 onion beds during planting. The drip tape had emitters spaced 12 inches apart and emitter flow rate of 0.22 gal/min/100 ft (Toro Aqua-Traxx, Toro Co., El Cajon, CA). The distance between the tape and the center of each double row of onions was 11 inches. The experiment used Vaquero onions that had been irrigated exclusively by SDI using well water until August 25, 2015. All seed was from the same lot, was planted on the same day, and cultural practice operations were identical across irrigation systems. Two double rows of onions were planted on 1.12-m beds. Each main irrigation plot consisted of three beds of onions 120 ft long. All observations were made in the central bed of each irrigation plot. Drip tape was laid at planting at 4-inch depth in the middle of the bed serving two double rows of onions, one pair to each side of the tape. The drip tape had emitters

spaced 12 inches apart and emitter flow rate of 0.5 L/h (Toro Aqua-Traxx, Toro Co., El Cajon, CA). The distance between the tape and the center of each double row of onions was 7 inches. Until the experiment commenced, all onions were irrigated automatically with well water to maintain the soil water tension (SWT) in the onion root zone below 20 cb (cb = kPa) (Shock et al. 2000b). Soil water tension was measured with four granular matrix sensors (GMS, Watermark Soil Moisture Sensors Model 200SS, Irrometer Co., Riverside, CA) installed at 8-inch depth in the center of the double row. Sensors had been calibrated to SWT (Shock et al. 1998). The most common onion cultivar, conventional cultural practices, and other methods used in these trials followed the guidelines for protocols evaluating microbial contamination hazards to fresh produce (Harris et al. 2012).

Two furrow irrigation treatments and two SDI treatments were established in the same onion field. The irrigation treatments were replicated five times in randomized complete blocks. Irrigation water sources were evaluated for the concentration of *E. coli* four times during the growing season. The final irrigations occurred on August 24 and 25. The onions were lifted manually on August 31.

E. coli movement in the soil was monitored during several irrigation cycles starting in early August. The same canal provided water for the drip and the furrow systems. Onions were irrigated at least weekly, typically for 8-9 hours per irrigation. Irrigation for onions drip-irrigated with canal water, drip-irrigated with well water, and furrow-irrigated with canal water occurred on the same days, while onions furrow-irrigated with enhanced canal water occurred on the following day, due to the logistics of the water supply.

The onions were managed to minimize yield reductions from weeds, pests, diseases, water stress, and nutrient deficiencies. For weed control, the following herbicides were applied: on March 26, Roundup PowerMax[®] at 24 oz/acre was broadcast; on April 28, GoalTender[®] at 0.09 lb ai/acre (4 oz/acre), Buctril[®] at 0.25 lb ai/acre (16 oz/acre), and Poast[®] at 0.25 lb ai/acre (16 oz/acre) were broadcast; on May 4, Prowl[®] H₂O at 0.83 lb ai/acre (2 pt/acre) was broadcast; on June 4, GoalTender at 0.14 lb ai/acre (6 oz/acre), Buctril at 0.25 lb ai/acre (16 oz/acre), and Poast at 0.25 lb ai/acre (16 oz/acre) were broadcast.

For thrips control, the following insecticides were applied: M-Pede[®] at 36 oz/acre and Aza-Direct[®] at 2 pt/acre on May 14, Movento[®] at 5 oz/acre on May 23 by ground application; Movento at 5 oz/acre and Aza-Direct at 2 pt/acre on June 4 by ground application; Agri-Mek[®] at 3.5 oz/acre on June 12 and 18 by ground application; Radiant[®] at 10 oz/acre on June 25 by ground application and on July 4 by aerial application; Lannate[®] at 0.9 lb ai/acre on July 15 and 25 by aerial application; and Radiant at 10 oz/acre on August 8 by aerial application.

For disease control, Badge[®] fungicide at 0.28 lb ai/acre (1 pt/acre) was broadcast aerially on June 4.

