SURVIVAL OF *ESCHERICHIA COLI* ON ONION DURING FIELD CURING AND PACKOUT

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Abstract

The Food and Drug administration has expressed concern that Onions (*Allium cepa*) irrigated with water contaminated with high rates of *Escherichia coli* could harbor *E. coli* on their surface or interior. On the other hand, since onions contain antimicrobial compounds and field conditions may not be conducive to *E. coli* survival, the *E. coli* population on the surface of onions might become negligible through the course of field curing. Further, the relationship between the *E. coli* in the irrigation water to the *E. coli* on onion bulbs after field curing, harvest, and packout has not been studied. To determine if *E. coli* should be of concern in onion production, we sought to measure the die-off of *E. coli* on onions between the last irrigation and harvest and the presence of *E. coli* on onions after packout. Well water was tested and had no *E. coli*; ditch water intentionally run across a pasture prior to use had 218 to > 2400 MPN of *E. coli*/100ml. Onions were sampled from those furrow irrigated (ditch water) and those drip irrigated (well water) starting at lifting 3 September 2013 for four consecutive weeks. At 0 and 28 days after lifting, both interior and exterior of the onions were tested for *E. coli*. At 7, 14, and 21 days after lifting, only the exterior of the onions was tested. None of the onions contained *E. coli* internally at 0 or 28 days after lifting. At lifting *E. coli* was present on the exterior of both the drip and furrow irrigated onions and seemed to be largely unrelated to the irrigation water. The exterior *E. coli* contamination decreased rapidly after lifting. After harvest and packout on 14 October 2013, no *E. coli* was detected on the onion bulb exteriors from either irrigation treatment. *E. coli* introduced into the onion field through furrow irrigation was not present on or in the packed out onion bulbs.

Introduction

Bacteria are ubiquitous in nature. Fresh produce can become contaminated in the field with bacteria that are potentially harmful to humans from many sources, including from irrigation water (Beuchat, 1996). Bacteria from water could possibly contaminate the exterior or interior of an onion. We sought to examine the survival and die-off of *E. coli* during field curing on

onions following furrow irrigation with water containing substantial amounts of *E. coli* (Shock et al., 2013).

There are many factors that affect the contamination and survival of *E. coli* in soil, water, and on fresh and minimally processed produce. Given that conditions are favorable, *E. coli* can survive in open environments (van Elsas 2011). However, most soils and aquatic environments have highly fluctuating conditions, causing reduced bacterial survival and growth. For example, the availability of water plays a key role in the survival of *E. coli*. Extreme water fluctuations have harsh effects on *E. coli* physiology and survival in the environment. Intense dry conditions can result in considerable cell death, whereas substantial flooding shifts cellular metabolism to anaerobic processes. Different soil properties, such as porosity, surface area, bulk density and macropore structure are also important factors for bacteria to percolate through the soil, with regard to adsorption and gravitational movement with water (Mankin et al. 2007).

In all habitats studied, namely soil and manure habitats, *E.* coli population sizes show progressive declines. However, under complex natural conditions, *E. coli* fate is not accurately predictable (van Elsas 2011).

The leaf environment of some plants has been shown to develop a biofilm that harbors human pathogens (Heaton and Jones 2008). *E. coli* applied to onion leaf tissue in the fall in Georgia at 1,000,000 CFU/100ml could be recovered up to 74 days after application (Islam et al. 2004), but the population declined logarithmically over time.

Like 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 may die off before harvest. 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. Bulbs grown with contaminated water may be just as clean as those grown with zero *E. coli* irrigation water by the time that they are lifted and cured. It is unknown to what extent *E. coli* are present on onion bulbs at lifting or to what extent they die off during field curing. We examined the extent that *E. coli* occurring on onion bulbs at lifting would spontaneously die off during field curing, and their presence on the packed out product.

Materials and Methods

"Vaquero" onions were grown at the Oregon State University Malheur Experiment Station, Ontario, Oregon, in Owyhee silt loam in a field that had no history of manure application over the last three decades (Shock et al. 2013). The soil texture consisted of 34% sand, 66% silt, and 0% clay. Most rows of onion were grown under drip irrigation using well water free of *E. coli.* Other rows of onions were furrow irrigated with water containing *E. coli.* Onions were grown identically in every other detail except for the water sources and irrigation systems. The seed was from the same lot, the seed was planted on the same day, and all operations were identical. Irrigation ceased on 27 August 2013.

Onions to be used for testing were lifted 3 September 2013 from the field by hand using sterile latex gloves and left on the soil surface to cure. Sterile gloves were changed at lifting between irrigation treatments to avoid cross-contamination. Five plots were marked with 90 successive bulbs in each row for the onions grown with well water using drip irrigation and five plots were

marked for the onions grown with furrow irrigated ditch water. The plots were numbered 1-10. Plots 1-5 were drip irrigated with well water with no *E. coli* and plots 6-10 were furrow irrigated with ditch water containing 218 to >2400 MPN *E. coli* per 100 ml of water (Shock et al. 2013). Fifteen onions from each plot were tested each week (Figure 1). In the first week, every sixth onion was picked up using sterile latex gloves, topped using sterilized scissors or knife, and placed into a double-bagged 13-gallon trash bag labeled accordingly. Care was taken to avoid cross contamination of onion samples. No attempt was made to remove the roots or remove the soil attached to the roots or adhering to the outer skins, as these are normally incidentally removed by the automated handling of onion bulbs during mechanical harvest and packout. The samples were taken to the lab immediately afterwards for peeling and analysis. In the second week, every fifth onion was picked up, etc. until all but 15 onions from a plot were picked up.

Figure 1. Onions were lifted on 3 September 2013. Onions were lifted using sterile gloves. They were also sampled using sterile gloves and knives, Oregon State University Malheur Experiment Station, Ontario, Oregon.

The final 15 onion bulbs from each plot were harvested 14 October 2013 into numbered sterilized wire baskets using sterile latex gloves and topped using sterilized knives. The bulbs were packed out of the wire baskets on 14 October 2013. New sterile gloves and a freshly sterilized packing table were used for every packout sample. Bulbs were placed into a double-bagged 13-gallon trash bags labeled accordingly. The harvest and packout removed the loose skin and most of the attached roots and soil.

Bulb exteriors tested for *E. coli*

In the laboratory, the roots, soil, skins, and outer peel of the 15 onions were removed from the bulbs and weighed. They were then thoroughly washed in one liter of water. A 10ml sample of the wash water was used to estimate a Most Probable Number (MPN) using IDEXX *Colilert*® *+Quanti-Tray/2000*® (IDEXX Laboratories, Westbrook, ME) of *E. coli* from the outside of the onions. The *E. coli* MPN per onion bulb exterior was calculated. For the sixth onion sample after packout, roots, soil, skins, and outer peel of the 15 onions were removed from the bulbs and weighed, but since the remaining skins and roots held less soil, the added clarity allowed a 100 ml sample of the wash water to be used to estimate the MPN.

Bulb interiors tested for *E. coli*

The outer skins and scales were peeled from all the onions in 15 bulb sample and the bulbs were placed on an aluminum tray. The outside of the peeled onions were disinfected with 70 % ethanol and placed on a sterilized aluminum tray. The alcohol was allowed to dissipate. A wedge was cut out of each onion and the wedges were placed in a sterilized zip lock food grade bag and mixed. A sterilized stainless steel beaker was filled with mixed onion wedges and the remainder of the onion wedge sample was placed in a refrigerator. The cut onions wedges in stainless steel beaker were macerated with a food processer (Waring commercial immersion blender; model WSB) in the stainless steel beaker. After maceration, 10 ml of the resulting onion suspension was placed in 90 ml of Universal Pre-enrichment broth (UPB, Accumedia, Nedgen Michigan) and sealed. The UPB broth was placed in an incubator for 48 hours at 35^{o} C.

Along with every batch of samples, an additional positive inoculated sample was placed in an additional flask containing UPB broth. A glass jar with 100 ml sterilized water had a package of Colisure (Idexx) added for the presence of *E. coli*. Five ml of the UPB was transferred to Colisure mixture and incubated for 24 hours at 35^{o} C. After 24 hours the Colisure was tested with UV light for the presence of *E. coli*.

Soil tested 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 in an oven at 50^{o} C, 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* in the soil water using IDEXX *Colilert*® *+Quanti-Tray/2000*® (IDEXX Laboratories, Westbrook, ME). Results were reported as MPN of *E. coli* per 100ml of soil water based on the water in the soil at the time of sampling.

Results

Due to intentional excessive contamination with *E. coli,* the top 5 cm of soil next to the furrow-irrigated bulbs had 4033 MPN of *E. coli* per 100ml of soil water one week prior to lifting (Shock

et al 2013). The top 5 cm of soil with drip irrigation with well water had 0 MPN of *E. coli* per 100ml of soil water next to the bulb one week prior to lifting. The results show that this *E. coli* was excluded from entering the onion. At lifting, no *E. coli* were internalized in any of the onions (Table 1). At the end of field curing the *E. coli* in the soil with intentional excessive contamination with *E. coli* had decreased to 611 *E. coli* per 100ml of soil water at the end of field curing, still without any internal *E. coli* in the bulbs (Table 1).

The furrow irrigated onions had received water up to in excess of 2400 MPN of *E. coli* per 100ml of water and the drip irrigated onions had been irrigated with well water containing 0 MPN *E. coli*/100ml of water (Shock et al. 2013). In spite of the marked differences in irrigation water, the furrow irrigated onions had no more exterior *E. coli* contamination at lifting than the drip irrigated onions (Table 1). The exterior *E. coli* contamination decreased rapidly after lifting. None of the onions contained *E. coli* internally at 0 or 28 days after lifting.

After packout, there was no difference in *E. coli* contamination on the onion exteriors between onions grown using drip irrigation and clean well water and onions grown with *E. coli* contaminated ditch water using furrow irrigation (Table 2).

Table 1. Tissue test of onions that were drip irrigated with well water and furrow irrigated with ditch water, Oregon State University, Malheur Experiment Station, Ontario, Oregon, 2013. The onions tested here retained the loose skins, dirt, and roots that are ordinarily removed during mechanical harvesting and packout (Figure 2).

Days from lifting	Treatment		Average *E. coli* next to the onion bulb (Aug. 27) MPN/100ml of soil water	Average weight of skins, peel, roots, and soil (g/bulb)	Average external *E. coli* per onion	*E. coli* inside the bulb
	Irrigation System	Water Source				
0	Drip	Well	0 (0)*	15	1,615 (3,570)*	0
	Furrow	Ditch	4,033 (4,165)	18	956 (1,397)	0
7	Drip	Well		23†	972 (2,166)	N/A
	Furrow	Ditch		26†	343 (353)	N/A
14	Drip	Well		12	18 (28)	N/A
	Furrow	Ditch		13	15 (17)	N/A
21	Drip	Well		14	0 (0)	N/A
	Furrow	Ditch		16	5.3 (5.6)	N/A
28	Drip	Well		14	0 (0)	0
	Furrow	Ditch		15	6.7 (6.7)	0

*standard deviation

†Weight affected by rainfall

Table 2. *E. coli* on onions following harvest and packout, Oregon State University, Malheur Experiment Station, Ontario, Oregon, 2013. In the packout the loose skins, dirt, and roots were removed from the bulbs (Figure 3).

Days from lifting	Treatment		Average *E. coli* next to the onion bulb on October 1 (MPN/100ml of soil water)	Average weight of skins, peel, roots, and soil on October 15 (g/bulb)	Average MPN external *E. coli* per onion on October 15
	Irrigation System	Water Source			
41	Drip	Well	0	6.2	0 (0)*
	Furrow	Ditch	611	7.6	0 (0)

*standard deviation

Discussion

E. coli decline in the soil

E. coli in the soil next to the furrow-irrigated onion bulbs declined from 4033 to 611 MPN/100 ml of soil water from August 27 to October 1. The soil environment included many factors reviewed by van Elsas et al. (2011) to be unfavorable for *E. coli* survival (oscillating temperature, high pH, low soil clay content, and aerobic conditions).

E. coli on the bulb exteriors

In a study by Islam et al (2005) it was reported that the persistence of E. coli O157:H7 in soil is dependent on the type of vegetable grown in the soil, with inactivation more rapid in soil in which onions are grown than in soil in which carrots are grown. Islam et al was also determined that *E. coli* O157:H7 cell numbers progressively declined on both carrots and onions with time, but did so more rapidly on onions. Generally, *E. coli* O157:H7 survived better on carrots than on onions. It should be noted that the levels of *E. coli* O157:H7 used in that study were far greater than what would likely be found on an agricultural field.

In the present study, the amount of *E. coli* present on the outside skins, roots, and soil of the onion bulb at lifting was not related to the history of onion irrigation (Table 1). Onions grown with furrow irrigation with a heavy load of *E. coli* in the irrigation water had no more external contamination at lifting than onions that were drip-irrigated with well water containing no *E. coli*. The source of the *E. coli* on the bulb exteriors at lifting was not determined and warrants additional study. The *E. coli* present on the bulb exteriors rapidly died off over the period of three weeks. The small amount of *E. coli* present on the outside of the onion bulbs after 3-4 weeks of curing in the furrow irrigated treatment may have been related to the soil on the onion samples (Figure 2) since there still were residual *E. coli* present in the surface soil following furrow irrigation.

Figure 2. Onions sampled 0, 7, 14, 21, and 28 days after lifting retained loose skins, soil, and a few roots, Oregon State University Malheur Experiment Station, Ontario, Oregon.

Figure 3. Onions lost most of the loose skins, soil, and roots during manual harvest and packout, October 2013, Oregon State University Malheur Experiment Station, Ontario, Oregon.

Figure 4. Onions typically lose most of the loose skins, dried roots, and attached soil during mechanical harvesting and packing operations (Photo courtesy of Duane Kido, TopAir Inc., Parma, Idaho).

The onions analyzed 0 through 28 days after lifting retained roots, loose skins, and attached dirt (Figure 2) and are not typical of onion bulbs that are mechanically harvested after curing because mechanical operations cause much of the loose skins, soil and roots to be dislodged from the bulbs. Onions packed out from this trial were relatively free from loose skins and soil (Figure 3) and no longer held *E. coli* on their exteriors (Table 2). The relatively clean appearance of these onions is typical of onions packed out by commercial operations. Field curing followed by mechanical harvesting and packing typically dislodges most of the loose skins and soil that adheres to the onion bulbs (Figure 4).

E. coli internalized in the onion bulbs

Although *E. coli* was present on the bulb exteriors at lifting, it was not internalized in any of the onion bulbs tested at lifting or at the end of the curing process. The onion skins and the outer layer may provide morphological barriers to *E. coli* infection. Tauxe et al. (1997) enumerate four broad factors that affect "the survival, growth, and inactivation of microorganisms on fresh produce". One of these factors is "the physiologic state of the plant tissue and its resistance to microbial metabolic processes." Tauxe et al. point out, "Normally, the exterior of produce acts as a physical barrier, preventing bacteria from penetrating into the interior." Dry bulb onion has such a barrier in its skin and outer layer, both of which are typically discarded prior to consumption.

Antimicrobial compounds in onion bulbs may be important in the resistance of bulbs to infection by *E. coli*. Onion chemistry inhibits human pathogenic organisms (Johnson and Vaughn, 1969; Elnima et al. 1983; Block 1985; Zohri et al. 1995; Sofos et al. 1998; Yin and Tsao 1999; Kyung and Lee 2001; Srinivasan et al. 2001; Griffiths et al. 2002; Indu et al. 2006; Islam et al. 2004; Islam et al. 2005; Block 2010; Ye et al. 2013). Onions share a common non-protein amino acid

class, *S*-Alk(en)yl-L-cysteine sulfoxides, with other *Allium* species (including garlic, elephant garlic, wild garlic, leeks, scallions, shallots, Chinese chives, and chives) as well as with the unrelated *Brassica* (cruciferous vegetables including broccoli, cauliflower, cabbage, Chinese cabbage, kale, turnip, swede, and kohlrabi) (Kyung and Lee 2001). This class of amino acids and its associated thiosulfate breakdown products have strong antimicrobial activities against a broad spectrum of human pathogenic bacteria (Srinivasan et al. 2001) as well as specific strains of *Aspergillus* fungi (Yin and Tsao 1999; Srinivasan et al. 2001). In Petri dish culture, crude extract of onion, is known to inhibit growth of the following human pathogens within 1.3 to 2.3 cm: the Gram-negative bacteria *Chromobacterium violaceum, E. coli, Enterobacter faecalis, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella paratyphi,* and *Salmonella typhi*; the Gram-positive bacterium *Bacillus subtilis*; and the fungi *Aspergillus flavus, Aspergillus fumigatus,* and *Candida albicans* (Srinivasan et al. 2001). Based on this broad-spectrum antimicrobial activity, it is likely that colonies of many bacteria do not normally survive beyond the skin of the onion.

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