MOVEMENT OF *ESCHERICHIA COLI* IN SOIL AS APPLIED IN IRRIGATION WATER

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Abstract

The US Food and Drug Administration (FDA) has proposed that If irrigation water exceeds 235 colony-forming units (CFU) of *E. coli* /100 ml in any one sample or 126 CFU/100 ml in the average of any five consecutive samples, growers would have to cease using that water in any way that directly contacts the surface of fresh produce (FDA 2013). The FDA has proposed that these *E. coli* levels are an indication of high risk of bacterial contamination of fresh onion (*Allium cepa* L.) bulbs regardless of the irrigation system. If onion irrigation exceeds 235 CFU, it is not known whether the contaminated water applied by furrow or drip irrigation actually reaches the onion bulb. Soil could filter *E. coli* and other bacteria before irrigation water reaches onion bulbs. “Vaquero” onions were grown on Owyhee silt loam. In our preliminary studies reported here, well water free of *E. coli* was applied to onions through drip irrigation or through furrow irrigation. A second water source was intentionally enriched with *E. coli* by being run across a pasture and recaptured prior to use. Furrow and drip irrigation were used to apply this water containing 218 to >2400 MPN/100ml for 11+ hours per irrigation. *E. coli* was monitored in the soil water at the end of irrigation cycles through direct sampling of the soil. Soil water was also sampled using sterile soil solution capsules (SSSC) to sample *E. coli* in the soil water that moved into place, to differentiate the movement of soil water from the soil water already in place. Soil water measurements were made adjacent to the water source, half way to the bulbs, and immediately adjacent to the onion bulbs. For furrow irrigation with ditch water the *E. coli* counts in the soil next to the onion bulbs was only 0% and 21% of the counts in the irrigation water following the first and second irrigations, respectively. During subsequent furrow irrigations, the *E. coli* counts in the soil water next to the onion bulbs exceeded the counts in the irrigation water. For drip irrigation with ditch water, the *E. coli* counts in the soil solution next to the onion bulbs remained very low. The soil water sampled by the SSSC adjacent to the onion bulbs drip-irrigated with ditch water also had very low *E. coli* counts.

Introduction

As a direct consequence of Public law 111-353-Jan. 4, 2011, on January 16, 2013, the Food and Drug Administration published Standards for the “Growing, Harvesting, Packing, and Holding of Produce for Human Consumption; Current Good Manufacturing Practice and Hazard Analysis and Risk-Based Preventive Controls for Human Food; Draft Qualitative Risk Assessment of Risk of Activity/Food Combinations for Activities (outside the Farm Definition) Conducted in a Facility Co- Located on a Farm; Availability; Proposed Rules” in the Federal Register (FDA, 2013) which we refer to here as the “proposed rules.”

The proposed rules place stringent testing requirements and use limitations on agricultural water that is applied to produce covered by the rules. In summary, covered produce is any fresh produce that is consumed raw more than 0.5% of the time. Fresh produce that will be processed prior to consumption is not covered. Covered produce includes fresh market onions, but does not include onions grown for processing. Agricultural water is defined as “Any applied water that comes into contact with the produce surface”, which FDA has clarified would include furrow and drip irrigation water when applied to onions.

From planting to harvest, agricultural water would need to be tested regularly: every week for surface irrigation water. Agricultural water would need to have fewer than 236 units (either colony-forming units, CFU, or most probable number, MPN) of *E. coli* per/100 ml in any one sample or 126 CFU/100 ml in the average of any five consecutive samples to be applied to the produce, and growers would not be allowed to use agricultural water that does not meet the standard for fresh produce.

In the Treasure Valley, irrigation systems mix relatively clean water with runoff water. This intermixing results in high counts of *E. coli* in irrigation water throughout large parts of the water distribution systems. The burdens of the proposed rules for onion growers in the Treasure Valley consist of the labor for sampling water weekly and record keeping, the cost of laboratory analysis, and any additional losses incurred as a result of not being allowed to use the water. 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.

In FDA 2013, proposed Sec. 112.3(c), “direct water application method” is defined to mean “using agricultural water in a manner whereby the water is intended to, or is likely to, contact covered produce or food-contact surfaces during use of the water.” As defined indirectly on page 3563, drip and furrow irrigation are “indirect water application methods”. But these possible indirect water application methods are not recognized for onion bulb production in the proposed rules.

This 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, maybe water with too high of a bacteria count would have a much lower count as it soaks through the soil and reaches the proximity of the onion bulb. We sought to determine whether or not water contaminated with *E. coli* applied by drip irrigation is filtered by the soil, greatly reducing the *E. coli* in the soil water that actually reaches onion bulbs. We also sought to determine whether or not contaminated water applied by furrow irrigation is filtered by the soil, greatly reducing the *E. coli* in the soil water that actually reaches onion bulbs.

Materials and Methods

The trial was conducted at the Oregon State University Malheur Experiment Station, Ontario, Oregon, in a field of Owyhee silt loam soil with no history of manure application over the last three decades. The location of the center of *E. coli* field trial was 43.98099°N - 117.02127°W. The test utilized 'Vaquero' onions planted for this purpose at 150 plants per acre (470 plants per hectare), where the onions were initially established exclusively with drip irrigation using well water and maintained with an irrigation criteria of 20 kPa soil water tension. Both the well water and the soil tested negative for the presence of *E. coli* prior to the imposition of the treatments. Details of the planting, irrigation, soil fertility, and fertilization are available elsewhere (Pinto et al., 2014).

E. coli movement in the soil was monitored during several full irrigations starting mid-summer using 1) drip irrigated with ditch water and 2) drip irrigated with well water (as a check) and 3) furrow with irrigated ditch water and 4) furrow irrigated with well water (as a check). The same well and ditch water was used for the drip and the furrow systems.

Irrigation water

The irrigation water was sampled for each system and water source every hour. The location of the ditch water source was 43.98091°N - 117.02271°W and the location of the well water source was 43.97804°N - 117.01512°W.

The furrow irrigated with ditch water with considerable *E. coli* contamination was setup with a siphon tube that siphoned the water into a basic plastic storage container. Affixed to the storage container was 2 inch lay-flat which carried the water to the head of the furrow. Attached to the lay-flat were two 5/8 inch ball valves for water flow control.

The furrow irrigated with well water was affixed with a one inch ball valve to control access to the well. A Nelson Rotator R33 sprinkler was attached to a Nelson 10 lb. pressure regulator with a #28 nozzle. The sprinkler head was captured in a plastic bag in order to create a metered emission similar to furrow irrigation.

The drip system with well water was already in place because that is how the field was being irrigated prior to this trial. In order to obtain the water samples needed, a Nelson 10 lb. pressure regulator was fitted to the drip tape system already in place. The drip taped utilized in the field that was used for this trial is Toro Aqua-Traxx EA5060822.

For the onions that were drip-irrigated with ditch water, an Ozawa injector pump outfitted with four heads was installed. Each head was capable of providing 12 gallons/hour of pressurized water from the ditch to supply water to the sand media filter. The sand media filter was filled with 30 grit crushed garnet rather than sand because the garnet requires less replacement and flushing and is capable of filtering to over 200 mesh. The installation of the sand media filter was atypical because media filters are commonly installed with a minimum of two filters, but due to very limited flow needed for the experiment, only one media filter was needed.

E. coli in the irrigation water

The well water was free of *E. coli.* Irrigation runoff water from a pasture was intentionally captured and added to the ditch water to assure high *E. coli* counts in the ditch water. The *E. coli* counts ranged from 218 to >2400 MPN/100 ml. Hereafter this water is referred to as having considerable *E. coli* contamination.

During each irrigation, water samples were taken every hour for 10 hours from three sources and numbered 1-30 accordingly. Well water sample was taken from a built-in secondary release valve before it branched into furrow or drip. Ditch drip water was sampled using the same release valve method. Ditch furrow water sample was taken from the spigot where the water was applied to the furrow. New sterile latex gloves were worn when handling the each water sample collection bottle to prevent contamination. Water samples were placed in sterile plastic bags and stored on ice in a cooler until they were later transferred to a refrigerator for overnight storage. Samples were taken to the laboratory first thing the next morning for analysis, 27 hours after the first sample was taken.

Sterile soil solution capsules

Sterile soil solution capsules (SSSC) were conceived of for the purpose of this trial. These soil water collectors are permeable fiber capsules filled with sterilized soil from the same field as where they would be installed. They are 2.2 cm in diameter, 7.3 cm in height not including the rubber stopper, and 8.6 cm in height when including the rubber stopper (Figure 1) made from the case of Watermark Soil Moisture Sensors (Irrometer Co. Inc., Riverside CA). The SSSC allows inflow of water along with *E. coli*.

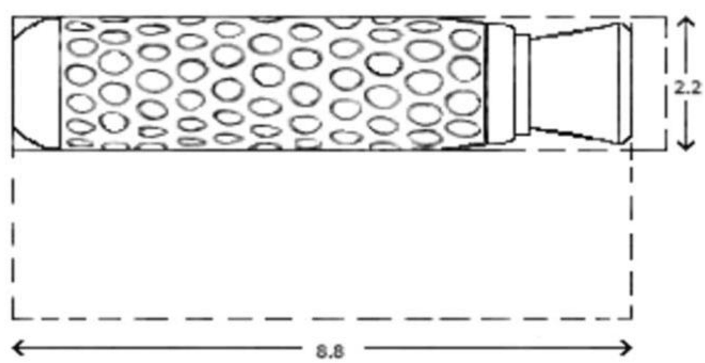

Figure 1. Sterile soil solution capsule (SSSC) dimensions. Distances are in cm.

To prepare the SSSC, three buckets and four aluminum baking pans were washed with bleach and rinsed with distilled water for sterilization. Once sterilized, latex gloves were worn at all times to prevent contamination of sterile items from contact with skin. If gloves came in contact with any non-sterile surface, those gloves were thrown in the garbage and new gloves were used. Note that these procedures were not performed in a cleanroom, so the environment itself was not sterile, but precautions were taken to keep contamination at a minimum. Three pounds of soil taken from the experiment site was sieved and then baked at 500 degrees Fahrenheit for one hour for sterilization. One of three buckets contained a bleach solution of 60 ml of bleach to four L of distilled water for a sterilization bath. The other two buckets contained distilled water to serve as successive rinses. The empty SSSC soaked in the bleach solution for not less than a minute and spent not less than one minute in each subsequent rinse bath. Once removed from the second rinse, empty SSSC were placed on a sterile aluminum pan and placed in the sun to dry for about 10 minutes in 38 degrees Centigrade. When finished drying, the soil water collector capsules were filled with 11 cubic centimeters of the sterilized soil.

The second time the experiment was run, the same methods were used for sterilization and filling of new soil water collector capsules; however, this time the capsules were run through a third distilled water rinse before they were set out to dry.

Field design

The tests with furrow irrigation were conducted on the station simultaneously in the same field using both clean well water and ditch water with considerable *E. coli* contamination. Water being applied was sampled hourly from both furrow irrigation water sources. Likewise, side by side tests with drip irrigation were conducted simultaneously using both clean well water and ditch water with considerable E. coli contamination. Measurements were replicated four times for each system and at three sampling distances between the edge of the water up to directly against the onion bulbs.

For the drip irrigated onions, three SSSC were placed in a staggered line, with one being placed right next the drip tape, one being placed next to the onion bulb, and one being placed directly in the middle of the drip tape and onion bulb (Figures 2 and 3). The same procedure was used for the furrow irrigated onions (Figures 4 and 5). Latex gloves were worn when the capsules were being placed out in the field, as well as when they were collected so as not to contaminate them. After the first irrigations, the number of SSSC was doubled, so that more soil was available for *E. coli* analyses (Figure 3).

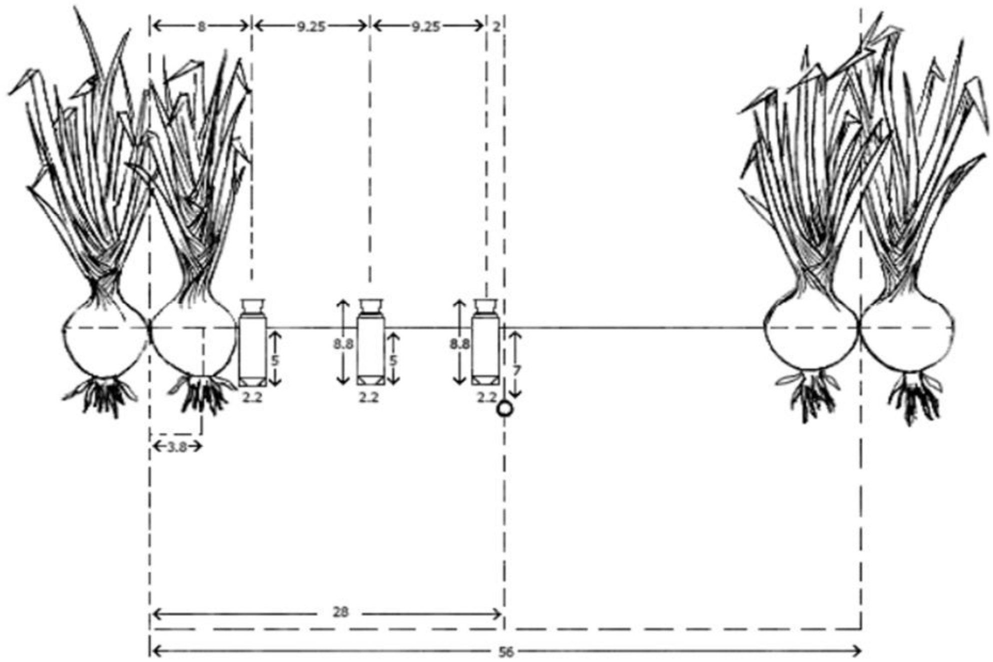

Figure 2. Placement of sterile soil solution capsules (SSSC) in the drip irrigated onions from the drip tape and bulbs. Distances are in cm.

Figure 3. Placement of sterile soil solution capsules (SSSC) from the drip tape to adjacent to the onion bulbs.

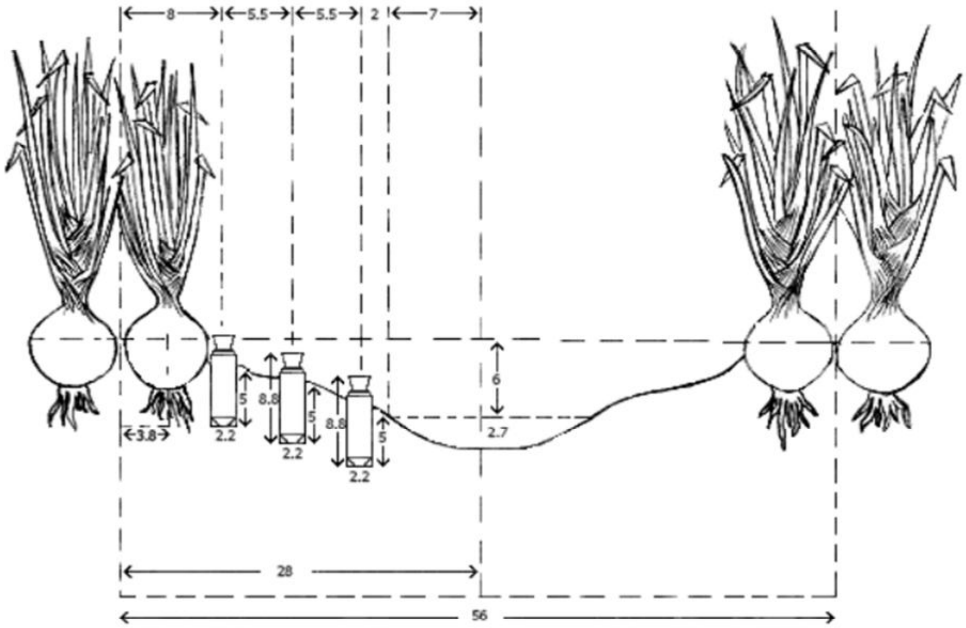

Figure 4. Placement of sterile soil solution capsules (SSSC) in the furrow irrigated onions. Distances are in cm.

Figure 5. Placement of sterile soil solution capsules (SSSC) from near edge of furrow irrigation water to adjacent to the onion bulbs.

E. coli in the soil water

Samples of *E. coli* in the soil water were taken two different ways, sampling the soil itself and capturing a sample of soil water inside the SSSC. At the end of each irrigation soil samples were taken 0 to 5 cm depth in a wedge 2.2 cm wide and 10 cm long parallel to the water sources and onion rows (Figures 6 and 7) analogous to the positions of the SSSC (Figures 2 and 4). To minimize cross contamination in collecting soils and SSSC, sample retrieval was grouped based on the irrigation system, water source, and sample position. Four samples were collected, followed by a change of latex gloves and sterilization of all equipment in 60 ml of bleach diluted in four liters of distilled water followed by rinsing in three successive baths of four liters of distilled water.

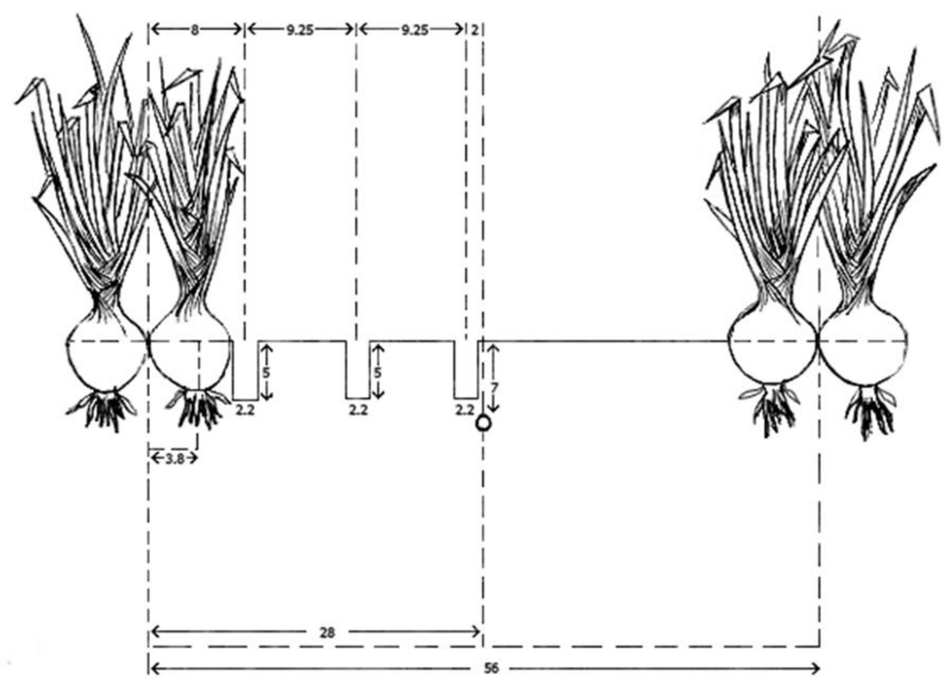

Figure 6. Location of soil samples in the drip-irrigated onions with respect to the drip tape and onion bulbs. Distances are in cm.

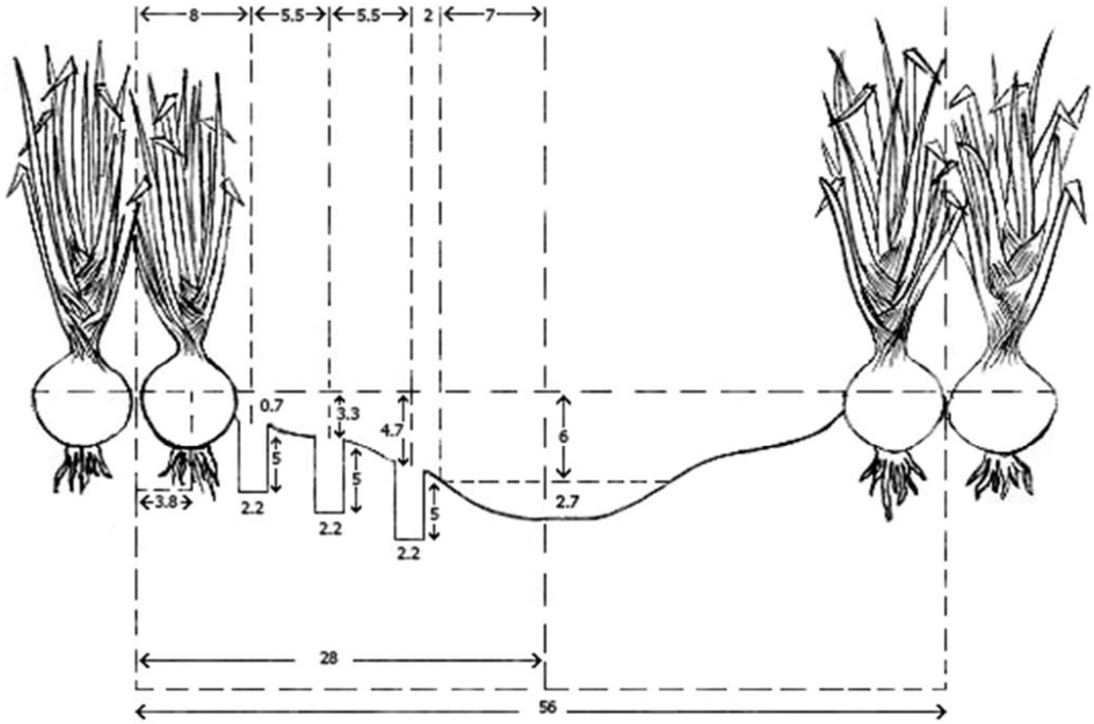

Figure 7. Location of soil samples in furrow-irrigated onions with respect to edge of the water and onion bulbs. Distances are in cm.

For each irrigation, the SSSC before the onset of irrigation and were collected at the end of the 10 to 11-hour irrigation. The capsules that contained sterilized soil took up water. At the end of the irrigation, each capsule was retrieved. Each SSSC was placed into a sterile two oz. Whirl-Pak labeled “Soil Solution” and its corresponding number. A sample of the soil located next to each capsule was also collected and placed in a sterile two oz. Whirl-Pak labeled “Soil” with its corresponding number. Samples were grouped according to their type, irrigation system and water source, placement in a field, and each group of Whirl-Pak bags was placed in a separate sterilized gallon-sized Ziploc bag to minimize cross contamination. All SSSC samples and soil samples were immediately stored on ice in a cooler, and then moved to a refrigerator for overnight storage. All samples were taken to the laboratory for analysis about 17 hours after the first sample was taken.

Water analyses for *E. coli*

Water samples were maintained under refrigeration until analyses. The Most Probable Number (MPN) of *E. coli* was determined using IDEXX *Colilert® +Quanti-Tray/2000®* (IDEXX Laboratories, Westbrook, ME).

Soil analyses for *E. coli*

Soil was sampled 0 to 5 cm deep from 20 random spots in the drip irrigated and furrow irrigated onion rows at harvest. Soil samples were refrigerated until analyzed. Part of each soil sample was weighed wet, dried, and weighed dry to determine the soil water content. Fifty g of each soil sample was diluted in 75 ml of water and shaken. Then 50 ml was removed and was used to estimate a Most Probable Number (MPN) of *E. coli* using IDEXX *Colilert® +Quanti-Tray/2000®* (IDEXX Laboratories, Westbrook, ME). Data were reported as Most Probable Number (MPN) of *E. coli* /100 ml of soil water. To determine the *E. coli* in the SSSC 10 g of soil from inside the capsule was diluted in 60 ml of water and shaken, and in other respects the analyses were the same as for the soil samples.

Environmental monitoring

Two Irrometer Watermark Monitor Data Loggers were set up with four Irrometer Model 200SS Watermark Soil Moisture Sensors, one air temperature sensor and one soil temperature sensor each. One unit was installed in a furrow system and the other unit in a drip irrigation system. The purpose of these Data Logger units was to record soil moisture content, soil temperature, and air temperature.

Results

Drip irrigation of onion with well water

Measurements made on the well water were all negative on 17 July and 31 July (Table 1). No *E. coli* was found in the soil adjacent to the wetting front in the irrigation furrows but a considerable amount was found in one spot between the edge of the wetting front and the onion bulbs (Table 2). This was apparently a random occurrence. The *E. coli* counts in the SSSC were low and none occurred next to the onion bulbs (Table 3).

Table 1. *E. coli* counts in the well water used to drip irrigate onions, Oregon State University, Malheur Experiment Station, Ontario, Oregon, 2013.

Sample number	17 July	31 July
	Hourly MPN of *E. coli*/100ml of irrigation water	
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0
Average	**0**	**0**

Table 2*. E. coli* counts in the soil where onions were drip irrigated with well water, Oregon State University, Malheur Experiment Station, Ontario, Oregon, 2013.

Position of the soil sample with respect to the water source and onion bulbs.	17 July	31 July
	Average *E. coli*/100ml soil water	
Next to drip tape	0 (0)*	0 (0)
In between	36 (72)	3,415 (6,596)
Next to onion bulb	0 (0)	69 (139)

*Standard deviation

Table 3. *E. coli* counts in soil solution using "sterile soil solution capsules" (SSSC) where onions were drip irrigated with well water, Oregon State University, Malheur Experiment Station, Ontario, Oregon, 2013.

Position of soil solution sample with respect to the water source and onion bulbs	17 July	31 July
	Average *E. coli*/100ml soil solution	
Next to drip tape	0 (0)*	3 (7)
In between	0 (0)	87 (173)
Next to onion bulb	0 (0)	0 (0)

*Standard deviation

Drip irrigation of onion ditch water

Trials were run on three dates with a drip irrigation system using *E. coli* contaminated water by capturing runoff water from a pasture. The water contained from 488 to >2400 MPN/100ml *E. coli* per 100 ml (Table 4). The *E. coli* in the soil adjacent to the drip tape was very high but the counts in the soil near the onion bulbs were low (Table 5). The *E. coli* counts captured by the SSSC next to the drip tape were very high but the counts midway to the bulbs were moderate and the counts adjacent to the bulbs were extremely low (Table 6).

Table 4. *E. coli* counts in the ditch water used to drip irrigate onions, Oregon State University, Malheur Experiment Station, Ontario, Oregon, 2013.

Sample number	17 July	21 August	27 August
	Hourly MPN of *E. coli*/100ml of irrigation water		
1	1203	920	1553
2	648	980	870
3	1119	1119	>2419
4	866	1203	>2419
5	1011	1732	913
6	1299	1203	>2419
7	1046	1413	>2419
8	613	1119	>2419
9	770	1413	>2419
10	488	1203	-
Average	**906**	**1231**	**>1984**

Table 5*. E. coli* counts in the soil where onions were drip irrigated with ditch water, Oregon State University, Malheur Experiment Station, Ontario, Oregon, 2013.

Position of the soil sample with respect to the water source and onion bulbs.	17 July	21 August	27 August
	Average *E. coli*/100ml soil water		
Next to drip tape	320 (373)*	6,189 (10,783)	3,281 (3,667)
In between	207 (271)	125 (252)	291 (346)
Next to onion bulb	0 (0)	0 (0)	57 (49)

*Standard deviation

Table 6. *E. coli* counts in soil solution using “sterile soil solution capsules” (SSSC) where onions were drip irrigated with ditch water, Oregon State University, Malheur Experiment Station, Ontario, Oregon, 2013.

Position of soil solution sample with respect to the water source and onion bulbs	17 July	21 August	27 August
	Average *E. coli*/100ml soil solution		
Next to drip tape	711 (1,423)*	12,426 (22,350)	12,769 (25,302)
In between	0 (0)	66 (68)	7,991 (10,491)
Next to onion bulb	0 (0)	11 (12)	0 (0)

*Standard deviation

Furrow irrigation of onion with well water

As mentioned above, the measurements made on the well water were all negative on 17 July and 31 July (Table 7). Very few *E. coli* were found in the soil adjacent to the wetting front in the irrigation furrows and none were found adjacent to the onion bulbs (Table 8). The *E. coli* counts in the SSSC were low and none occurred next to the onion bulbs (Table 9).

Table 7. *E. coli* counts in the well water used to furrow irrigate onions, Oregon State University, Malheur Experiment Station, Ontario, Oregon, 2013.

Sample number	17 July	31 July
	Hourly MPN of *E. coli*/100ml of irrigation water	
1	0	0
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0
Average	**0**	**0**

Table 8. *E. coli* counts in the soil where onions were furrow irrigated with well water, Oregon State University, Malheur Experiment Station, Ontario, Oregon, 2013.

Position of the soil sample with respect to the water source and onion bulbs.	17 July	31 July
	Average *E. coli*/100ml soil water	
Next to furrow	0 (0)*	90 (95)
In between	0 (0)	7 (7)
Next to onion bulb	0 (0)	0 (0)

*Standard deviation

Table 9. *E. coli* counts in soil solution using “sterile soil solution capsules” (SSSC) where onions were furrow irrigated with well water, Oregon State University, Malheur Experiment Station, Ontario, Oregon, 2013.

Position of soil solution sample with respect to the water source and onion bulbs	17 July	31 July
	Average *E. coli*/100ml soil solution	
Next to furrow	0 (0)*	37 (31)
In between	0 (0)	139 (278)
Next to onion bulb	0 (0)	0 (0)

*Standard deviation

Furrow irrigation of onion with ditch water

Trials were run on four dates with a drip irrigation system using *E. coli* contaminated water by capturing runoff water from a pasture. The water contained from 218 to >2400 MPN/100ml *E. coli* per 100 ml (Table 10). The *E. coli* in the soil adjacent to the wetting front had high counts following the second furrow irrigation and a high level of contamination penetrated next to the onion bulbs by the third irrigation (Table 11). The *E. coli* counts captured by the SSSC next to the wetting front were high after the first irrigation and penetrated to the soil adjacent to the bulbs by the second furrow irrigation (Table 12).

Table 10. *E. coli* counts in the ditch water used to furrow irrigate onions, Oregon State University, Malheur Experiment Station, Ontario, Oregon, 2013.

Sample number	17 July	31 July	21 August	27 August
	Hourly MPN of *E. coli*/100ml of irrigation water			
1	665	na	1203	>2419
2	658	1733	866	1732
3	1203	2419	980	>2419
4	524	1299	1732	>2419
5	727	1203	980	>2419
6	1299	1986	648	>2419
7	866	344	1299	>2419
8	579	218	980	>2419
9	517	344	816	>2419
10	488	1553	920	
Average	**753**	**1110**	**1043**	**>2343**

Table 11. *E. coli* counts in the soil where onions were furrow irrigated with ditch water, Oregon State University, Malheur Experiment Station, Ontario, Oregon, 2013.

Position of the soil sample with respect to the water source and onion bulbs.	17 July	31 July	21 August	27 August
	Average *E. coli*/100ml soil water			
Next to furrow	66 (132)*	1,279 (485)	17,139 (5,078)	4,356 (2,126)
In between	128 (178)	365 (251)	16,917 (3,694)	2,160 (903)
Next to onion bulb	0 (0)	234 (211)	16,900 (12,023)	4,033 (4,165)

*Standard deviation

Table 12. *E. coli* counts in soil solution using "sterile soil solution capsules" (SSSC) where onions were furrow irrigated with ditch water, Oregon State University, Malheur Experiment Station, Ontario, Oregon, 2013.

Position of soil solution sample with respect to the water source and onion bulbs	17 July	31 July	21 August	27 August
	Average *E. coli*/100ml soil solution			
Next to furrow	422 (465)*	3,837 (924)	6,664 (7,254)	17,853 (17,046)
In between	0 (0)	4,529 (2,809)	1,768 (1,044)	10,077 (5,451)
Next to onion bulb	0 (0)	1,225 (1,349)	560 (404)	22,495 (15,917)

*Standard deviation

Discussion

The observations are preliminary in that relatively few observations were conducted. The number of monitored irrigations was limited due to the cost of the *E. coli* analyses. A fully replicate field trial with repeated observations at many locations on each irrigation furrow is warranted, but beyond the current financial capabilities.

In the FDA proposed rules "direct water application method" was defined as "using agricultural water in a manner whereby the water is intended to, or is likely to, contact covered produce or food-contact surfaces as opposed to "indirect water application methods". Although both furrow or drip irrigation might be considered an "indirect water application method" the furrow irrigation observed here resulted in high *E. coli* MPN in the soil water adjacent to the onion bulbs while the drip irrigation of onion did not.

The *E. coli* found in Table 2 suggests that there is at least one important source of *E. coli* other than the soil and irrigation water in this production system. The soil and water both tested negative in the beginning of this trial.

The high variability of *E. coli* in the soil water and in the SSSC suggests that water movement and *E. coli* transport in the water varies greatly by the spot sampled. A much number of sampled locations would need to pooled before analysis to reduce the variance in the data.

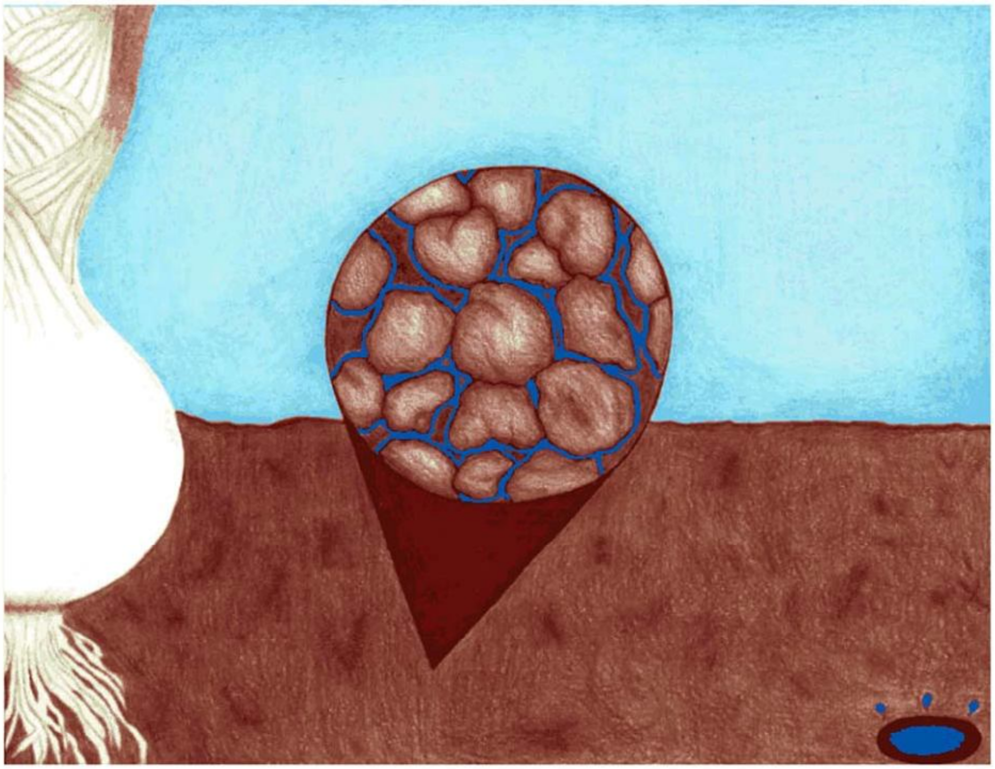

Figure 8. Diagram of water movement toward the onion bulb from a buried drip tape.

It is possible that the low movement of E. coli to soil positions adjacent to the onion bulbs was due to water movement by non-saturated capillary flow of water around the soil particles (Figure 8). It is not known whether onion irrigation with contaminated water actually increases the E. coli count on lifted onions or increases the internalization of E. coli in onion bulbs. Onion bulbs drip-irrigated with well water and furrow-irrigated with ditch water were intensively examined internally and externally for E. coli at the end of the trial and subsequently during curing and packout (Shock et al. 2013).

References

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