URAN at 20 lb N/acre was applied through the drip tape weekly starting May 28 and ending June 24, totaling 100 lb N/acre.

Furrow-irrigation with canal water

Lay-flat hose (2-inch diameter) was placed along each irrigation replicate to deliver water from the canal to the field. The primary lay-flat was connected to a two-way splitter, which itself was connected to a 0.8 inch PVC pipe. The PVC pipe was directly connected to the source canal, with water flow controlled by a plate in the canal. The inlet plate was opened partially to allow

water to flow through the system to irrigate the onions, and simply closed to cease irrigation. Attached to the lay-flat at the top of each respective onion bed were valves for water flow control. The system delivered approximately $\frac{3}{4}$ gal/min/furrow.

Furrow-irrigation with enhanced canal water

The *E. coli* enhanced canal water irrigation was set up identically to the canal water irrigation described above, the primary difference being that burlap bags with cattle manure were placed in with the irrigation canal above the inlet pipe to try to ensure a higher level of *E. coli* in the irrigation water. Tins were placed in the canal and directed the runoff water to flow by the inlet pipe in an effort to ensure high *E. coli* content in the irrigation water. The system delivered approximately $\frac{3}{4}$ gal/min/furrow.

Subsurface drip-irrigation with well water

The SDI system with well water was already in place prior to this trial. Due to the completely randomized placement of the other irrigation systems in the onion field, additional plumbing was needed to reattach the drip-irrigated plots. In order to obtain the water samples needed, a water release valve was attached to the drip distribution tubing already in place. The SDI irrigation system delivered 3.5 gal/min to the 5 replicates.

Subsurface drip-irrigation with canal water

This SDI system utilized the drip tape already in place from the initial irrigations and required new plumbing to canal water. The valves at the top of each replicate were switched from the main well water line to the canal water line after water filtration. A 0.5-horsepower, gas-powered pump delivered water from the canal to a filter station at 240 kPa. Two sand media filters (Toro Aqua-Clean Model 2-18, The Toro Co., San Diego, CA) were filled with 30-grit crushed garnet because the garnet requires less replacement and flushing and is capable of filtering to over 200 mesh. The filter station was set to automatically enter a back flush cycle every 30 minutes. The system was calibrated to deliver approximately 5 gal water/minute to the field at 12 psi and the system delivered 3.5 gal/min to the 5 replicates.

***E. coli* in the irrigation water**

The irrigation water was sampled hourly from each of the four irrigation systems for the first 8 hours of irrigation per treatment per irrigation. Furrow irrigation samples were collected from the valves where the water entered the furrow. Drip samples were collected by installing a water release valve directly into the 0.8 inch hoses that carried the water to the drip tapes. All samples were collected from the water inlets of the first replicate. Sample kits were used to collect each sample. These kits included a sterilized sample bottle, one pair of sterilized nitrile gloves, and a resealable plastic bag to label and store the bottle in. Water samples were kept refrigerated until analysis and were analyzed within 24 hours of collection.

The IDEXX Colilert[®] +Quanti-Tray/2000[®] system (IDEXX Laboratories, Westbrook, ME) was used to quantify generic *E. coli* concentrations in the samples. The Colilert system has been approved as a water-quality testing system by the U.S. Environmental Protection Agency (1999) and the manufacturer's directions were followed. Briefly, a reagent pack that contains an enzyme substrate and a nutrient broth was added to 100 ml of a water sample. The substrate reacts with glucuronidase, an enzyme that is present in 95% of *E. coli* strains (with the group containing O157:H7 being an exception). Aliquots of each sample are then placed in wells of Quanti-Tray/2000 trays. The presence of *E. coli* is indicated by fluorescence. The most probable

number (MPN) of *E. coli* per 100 ml of water was determined from the number of positive wells for each sample (Edberg et al. 1988, 1990) If all sample wells are positive, MPN values are reported as over 2420 MPN/100 ml. Quantification would then require testing serial dilutions of the original sample. The lower limit of detection was 1 MPN/100 ml.

Comparisons of the MPN of *E. coli* per 100 ml of water between treatments and over irrigation dates were made using analysis of variance, omitting the treatment that used drip irrigation with well water where *E. coli* was not detected.

Soil water samples

Soil samples were replicated five times for each irrigation system and at three sampling distances between the edge of the water in the furrow irrigation or next to the drip tape up to directly against the onion bulbs (Fig. 1 and 2). Soil sampling was conducted the morning following irrigation. To sample the soil, sterile, plastic putty knives were used to extract a wedge of soil 2 inches deep, 1 inch wide, and 4 inches long parallel to the water sources and onion rows. To minimize cross contamination in collecting soils, sample retrieval was grouped based on the irrigation system, water source, and sample position. The samples for a specified treatment and position were collected in all replicates, followed by a change of latex gloves and sterilization of all equipment in 60 ml of bleach diluted in 4 L of distilled water followed by rinsing in three successive baths of 4 L of distilled water.

Soil samples were immediately placed in a cooler on ice, transported to the laboratory, refrigerated until analyzed, and analyzed as soon as possible. When possible the analyses were started the day that the samples arrived in the laboratory. Part of each soil sample was weighed wet, dried, and weighed dry to determine the soil water content. Separately, 50 g of each soil sample was diluted in 75 ml of distilled water and shaken. Then 50 ml of water was removed and was used to estimate the MPN of generic *E. coli* in the soil water using IDEXX *Colilert* + *Quanti-Tray/2000* (IDEXX Laboratories, Westbrook, ME) (Edberg and Edberg, 1988). Results were expressed in terms of MPN/100ml of soil water.

The MPN of generic *E. coli* in the soil water was compared between treatments, soil sampling positions, and irrigations. Where *E. coli* was not detected, the MPN per 100 ml was assumed to be 1, the detection limit of the procedure for statistical analyses.

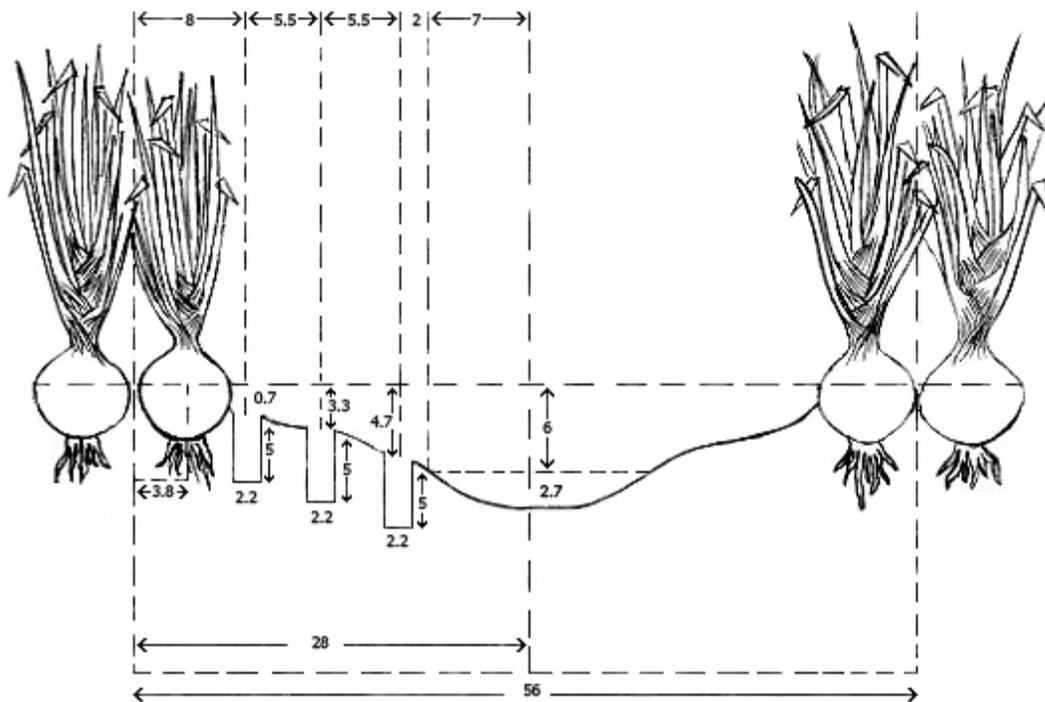


Figure 2. Location of soil samples in furrow-irrigated onions with respect to edge of the water and onion bulbs. The distances are in cm (2.54 cm = 1 inch).

Sampling onion before and after lifting

Onions were lifted on August 31, 2015, 7 days after the last irrigation. Onions were sampled for *E. coli* at four time intervals: 1) August 27, 3 days after the last irrigation; 2) September 2, 3; 3) September 9, 10; and 4) September 16, 17.

On each date, 3 samples of 20 onions were collected from each plot. No onions were discarded. Onions were topped and placed in wire baskets and then placed in plastic storage bags. To minimize potential inadvertent cross contamination, workers wore gloves, and used sterilized knives and baskets in the field. Knives and baskets were sterilized with bleach and workers exchanged gloves as they moved from plot to plot. Because we anticipated that irrigation treatments would have different amounts of *E. coli*, we sampled them in order of expected *E. coli* levels, going from low to high (i.e., drip-irrigated with well water, drip-irrigated with canal water, furrow with canal water, furrow with *E. coli*-enhanced canal water). Within irrigation treatments, we expected treated onions to have lower *E. coli* levels than untreated onions. Therefore, we sampled the treated plots first.

Onions were transported to Western Laboratories, Inc. in Parma, Idaho for *E. coli* analysis. In the laboratory, the roots, small remnants of soil, skins, and outer peel of the onions were removed from the bulbs and weighed. The skins, peels, roots, and soil were then thoroughly washed in 1 L of water. A 100-ml sample of the wash water was used to estimate a MPN of generic *E. coli* present on the exteriors of the onions, using IDEXX Colilert +Quanti-Tray/2000 (Edberg and Edberg 1988, Edberg et al. 1990). The *E. coli* MPN per onion bulb exterior was then calculated based on the number of onions in each sample. Bulb interiors sampled August 31 were tested for

internal *E. coli* as described below.

Sampling onions for *E. coli* contamination at packout

Four boxes of onions were harvested on September 22 from each of the five plots of bulbs drip-irrigated with well water and each of the plots of bulbs furrow-irrigated with enhanced canal water. Sampling was done using sterile rubber gloves, wire basket, and knife. Baskets and knives were sterilized and gloves were changed between treatments. The 4 boxes were filled from each irrigation treatment in each of the 5 replicates, for a total of 40 boxes. Representative onion sampling was achieved by pulling every sixth onion, then going back and pulling every fifth onion, and so on until the boxes were full. The sampled onions remained in the field for 8 days until September 30, when storage containers were transferred into a cold air storage unit. The storage conditions were monitored and maintained similar to those used for commercial onion storage. Onion storages are automated to use ambient air to cool the bulbs to 1°C (33.8°F) as opportunistically feasible.

On November 16 and 17, 2015, 3 samples of 20 bulbs each were packed out from each box. New sterile gloves and a freshly sterilized packing table were used individually for each “packout” sample. Onions were packed out in the following order: onions from the drip-irrigated then furrow-irrigated boxes. This order was chosen to minimize cross contamination by handling presumably the “cleanest” onions first.

Results and Discussion

Irrigation water

The MPN of *E. coli* per 100 ml and date in the hourly water samples of irrigation canal water varied over time (Table 1). The variation was expected since the canal water source influenced was continuously changing by the runoff return flow from various upstream neighboring fields. The irrigation treatment with “enhanced” *E. coli* had significantly greater MPN of *E. coli* per 100 ml during the first, second, and third irrigations.

During the first irrigation, there were significant differences in the water between all of the treatments, with the enhanced treatment having the highest *E. coli* MPN, followed by the drip-irrigated with canal water, then the furrow-irrigated with canal water, and the drip-irrigated with well water. The drip-irrigated treatment had no detectable *E. coli*. Significant differences were less clear during subsequent irrigations.

Subsurface drip irrigation with well water

E. coli was not detected in the hourly samples of the well water for four irrigations. The corresponding soil had no detectable *E. coli* at any of the three sampling positions, except for the soil samples collected on August 12, which showed very low amounts next to the drip tape (5.4 MPN *E. coli*/100ml soil water) and halfway between the tape and onion bulbs (3.1 MPN *E. coli*/100ml soil water) (Table 2).

Table 1. *E. coli* levels in irrigation water of four different irrigation treatments at the Malheur Experiment Station, Ontario, OR August 2015. Collections were taken hourly for 7-8 hours at each irrigation event. Enhanced *E. coli* treatments were made by running irrigation across a pasture before flowing back in the irrigation canal the day following other irrigation treatments. Samples were collected where water the entered field, except for the upstream collections, which were collected upstream of the pasture return flow to determine how *E. coli* levels were enhanced.

Irrigation treatment	Collection	Irrigation 1		Irrigation 2		Irrigation 3		Irrigation 4	
		Aug. 5, 6		Aug. 10, 11		Aug. 17, 18		Aug. 24, 25	
		Mean	Std. dev	Mean	Std. dev	Mean	Std. dev	Mean	Std. dev
Furrow	At field	8 A*	9	-		1560 A	1087	302 A	106
Furrow-enhanced	At field	775 B	325	1392	942	5042 A	7133	300 A	341
Enhanced	Upstream	608 -	526	540	716	461 -	na	110 -	33
Drip, well	At field	nd ¹ C	na ²	-		nd B	na	nd B	na
Drip, canal	At field	13 D	3	-		2098 A	2390	330 A	66

* Means followed by different upper case letters are statistically different. The standard deviations (Std. dev) are an indication of how variable the data are. Larger standard deviations indicate greater variation in the data. Upstream measurements were not included in the analyses.

¹nd, not detected.

²na, not available.

Table 2. Mean *E. coli* content in soil from different irrigation treatments and taken from different locations within the onion bed. Capsules were placed next to the water source (furrow or drip tape), next to onion bulbs, or midway between these locations. Malheur Experiment Station, Ontario, OR, August 2015.

Locations on the onion bed	Furrow irrigated with canal water		Furrow irrigated with canal water, enhanced <i>E. coli</i>		Drip irrigated with well water		Drip irrigated with canal water	
	Mean	Std. dev.	Mean	Std. dev.	Mean	Std. dev.	Mean	Std. dev.
1st Irrigation								
Water source	29	53	34	39	nd ¹	na ²	7	3
Middle	3	5	27	21	nd	na	1	1
By onion bulb	6	13	2	3	7	15	1	3
2nd Irrigation								
Water source	5	3	234	417	25	55	4	4
Middle	6	5	3914	8393	nd	na	4	5
By onion bulb	0.6	1	19	22	0.20	0.5	1	1
3rd Irrigation								
Water source	444	730	2163	2010	4	8	54	53
Middle	24	18	442	396	3	5	3	3
By onion bulb	2	1	31	18	nd	na	0.8	0.8
4th Irrigation								
Water source	80	128	53	58	nd	na	18	16
Middle	9	7	19	25	0.2	0.5	2	1
By onion bulb	3	5	0.6	0.9	nd	na	3	4

¹nd, not detected; ²na, not available.

After the first irrigation the amount of *E. coli* in the soil water varied significantly by treatment ($P = 0.01$). During the first irrigation, the enhanced *E. coli* had the highest level of *E. coli*, followed by the furrow-irrigation treatment and the drip-irrigated with canal water. Well water had the lowest soil water *E. coli* levels, although these were not statistically different from the soil water in the treatment drip-irrigated with canal water. Following the first irrigation, soil water *E. coli* levels generally decreased from the water source to the onion bulb across the onion beds ($P = 0.01$). In terms of location effects, the normal canal water treatment tended to have high *E. coli* next to the water source but significantly lower levels at the other two locations

towards the onion bulbs.

In the second irrigation, all other treatments had significantly lower *E. coli* levels than the enhanced treatment ($P = 0.01$). As in the first irrigation, the highest *E. coli* levels were next to the water source, with the other two locations having significantly lower *E. coli* levels, except for the enhanced furrow-irrigated treatment. In the enhanced treatment the highest soil water *E. coli* was between the water source and the onion bulbs.

In the third irrigation, all irrigation treatments were significantly different from one another, with the enhanced treatment having the highest levels and the well water the lowest ($P = 0.0001$).

The collection locations also differed from one another, with the lowest levels next to the onion bulbs and the highest levels next to the water source ($P = 0.0001$). The interaction effects among irrigation treatments and sampling positions were also significant ($P = 0.01$).

In the fourth irrigation, the results were similar to the second irrigation, with the well water treatment having significantly lower levels than the other three treatments ($P = 0.0001$). The samples collected next to the water source had the highest *E. coli* levels ($P = 0.0001$). There was no difference between the middle samples and the samples next to the bulbs.

Subsurface drip irrigation with canal water

The *E. coli* counts in the canal water (used for both this treatment and the furrow-irrigated treatment below) varied substantially during and among irrigations, as should be expected since the irrigation water source received runoff water from many growers upstream. Concentrations of *E. coli* were higher during the second irrigation. The soil was sampled four times throughout the season, and concentrations of *E. coli* were always highest in the soil water near the drip tape and decreased as the water flowed towards the onion bulbs (Table 2).

Furrow irrigation with canal water

The furrow irrigation system was carefully managed, as is typical for onion production in southeastern Oregon, to avoid water reaching directly onto the shoulders of the onion bulbs. *E. coli* counts in the canal water varied substantially during and among irrigations but was not statistically different from the canal water used in the drip-irrigated treatment. On some dates, the water had relatively few *E. coli* counts while on other dates some samples exceeded the testing limits (Table 1). *E. coli* in the soil water consistently decreased as water moved laterally through the soil, regardless of the initial contamination level (Table 2).

Furrow irrigation with enhanced canal water

The attempt to enhance the *E. coli* counts by running irrigation water across a pasture and reintroducing the runoff water into furrow irrigation resulted in widely variable enhancement of *E. coli* among and within irrigations (Table 1). The “enhanced” treatment had significantly higher MPN’s than the canal water during the first, third, and fifth irrigations. Even with elevated concentrations of *E. coli* in the water, the same trend developed once the water entered the soil: the highest concentrations tended to be adjacent to the wetting front, and the lowest concentrations tended to be near the onion bulbs (Table 2).

The strategy to enhance furrow irrigation water with *E. coli* from pasture runoff presented logistical difficulties. Initially, irrigations were conducted on the same day as the other three irrigation systems. Due to logistic difficulties, irrigations were changed to the day following the other treatments, and the baseline of bacterial content in the irrigation canal enhanced with the

pasture runoff also varied from day to day. In spite of fresh bovine manure in the pasture, the water pathways providing pasture runoff did not consistently enrich the water as planned.

Soil filtration of *E. coli*

The analysis of variance of the \log_{10} of the MPN of soil water *E. coli* showed significant statistical differences ($P = 0.01$) for irrigation treatments, soil sampling positions, irrigations, the interaction of treatments with positions, and the interactions of treatments with irrigations. The interaction of treatments with irrigations and positions and the interaction of positions with irrigations were not statistically significant. In every set of soil samples except those drip irrigated with well water, the data showed that *E. coli* concentrations in the soil water decreased as water moved laterally through the soil. There were no significant differences in the performance of the soil in filtering *E. coli* between the drip-irrigated system with canal water and furrow-irrigated treatment with canal water. The soil filtering described here is for lateral flow of water, in some ways analogous to previous published research on soil filtering of bacteria under vertical flow of water in soil and water columns (Jamieson et al. 2002; Mankin et al. 2007; Safacoust et al. 2011, 2012; Semenov et al. 2009; Unc and Goss 2004). Regardless of initial counts, concentrations typically were highest near the drip tape for SDI or water's edge of furrow irrigation, lower at the points halfway between the drip tape/furrow and onion bulbs, and lowest at spots adjacent to the onions. Collectively, the data suggest that the soil is acting as a natural filter to remove bacteria and possibly other biological contaminants, either through physical, biological, or chemical means, under the trial conditions.

It was probable that the low amounts of *E. coli* found at soil positions adjacent to the onion bulbs were due at least in part to water movement by nonsaturated capillary flow of water around the soil particles, leaving the bacteria behind on the soil particles nearer to the water sources.

Onion external *E. coli* contamination

E. coli was detected on onion exteriors sampled before lifting (Table 3). Most samples had no *E. coli*, some had minor levels (<126 MPN), and a few had high levels. These results occurred across all four irrigation treatments. For example, the high mean in the well water treatment was from a single sample with 13,950 MPN per bulb. This variation suggests there can be random, variable amounts of *E. coli* that can be found on onion exteriors. None of the onion interiors from this sampling were contaminated with *E. coli*.

Table 3. *E. coli* levels on onion exteriors before and after lifting August 31, 2016. Values are the MPN of *E. coli* per onion bulb.

Irrigation system	Water source	Collection date	Mean	Standard error
Furrow	Canal	27 August	963.25	570.82
Furrow	Canal	3 September	0.13	0.13
Furrow	Canal	10 September	2.76	2.76
Furrow	Canal	17 September	20.61	19.33
Furrow	Enhanced canal	27 August	279.80	141.17
Furrow	Enhanced canal	3 September	132.62	132.41
Furrow	Enhanced canal	10 September	1.07	1.07
Furrow	Enhanced canal	17 September	0.20	0.15
Drip	Well	27 August	931.99	929.86
Drip	Well	2 September	nd ¹	na ²
Drip	Well	9 September	nd	na
Drip	Well	16 September	24.49	24.35
Drip	Canal	27 August	9.31	5.89
Drip	Canal	2 September	nd	na
Drip	Canal	9 September	nd	na
Drip	Canal	16 September	0.97	0.90

¹ nd, not detected, ² na, not available

Onion internal *E. coli* content

Although 60 bulbs from every replicate of all four treatments were sampled on 16 September, *E. coli* was not detected in any of the onion samples. The follow-up sampling of 4 60-bulb samples from every replicate of the furrow-irrigated treatment with enhanced *E. coli* and from the drip-irrigated well water check treatment also had no detectable presence of *E. coli*. In spite of the generic *E. coli* in the soil water immediately adjacent to the onion bulbs, *E. coli* was not internalized with either subsurface drip or furrow (flood) irrigation. These results differed from those of Solomon et al. (2002) where *E. coli* O157:H7 delivered through flood or sprinkler

irrigation was internalized by lettuce. Different plant species are expected to have variable susceptibility to the internalization of human pathogens (Hirneisen et al. 2012). Onion may have some resistance to *E. coli* infection of the bulb via the roots or skin.

Food Safety Regulation

As a direct consequence of the Food Safety Modernization Act (FSMA), Public Law 111-353-Jan. 4, 2011, on November 27, 2015 the U.S. Food and Drug Administration (FDA) published *Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption; Final Rules* in the Federal Register, which are referred to here as the “final rules” (U.S. FDA 2015). The FSMA is the first major federal reevaluation of food safety standards since 1938. It charges the FDA with ensuring the safety of the U.S. food supply by acting preventively rather than reactively to foodborne illness outbreaks. The FSMA rules cover many aspects of the growing and handling of produce, in particular placing stringent testing requirements and use limitations on agricultural water that is applied to any produce covered by the rules (U.S. FDA, 2015). Agricultural water is defined in the final rules “as water used in covered activities on covered produce where water is intended to, or is likely to, contact covered produce or food-contact surfaces, including water used in growing activities (including irrigation water applied using direct water application methods, water used for preparing crop sprays, and water used for growing sprouts) and in harvesting, packing, and holding activities (including water used for washing or cooling harvested produce and water used for preventing dehydration of covered produce).” Further, the quality of agricultural water intended for irrigation would have to be consistent with criteria based on the U.S. Environmental Protection Agency microbial quality standards for recreational water in order to be used (U.S. FDA 2015). The FDA has adopted generic *E. coli* as the indicator for determining agricultural water quality, although many strains of the bacterium are not pathogenic (Edberg et al. 2000, U.S. FDA 2013, 2014).

In the 2015 final rules, the FDA has set standards for permissible water quality as a rolling geometric mean of generic *E. coli* of up to 126 colony forming units (CFU)/100 ml of irrigation water with a statistical threshold value of up to 410 CFU/100 ml, based on 20 samples for surface irrigation water. These standards are a significant revision to 2013 proposed rules, which would have set a rolling geometric mean of generic *E. coli* of up to 126 CFU/100 ml of irrigation water and if a single sample exceeded 235 CFU/100 ml, irrigation would have to cease until the water supply was in compliance (U.S. FDA 2013). Under the final rules, for fresh produce irrigated with water beyond these thresholds, there would be an allowance for natural die-off of bacteria, assuming a 0.5 log per day die off from last irrigation for up to 4 days before harvest. If the water quality profile at the last irrigation exceeds the regulated thresholds, the crop cannot be harvested until sufficient time has passed for the *E. coli* levels to come into compliance (U.S. FDA 2015). Dry bulb onions in the Treasure Valley cure in the field for 2-4 weeks following the last irrigation before they are actually harvested; this would effectively eliminate most all *E. coli* delivered from irrigation water.

In the FSMA, the inclusion of proposed rules for agricultural water standards is an attempt to prevent disease outbreaks of human pathogenic organisms due to the consumption of contaminated produce. Lettuce, sprouts, cabbage, apple cider, apple juice, and tomatoes are the primary produce identified as contaminated with *E. coli* before purchase (Rangel et al. 2005). In the FSMA, sprouts have different standards than other produce. The FDA is developing special guidance for other potentially high-risk crops, such as leafy green vegetables, melons, and tomatoes (U.S. FDA 2015). All other vegetables, including dry onion bulbs, presently are being

considered together. This “other” category includes six known sources of outbreaks of foodborne illness: almonds, green onions, raspberries, peas, peppers, and squash (U.S. FDA 2013). Of these six known sources, hot peppers and green onions from Mexico account for all but 3.2% of the estimated human health cost (calculations by Shock, B.M., based on the data in U.S. FDA 2013).

Our results indicate that generic *E. coli* from surface irrigation water is not likely to contaminate dry bulb onions. Despite the low risks to human health, onion growers in the Treasure Valley will incur significant costs in complying with the FSMA produce safety rules. These burdens will consist of the labor for water sampling and record keeping, the cost of laboratory analysis, and any additional costs for potential water quality remediation procedures. Losses from the proposed rules to the community could extend to lost investment in onion production equipment, onion storage buildings and packing facilities, and potential loss of employment and property values.

Conclusions

The silt loam tended to filter *E. coli*, retaining most of the bacteria close to where the water entered the soil irrespective of the irrigation system. Under both furrow and subsurface drip irrigation, a fraction of the *E. coli* from the irrigation water was delivered to the soil water immediately adjacent to the onion bulbs. No *E. coli* was detected inside of the onion bulbs from any irrigation treatment. Current subsurface drip or furrow (flood) irrigation practices in the Treasure Valley of Idaho and Oregon do not appear to pose a significant risk for bacterial contamination of dry bulb onion grown on silt loam.

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