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

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Abstract

The U.S. Food and Drug Administration (FDA) remains concerned that onions (*Allium cepa*) irrigated with water containing high rates of the bacterium *Escherichia coli* could retain *E. coli* on their surface or interior at time of harvest. To determine if *E. coli* should be of concern in onion production, we measured the die-off of *E. coli* on onions between the last irrigation and harvest and the presence of *E. coli* on onions after packout. Two experiments were performed to test *E. coli* die-off. The first experiment isolated the onions on a lined wooden table, and the second experiment kept the onions in a field setting. Onions were coated with a manure solution, then set to cure for several weeks. Results of both trials showed rapid decline of *E. coli* after the first 2 weeks, followed by a steady decline for most of the trial. *E. coli* never completely died off, but was substantially reduced from initial levels. Die-off rates were lower than the proposed FDA allowance, but they would be sufficient for assuring enough die-off of bacteria from the time of last irrigation until harvest under normal field conditions.

Introduction

Bacteria are ubiquitous in nature. Fresh produce can become contaminated in the field with potentially human pathogenic bacteria 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 determine the survival and die-off of *E. coli* during field curing on onions following exposure to substantial amounts of *E. coli* via a manure solution.

There are many factors that affect the contamination and survival of *E. coli* in soil, water, and on fresh and minimally processed produce. Given favorable conditions, *E. coli* can survive in open environments (van Elsas et al. 2011). However, most soils and aquatic environments have highly fluctuating conditions that reduce 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 affecting bacterial adsorption and gravitational movement with water (Mankin et al. 2007).

Onion bulbs may acquire *E. coli* contamination in the field from various sources. *E. coli* from irrigation water that contacts onion bulbs during the growing season may not be internalized and

may die off before harvest. The onset of external contamination due to high amounts of *E. coli* in irrigation water has been researched in an accompanying study in this report (Shock et. al 2015). Bulbs grown with contaminated water may be just as clean as those grown with *E. coli*-free irrigation water by the time that they are lifted and cured. Other *E. coli* may possibly land on the bulbs by chance while they are growing and curing. 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* survives on onions after lifting, and analyzed the resulting die-off rates.

Materials and Methods

Two independent experiments were performed. The first experiment focused on researching the survival potential of *E. coli* exclusively on individual onion bulb exteriors. The second experiment looked at die-off rates in a field setting, as close to normal operations as possible and using bulked samples similar to those used as part of GAP testing.

Experiment I

Onions were lifted 12 September 2014 from the field by hand, using sterile latex gloves, and left on the soil surface to cure. Sterile gloves were changed between irrigation treatments to avoid cross-contamination. Sampling began on 24 September 2014. We collected approximately 120 onions of medium size from Replicate 1 of the *E. coli* trial onion field where the onions were drip-irrigated with well water. The onions were topped, placed into a wire basket, and then transferred to a wooden tote for transport to the station. Sterilized knives, baskets, and gloves were used during this process. At the station, cow manure and well water were mixed together to create a "homemade" *E. coli* solution to coat the onions with so that they had excessive contamination levels initially. In batches of approximately 10, the collected onions were dipped bottom-first into the solution. The solution coated at least halfway up the onion bulbs, but no onions were fully submerged. This treatment is similar to how onions would be exposed to irrigation water in the field. Onions were bathed for 1 min. The coated onions were placed upright (or as close as possible) on lined, wooden picnic tables (Fig. 1). Each week, a random selection of 10 onions was placed in individual gallon-sized ziploc bags labeled 1-10, and taken to Western Laboratories, Inc. (Parma, ID) for analysis.

Experiment II

A small plot of approximately 3,000 onions at a neighboring commercial field was used to begin this experiment. These onions had been furrow irrigated with standard ditch water. The onions were lifted approximately 2 weeks before the trial began. Since the onions had previously been lifted, workers went to the field on 6 October and pushed the onions back into the soil, so that the root system was below the surface. Similar to the first experiment, a solution created by mixing cow manure with water was used as the *E. coli* "treatment." Flags were placed starting at the east end of the field, skipping the first 25-30 ft of onions, to denote which onions would be treated and untreated. Workers used new paintbrushes to apply the *E. coli* solution to the above-ground exterior of the onions bulbs, making sure to evenly coat the exposed surface and avoid getting the solution into the necks of the onions. On 7 October, 360 onions were collected for analysis. We randomly sampled 5 sets of 60 onions from the treated plot, and 1 set of 60 onions from the untreated plot, using new gloves and sterilized equipment after each set. The onions were topped

and placed into black garden trash bags, and labeled set 0 to set 5 (with set 0 being the untreated onions). They were then transported to Western Laboratories, Inc. for analysis.

The remaining onions had to be moved from the neighbor's field and back to the Malheur Experiment Station. On 7 October, workers used sterilized equipment and pulled the remaining onions, placed them into wire baskets, and dumped them into new gunny sacks that were loaded into a pickup truck and transported to the station. An empty field at the southwest area of the station was used to conduct the remainder of experiment. There, the onions were placed on the ground in an upright position in designated, flagged areas according to the *E. coli* treatment (Fig. 1). Every following Monday, 5 sets of 60 onions from the treated plot and 1 set of onions from the untreated plot were randomly sampled for analysis. They followed the same sampling procedure as the first set.

Figure 1.Onions as they were laid out on a table for Experiment 1 (left) and in the field at the station for Experiment 2 (right). For Experiment 2, onions were placed according to their treatment: pink flags marked the treated onions, blue flags indicated the untreated onions, and the white flags indicated "backup" untreated onions. Malheur Experiment Station, Oregon State University, Ontario, OR, 2014.

Bulb exteriors tested for *E. coli*

In the laboratory, the roots, soil, skins, and outer peel of the 60 onions were removed from the bulbs and weighed. They were then thoroughly washed in 1 liter of water. A 10-ml 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.

Bulb interiors tested for *E. coli*

The outer skins and scales were peeled from all the onions in the 60-bulb samples, and the bulbs of each sample were placed on a separate aluminum tray. The outsides of the peeled onions were disinfected with 70% ethanol and placed on sterilized aluminum trays. The alcohol was allowed

to dissipate. A wedge was cut out of each onion and the wedges were placed in a sterilized ziplock 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 onion wedges in the beaker were macerated with a food processer (Waring commercial immersion blender; model WSB). After maceration, 10 ml of the resulting onion suspension was placed in 90 ml of universal pre-enrichment broth (UPB, Accumedia, Neogen, Lansing, MI) and sealed. The UPB 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. 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 the Colisure mixture and incubated for 24 hours at 35^{o}C. After 24 hours the Colisure mixture was examined under UV light for the presence of *E. coli*.

Results

Experiment I

The manure solution used to coat the onions had an initial *E. coli* count of 2.95 x 10^{7} MPN/100 ml, which is several orders of magnitude greater than observed in irrigation water. Even with these extraordinarily high initial *E. coli* levels, there was a significant progressive decrease in the average contamination of *E. coli* on the individual onion bulbs. Unfortunately, data from the initial set of onions was not resolved enough to be useful. However, starting from the second set on 29 September, by 22 October, over a 3.5-log average reduction occurred. Onions testing negative for *E. coli* contamination began occurring on 15 October. Average contamination levels began increasing after 22 October, for unknown reasons (Table 1, Fig. 2).

Table 1. The log values of the weekly results of the first *E. coli* die-off trial, that examined *E. coli* levels on individual onion bulbs. Onions were randomly selected each week. An error occurred for onion number 7 on 5 November, and that data was discarded. Malheur Experiment Station, Oregon State University, Ontario, OR. Data are expressed as log10 values. For example, a value of 4.38 = $1x10^{4.38}$= 23,998, 4.00 = 10,000, 3.00 = 1,000.

	Log values of *E. coli* /onion bulb							
Onion #	24-Sep	29-Sep	1-Oct	8-Oct	15-Oct	22-Oct	29-Oct	5-Nov
1	>5.38	4.38	5.89	1.83	1.57	0.00	2.72	0.00
2	>5.38	5.54	3.21	5.49	4.93	0.00	3.46	3.44
3	>5.38	4.96	4.74	3.81	4.32	0.00	3.85	3.75
4	>5.38	4.19	4.41	1.86	0.00	1.00	2.00	3.00
5	>5.38	6.02	4.11	0.70	1.18	3.85	2.06	3.94
6	>5.38	6.30	3.71	3.81	1.97	0.70	0.00	3.78
7	>5.38	2.17	3.86	3.94	1.57	2.17	2.03	-
8	>5.38	3.59	2.97	4.08	4.77	1.62	2.63	1.41
9	>5.38	5.06	5.71	2.64	5.24	0.70	1.31	2.25
10	>5.38	6.78	4.27	4.08	0.00	1.31	1.31	4.53
Average log MPN/onion	N/A	4.90	4.29	3.22	2.56	1.14	2.14	2.90
Standard Deviation	N/A	1.38	0.96	1.43	2.06	1.20	1.12	1.64
Standard Error	N/A	0.436	0.303	0.451	0.651	0.380	0.353	0.479

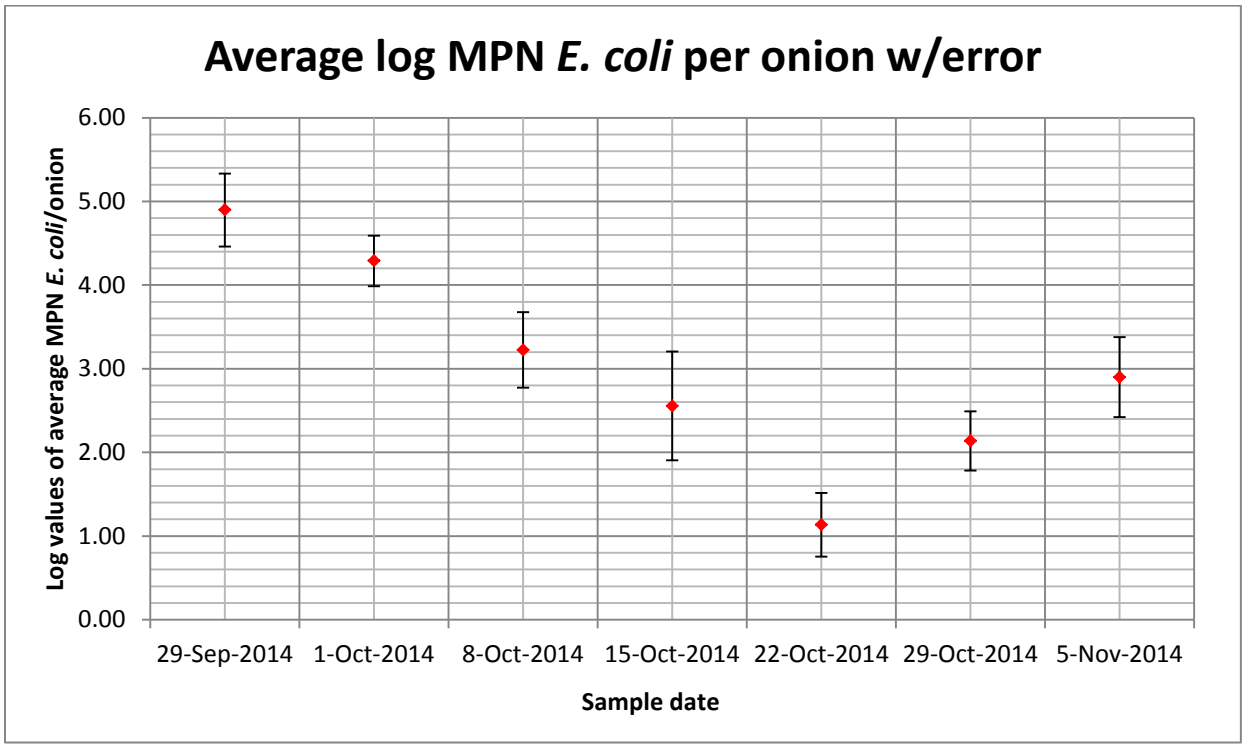

Figure 2. Average log values for each week of generic E. coli *per individual onion bulb in Experiment 1. Mean values are shown with standard error bars to indicate variation in the data. Malheur Experiment Station, Oregon State University, Ontario, OR, 2014.*

Experiment II

This experiment also showed a progressive decline in *E. coli* contamination initially. After 2 weeks, average *E. coli* levels per bulb had been reduced by almost a 3-log factor (Table 2, Fig. 3). The initial manure solution used in this trial of 7×10^7 MPN/100 ml was even higher than the amount used in Experiment 1. Average *E. coli* levels began increasing after 20 October, for unknown reasons, but dropped significantly by the last week. The untreated onions tested negative all weeks except for 20 October, where they showed minimal contamination. Though contamination levels increased near the end of the trial, they never reached the initial levels. Internalization of *E. coli* occurred only on 20 October, in sample sets Treated 1, Treated 2, and Treated 3. Bacterial detection on the untreated onions and internally on treated onions may have been from sampling onions with other unrelated decay rots that could have provided a suitable environment for *E. coli.* Every other sample for this trial tested negative.

Table 2. The log values of the weekly results of the second *E. coli* die-off trial. Each set represents 60 randomly selected onions. Malheur Experiment Station, Oregon State University Ontario, OR, 2014.

	Average log MPN *E. coli*/onion bulb					
Set:	6-Oct-2014	13-Oct-2014	20-Oct-2014	27-Oct-2014	3-Nov-2014	10-Nov-2014
Untreated	0.00	0.00	0.40	0.00	0.00	0.00
Treated 1	4.43	3.39	1.48	3.12	2.61	1.46
Treated 2	3.65	3.21	1.48	3.10	2.64	1.25
Treated 3	3.90	3.01	1.53	2.52	2.29	0.14
Treated 4	4.82	2.17	0.89	1.71	3.06	3.02
Treated 5	3.92	2.72	0.70	1.64	3.21	0.39
Mean (Treated)	4.14	2.90	1.22	2.42	2.76	1.25
Std. Dev.	0.475	0.478	0.391	0.721	0.372	1.136
Standard Error	0.212	0.214	0.175	0.322	0.166	0.508

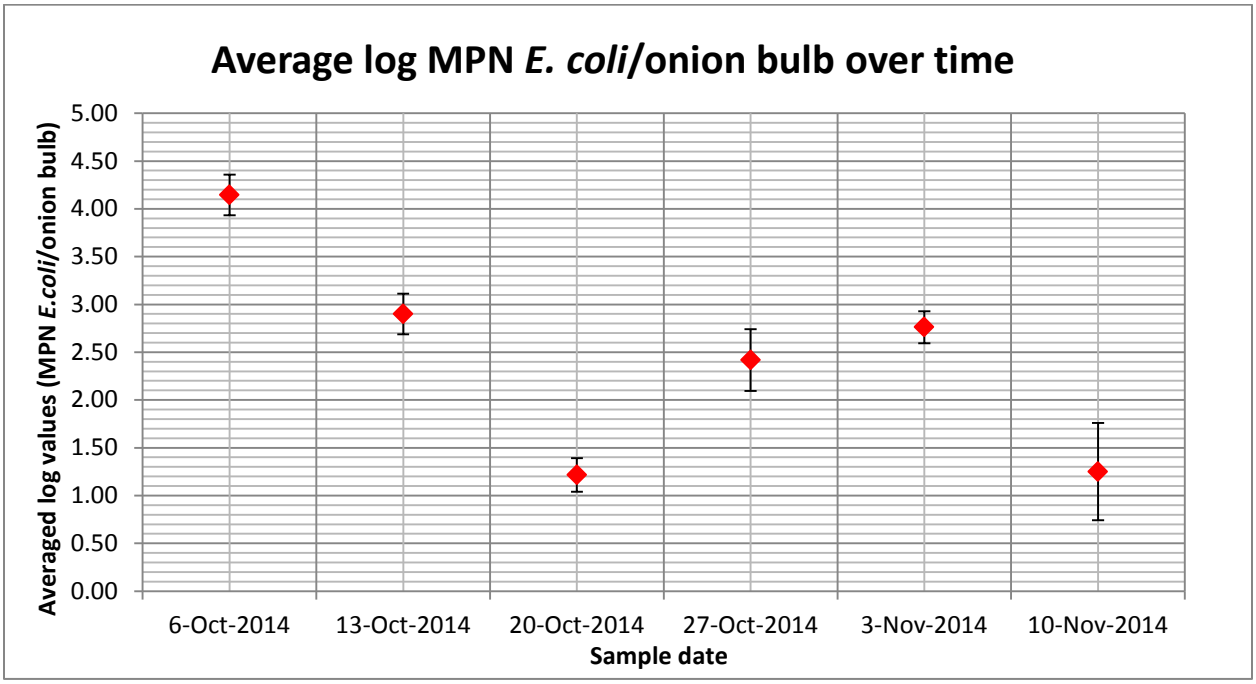

Figure 3. Average log values for each week of generic E. coli *per onion bulb based on 60-bulb samples in Experiment 2. Mean values are shown with standard error bars to indicate variation in the data. Malheur Experiment Station, Oregon State University, Ontario, OR, 2014.*

Discussion

Experiment I

The *E. coli* present on the bulb exteriors died off consistently for most of the trial period. Over the course of 24 days, we saw over a 3.5-log reduction in contamination levels. The largest reduction occurred between 15 and 22 October, about 3-4 weeks after the trial began. The onion skins from the samples taken on 24 September were not diluted enough at the lab to obtain useful results, so actual levels on those bulbs were unknown. Individual contamination levels varied significantly each week, but overall the average continually decreased. Average contamination levels actually began increasing during the last 2 weeks of the trial. This increase may have been associated with several days of rain during that week, with the additional moisture fostering the development of *E. coli* populations. Other factors may have been responsible for this, including colder weather, severe wind storms, animals contacting the onions, or any unintentional contact with unsterilized surfaces between sampling and delivery to the lab. *E. coli* was not internalized in any of the composite tests ran on the samples. The daily die-off rate from the beginning of the trial until the 22 October sample was 0.15 log units per day.

Experiment II

The untreated onion bulbs had no or very minimal *E. coli* present every week. *E. coli* present on the treated bulb exteriors died off consistently for the first 2 weeks of the trial. Over these 2 weeks, an approximately 3-log reduction occurred, averaged over the five sets of treated onions

sampled each week. The variation and standard error were both much smaller in this trial compared to the first experiment. The daily die-off rate in this experiment up to the 20 October sample was 0.21 log units per day. After the week of 20 October, the average contamination levels began increasing to values approaching 3-log, but dropped back down significantly during the last week. Because the untreated onions never showed an increase in contamination, it may be that at some point the bacteria on the treated onions found the right conditions to repopulate. As with Experiment 1, rain during this time may have provided conditions for *E. coli* populations to grow. Other factors may also have been responsible for this, including cooler weather, dust carried from wind storms, animals contacting the onions, or any unintentional contact with unsterilized surfaces between sampling and delivery to the lab. Quail were seen walking through the onion beds on one occasion, and the field was located adjacent to a natural windbreak, where many small animals likely lived. Onions tested positive for *E. coli* internalization only 1 week. The technician that ran the tests mentioned a correlation between the presence of rotted onions in the sample set with a positive result. Although rotted onions were thrown out during the sampling process, it is possible some made it through initial screening. The onion skins and the outer layer may provide morphological barriers to *E. coli* infection.

In both experiments, *E. coli* died off consistently and substantially at the beginning of the trials. This suggests that *E. coli* survival on onions is greatly limited in standard conditions. Even excessively high contamination levels dropped to acceptable levels in just 2 weeks. