DRIP VS. FURROW IRRIGATION IN THE DELIVERY OF *ESCHERICHIA COLI* TO ONIONS

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A Tribute to the Career of Terry Howell, Sr.

ABSTRACT. *Surface irrigation systems that reuse water may deliver bacteria to produce destined for fresh consumption. 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. Water was sampled hourly for* E. coli, *and the lateral movement of* E. coli *in the soil solution was tracked by soil samples following irrigations. Onion bulbs were sampled for* E. coli *contamination. The most probable numbers of E. coli in water and soil water were determined using IDEXX Colilert® and Colisure®, respectively, and Quanti-Tray/2000®. Under both furrow and subsurface drip irrigation, a fraction of the* E. coli *was delivered to the soil 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. No* E. coli *was detected inside of the onion bulbs from any irrigation treatment. Current subsurface drip or furrow (flood) irrigation practices do not appear to pose a significant risk for bacterial contamination of dry bulb onion grown on silt loam.*

Keywords. *Food safety, Water quality, Subsurface drip irrigation, Furrow irrigation, Onions, Allium cepa, Soil filtration of* E. coli*, Lateral water movement,*

The transmission of foodborne pathogens from fresh produce to humans has been documented and is of increasing concern (De Roever, 1998; Bernstein, 2011, 2013; 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 (Steele and Odumeru, 2004; Oliamat and Holley, 2012). 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 readily be contaminated by contaminated irrigation water in Nigeria.

The movement and persistence of fecal bacteria in soil has been the subject of substantial investigation, largely from the point of view of protecting groundwater quality (Jamieson et al., 2002). Bacterial survival is promoted by soils with high moisture retention, fine soil particle size, cool temperature, and neutral to slightly alkaline pH. Bacterial survival in soil is also favored by the application of manure and the specific crop's roots (Islam et al., 2005; Hirneisen et al., 2012). *Escherichia coli* persisted for up to several months in soil composted with manure and irrigated with water containing *E. coli*; however, the persistence of *E. coli* in the soil depended on the type of vegetable grown in the soil, with inactivation being more rapid in soil in which onions were grown (Islam et al., 2005).

Vertical movement of bacteria through the soil is favored by the volume of leachate, preferential flow, and coarse soil particle size and is reduced by adsorption and filtration by soil particles (Jamieson et al., 2002). Unc and

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Goss (2004) reviewed articles that showed that after water infiltrates the soil, the filtration of bacteria depends on the physical configuration of the soil, the soil chemistry, and the size of the bacteria relative to the diameter of soil pores. The path followed by water determines the direction of transport of bacteria. A study to understand the fate and transport of *E. coli* in soil columns showed that both the soil texture and structure affected the transport properties of the soil including dispersivity and attachment and straining coefficients (Safacoust et al., 2012).

Semenov et al. (2009) found limited *E. coli* movement in the field beyond the application layer with surface application of *E. coli.* Recovery rates of *E. coli* from water flowing through soil columns were tested for two different soil types, two different flow rates, and two different methods of preparing the soil for the columns (repacked or physically and biologically weathered) (Safacoust et al., 2011). In all eight instances, the *E. coli* recovery diminished with increasing soil depths of 15, 30, and 45 cm. Testing silt loam soil and sand for their sorption capacity (*E. coli* attached to soil, hence filtered out of the water) showed that the silt loam soil had a much greater capacity for sorption of *E. coli* than sand (Mankin et al., 2007). The sorption capacity for both increased as the *E. coli* concentration increased.

Kouznetsov et al. (2004) compared surface irrigation with SDI at 25 cm depth for microbial transport and survival in soil columns and concluded that SDI had lower pathogen survival in the soil and less likelihood to transmit pathogens to fresh produce. Although SDI has the potential to reduce produce contamination, drip tape placement, drip emitter flow rate, and irrigation scheduling practices need to be considered to realize this potential. Furrow irrigation was compared with SDI for the contamination of lettuce with bacteriophages and SDI resulted in greater contamination (Choi et al., 2004). Although the drip tape was buried 20 cm deep in the lettuce beds, water from the drip system flowed to the soil surface and directly wetted the lettuce stems (Choi et al., 2004). By placing drip tape directly over the top of potato beds, pathogens were transported directly to the soil and potatoes below the drip emitters (Forslund et al., 2011).

Internalization of human pathogens into roots of fresh produce has been reviewed (Hirneisen et al., 2012). *E. coli* from soil can be internalized from lettuce and move throughout the plant (Solomon et al., 2002b). Internalization of *E. coli* in lettuce can occur through sprinkler or flood irrigation transmission of the bacteria (Solomon et al., 2002a). The potential for internalization of a human pathogen via the root system seems to be specific to the pathogen strain, crop species and cultivar, plant development stage, and growth medium (Hirneisen et al., 2012).

In laboratory studies, the decline in *E. coli* population in soil is exponential with a half-life of three to four days. The rates of decline are more rapid under higher temperature and low moisture conditions (Ogden et al., 2001). Populations of *E. coli* decline more rapidly in muck soils than sand, more rapidly in aerobic than anaerobic conditions, more rapidly in drier soil than saturated soil, and more rapidly in soil with less versus greater organic material (Tate III, 1978). Under field conditions the rate of decline is greater in the presence of onion root systems than carrot root systems, and the population decline is influenced by the type of manure added to the soil (Islam et al., 2005). Logarithmic declines of *Salmonella* and *E. coli* occur in two Oregon soils planted to onion following heavy inoculation (Emch and Waite-Cusic, 2016). A review by Jamieson et al. (2002) describes why bacterial population trends in soil are complicated; bacterial populations are affected by many factors including soil mineral content, soil texture, organic material, temperature, moisture status, aeration, and competing organisms. In special environmental conditions *E. coli* populations in soil can increase and become a source of *E. coli* to water bodies (Ishii et al., 2006).

Agriculture in the Treasure Valley of eastern Oregon and southwestern Idaho is highly diversified, with the production of numerous crops and large numbers of cattle (Shock et al., 2000a; Shock and Shock, 2012; USDA Economic Research Service, 2011a, 2011b). Dry bulb onions are the most economically valuable crop grown in the region. Onions are grown on 9,000 ha in the valley, with an annual farm gate value of $150 million. Onions would be subject to the Food Safety Modernization Act (FSMA) rules for fresh produce (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 Food Safety Modernization Act 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 subsurface drip irrigation 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 are 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 CFU/100 mL could be recovered up to 74 days after application (Islam et al., 2005), but the population declined logarithmically over time. *Salmonella* and *E. coli* 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. The *E. coli* from irrigation water that reached the onion bulbs when they are growing in the field were not internalized and died before bulb 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. After onions were lifted and cured, bulbs grown with contaminated water had just as low external contamination as those grown with no detectable *E. coli* in the irrigation water (Shock et al., 2013b). It is unknown to what extent *E. coli* present in irrigation water could reach onion roots and bulbs and ultimately be a human health risk internal to marketed onion bulbs.

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 and reaches the proximity of the onion bulb. The 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

The trial was conducted at the Oregon State University Malheur Experiment Station, Ontario, Oregon, in a field of silt loam soil. The location of the center of the *E. coli* field trial was 43° 58' 45.20" N - 117° 1' 9.49" W. The experiment utilized 'Vaquero' onions that had been planted 12 March 2014 and irrigated exclusively by SDI using well water until 28 July 2014. The seed was all from the same lot, the seed 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 30 m long. All observations were made in the central bed of each irrigation plot. Drip tape was laid at planting at 0.07-m 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 0.3 m apart and emitter flow rate of 0.5 L/h (Toro Aqua-Traxx, Toro Co., El Cajon, Calif.). The distance between the tape and the center of each double row of onions was 0.28 m. 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 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, Calif.) installed at 20-cm 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* five times during the growing season. The final irrigation occurred on 5 September. The onions were lifted manually on 21 August and 10 September 2014.

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 on a weekly basis, typically for 11.5 h 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 same day or the following day, depending on the logistics of the water supply.

FURROW-IRRIGATION WITH CANAL WATER

Lay-flat hose (5 cm 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 2-cm 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 3 L/min per furrow or 45 L/min to the five replicates.

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 was the addition of pasture runoff at the experiment station mixed in with the canal water above the inlet pipe to try to ensure a higher level of *E. coli* in the irrigation water. The pasture was grazed by cattle prior to each pasture irrigation. The amount of pasture irrigation water runoff and its level of contamination varied continuously. Tins placed in the canal directed the runoff water to flow by the inlet pipe to try to ensure high *E. coli* content in the irrigation water. The system delivered approximately 3 L/min per furrow or 45 L/min to the five replicates.

SUBSURFACE DRIP-IRRIGATION WITH WELL WATER

The SDI system with well water was already in place because that is how the field had been irrigated 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 12.5 L/min to the five 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, Calif.) were filled with 30 grit crushed garnet as 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 min. The system was calibrated to have the capability to deliver approximately 20 L of water per minute to the field at 83 kPa, and the system delivered 12.5 L/min to the five replicates.

E. COLI IN THE IRRIGATION WATER

The irrigation water was sampled hourly from each of the four irrigation systems for the first 8 h 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 2.54-cm 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 h of collection.

The IDEXX Colilert® +Quanti-Tray/2000® system (IDEXX Laboratories, Westbrook, Maine) 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). 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 which 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; Edberg et al., 1990). If all sample wells are positive, MPN values are reported as >2420 MPN per 100 mL. Quantification would then require testing serial dilutions of the original sample. The lower limit of detection was 1 MPN per 100 mL.

Comparisons of the most probable number (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. Samples that had MPN >2419.6 per 100 mL were assumed to have MPN 2419.6 per 100 mL for statistical comparison purposes.

SOIL WATER SAMPLES

Soil samples were replicated five times for each irrigation system and at three sampling distances between

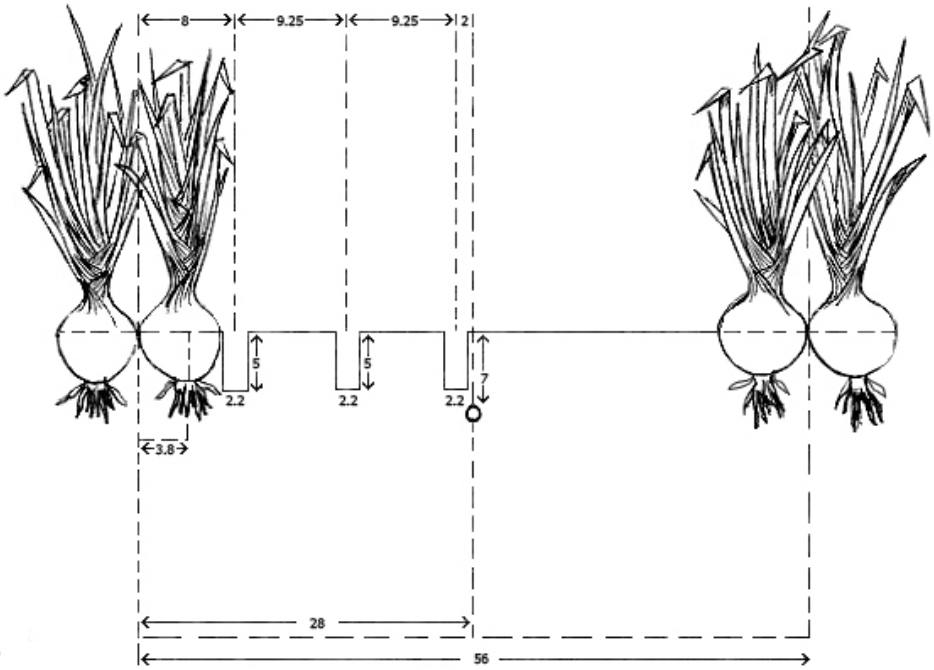

Figure 1. Location of soil samples in the drip-irrigated onions with respect to the drip tape and onion bulbs. The drip tape (o) was placed 7 cm deep in the center of the onion bed. Distances are in cm.

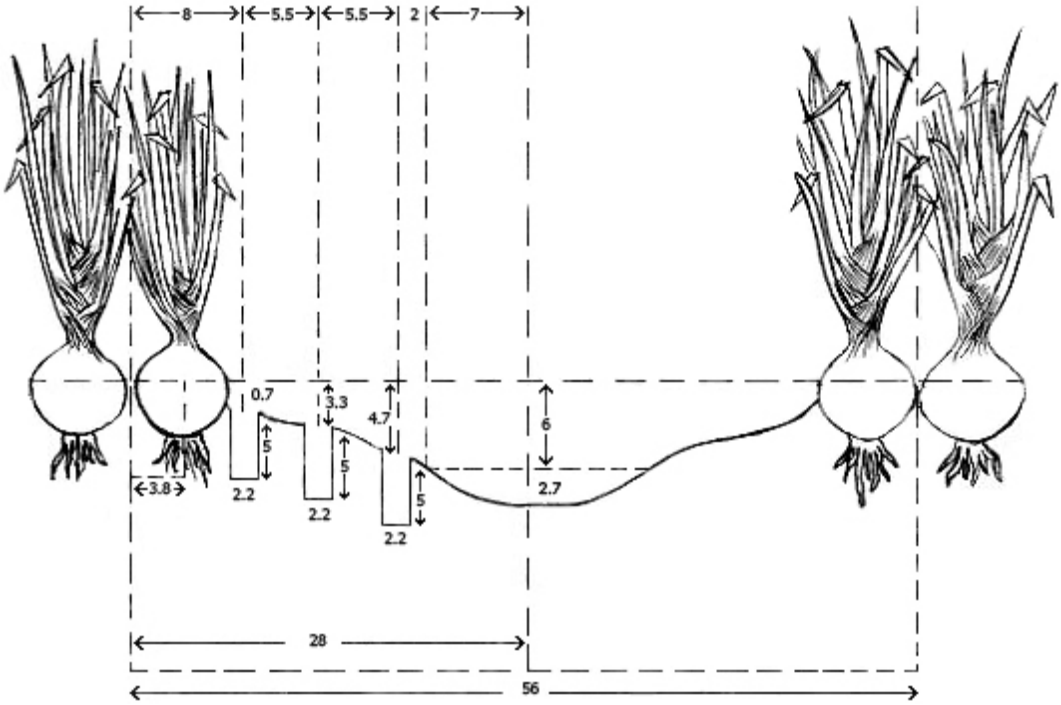

Figure 2. Location of soil samples in furrow-irrigated onions with respect to edge of the water and onion bulbs. Distances are in cm.

the edge of the water in the furrow irrigation or next to the drip tape up to directly against the onion bulbs (figs. 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 5 cm deep, 2.2 cm wide, and 10 cm 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 the water was removed and was used to estimate a Most Probable Number (MPN) of generic *E. coli* in the soil water using IDEXX *Colilert*® + *Quanti-Tray/2000*® (IDEXX Laboratories, Westbrook, Maine) (Edberg and Edberg, 1988). Results were expressed in terms of MPN/100 mL 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. Most probable number data were transformed to $\log_{10}$. Data were subjected to analysis of variance with treatments being the main plots, sampling positions as split plots, and irrigations as recurring measurements over time.

SAMPLING ONIONS FOR INTERNAL *E. COLI* CONTAMINATION

Onions were lifted after 24 and 55 d of the onset of the irrigation treatments for internal *E. coli* contamination. The first set of samples consisted of 60 bulbs from each replicate of all four treatments. The bulbs were lifted on 21 August, were allowed to cure in the field for three weeks, were crated, and were analyzed for *E. coli* on 16 September. The remainder of the onions were lifted on 10 September and cured in the field until 2 October. The second sampling consisted of 4 sets of 60 bulbs from every plot of the treatments considered to have the greatest and least risk of contamination: those furrow-irrigated with enhanced canal water and those irrigated with well water using subsurface drip irrigation. On 2 October the four subsamples of 60 bulbs from every replicate of these two treatments were crated and moved to a conventional onion storage until 4 November when they were packed out for analyses. Onion storages are automated to use ambient air to cool the bulbs to 1°C as opportunistically feasible.

Packout involved the removal of loose skins and roots that are ordinarily removed from onion bulbs in conventional harvesting and pack out operations. Packed out bulbs were placed into double-bagged 30-gal plastic bags labeled accordingly to the treatment and replicate. In some cases, fewer than 60 bulbs were available to packout, so a tally was kept for the number of bulbs in each bag and transported to the laboratory. The outer skins and scales were peeled from all the onions in each sample. To test for the presence of internal *E. coli* the peeled bulbs were placed on an aluminum tray. The outsides of the peeled onions were disinfected with 70**%** ethanol, and then the bulbs were placed on a sterilized aluminum tray. The

alcohol was allowed to dissipate. A wedge was cut out of each of 60 onions, and the wedges were placed in a sterilized stainless steel beaker and macerated with a food processer (Waring commercial immersion blender; model WSB). After maceration, 10 mL of the resulting onion suspension was placed in a flask containing 90 mL of Universal Pre-enrichment broth (UPB, Accumedia, Nedgen, Mich.) and sealed. Along with every batch of samples, an additional positively inoculated sample was placed in an additional flask containing UPB broth. The UPB broth was placed in an incubator for 48 h at 35°C.

A glass jar with 100 mL sterilized water had a package of Colisure (IDEXX) added as a substrate to test for the presence of generic *E. coli*. Five mL of the UPB was transferred to the Colisure mixture and incubated for 24 h at 35°C. After 24 h the Colisure samples were examined under UV light for fluorescence, indicating the presence of generic *E. coli* (Edberg and Edberg, 1988; Edberg et al., 1990).

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 was continuously changing, as influenced 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, third, and fifth irrigations. The source of the enhancement was pasture runoff water immediately adjacent to the onion experimental site, and the concentration of *E. coli* in the runoff was not controlled.

SUBSURFACE DRIP IRRIGATION WITH WELL WATER

E. coli was not detected in the hourly samples of the well water for five irrigations. The corresponding soil had no detectable *E. coli* at any of the three sampling positions,

Table 1. Concentration of *E. coli* in the water of four different systems used to irrigate onions.

Irrigation System, Water Source	Time after Irrigation Onset (h)	*E. coli* (MPN/ 100 mL)					
		5 Aug.	12 Aug.	19 Aug.	26 Aug.	4 Sept.	Mean
Subsurface drip irrigation, well water	0	nd[a]	nd	nd	nd	nd	
	1	nd	nd	nd	nd	nd	
	2	nd	nd	nd	nd	nd	
	3	nd	nd	nd	nd	nd	
	4	nd	nd	nd	nd	nd	
	5	nd	nd	nd	nd	nd	
	6	-	nd	nd	nd	nd	
	7	-	nd	nd	nd	nd	
	8	-	nd	nd	nd	nd	na[b]
Subsurface drip irrigation, canal water	0	141.4	>2419.6	547.5	85.7	88.2	
	1	98.7	1986.3	248.1	79.4	85.7	
	2	79.8	1986.3	131.7	71.2	107.6	
	3	55.4	1553.1	90.6	50.4	121.1	
	4	-	>2419.6	129.6	55.4	71.4	
	5	-	1732.9	222.4	33.6	214.1	
	6	-	1299.7	222.4	20.1	41.4	
	7	-	1119.9	101.7	22.8	34.5	
	8	-	1203.3	137.4	28.5	71.4	
	Mean	94	1663	203	50	93	420
Furrow irrigation, canal water	0	113.7	>2419.6	344.1	59.1	122.3	
	1	148.3	1299.7	131.7	47.1	119.8	
	2	86.5	1119.9	127.4	67	117.8	
	3	77.6	816.4	133.3	61.3	107.6	
	4	54.5	1299.7	235.9	30.9	101.4	
	5	60.5	>2419.6	248.9	29.8	86.7	
	6	-	1299.7	191.8	24.3	39.1	
	7	-	517.2	123.6	25.6	49.6	
	8	-	1203.3	86.2	29.2	26.2	
	Mean	90	1377	180	42	86	355
		5 Aug.	12 Aug.	20 Aug.	27 Aug.	5 Sept.	
Furrow irrigation, enhanced	0	1732.9	>2419.6	547.5	201.4	>2419.6	
	1	>2419.6	866.4	579.4	325.5	>2419.6	
	2	1986.3	1119.9	1119.9	270	>2419.6	
	3	>2419.6	980.4	488.4	261.3	>2419.6	
	4	1553.1	1553.1	920.8	198.9	>2419.6	
	5	>2419.6	1553.1	435.2	285.1	>2419.6	
	6	-	1203.3	410.6	125.9	>2419.6	
	7	-	686.7	488.4	101.7	-	
	8	-	1553.1	272.3	85.7	-	
	Mean	2089	1326	585	206	2420	1325
Overall mean		758	1455	323	99	866	700

LSD (0.05) treatments = 141
LSD (0.05) irrigations = 199
LSD (0.05) treatments × irrigations = 282

[a] nd, not detected.
[b] na, not available

except for the soil samples collected on 12 August, which showed very low amounts next to the drip tape (5.4 MPN *E. coli*/ 100 mL soil water) and halfway between the tape and onion bulbs (3.1 MPN *E. coli*/ 100 mL soil water) (table 2).

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 was receiving 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, avoiding water reaching directly onto the shoulders of the onion bulbs. *E. coli* counts in the canal water varied substantially during and among irrigations, but were not statistically different from the canal water used in the drip-irrigated treatment. Some dates the water had relatively fewer *E. coli* counts while other dates showed some samples exceeding 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. 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 highly 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; Unc and Goss, 2004; Mankin et al., 2007; Semenov et al., 2009; Safacoust et al., 2011, 2012). Regardless of initial counts, concentrations were typically 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

Table 2. Concentration of *E. coli* in the soil water (MPN/100 ml of water) following four different onion irrigation treatments.

Irrigation System, Water Source	Position of the Soil Sample with Respect to the Water Source and Onion Bulbs	Mean *E. coli* MPN/ 100 mL Soil Water			
		12 August	20 August	27 August	6 Sept.
Subsurface drip irrigation, well water	Next to the drip tape	5.4 (12.1)[a]	nd[b]	nd	nd
	In between	3.1 (6.9)	nd	nd	nd
	Next to the onion bulb	nd	nd	nd	nd
Subsurface drip irrigation, canal water	Next to the drip tape	209 (298)	582 (642)	2470 (3260)	675 (1500)
	In between	31 (36.4)	114 (157)	282 (165)	nd
	Next to the onion bulb	12 (19.0)	18 (20.6)	168 (159)	28 (53.8)
Furrow irrigation, canal water	Next to the wetting front	342 (244)	1470 (321)	656 (710)	1014 (1653)
	In between	57 (49.6)	886 (780)	463 (842)	123 (50.8)
	Next to the onion bulb	31 (42.9)	579 (702)	113 (132)	8.50 (12.8)
Furrow irrigation, enhanced	Next to the wetting front	136 (109)	1100 (269)	41.5 (53.0)	215 (99.4)
	In between	25.4 (29.8)	326 (211)	20.0 (34.6)	229 (260)
	Next to the onion bulb	7.5 (11)	441 (703)	4.4 (9.9)	24.6 (24.0)

[a] Standard deviation.
[b] nd, not detected.

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 non-saturated capillary flow of water around the soil particles, leaving the bacteria behind on the soil particles nearer to the water sources.

ONION INTERNAL *E. COLI* CONTENT

In spite of sampling 60 bulbs from every replicate of all four treatments on 21 August, *E. coli* was not detected in any of the onion samples. The 10 September sampling of four 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 were unlike those of Solomon et al. (2002a) 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 10 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. Food and Drug Administration, 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. Food and Drug Administration, 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. Food and Drug Administration, 2015). The FDA has adopted generic *Escherichia coli* as the indicator for determining agricultural water quality, although many strains of the bacterium are not pathogenic (Edberg et al., 2000; U.S. Food and Drug Administration, 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* ≤126 colony forming units (CFU) / 100 mL of irrigation water with a statistical threshold value ≤ 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* ≤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. Food and Drug Administration, 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 four 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. Food and Drug Administration, 2015). Dry bulb onions in the Treasure Valley cure in the field for 2 to 4 weeks following the last irrigation before they are actually harvested. This length of time 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. Food and Drug Administration, 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. Food and Drug Administration, 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. Food and Drug Administration, 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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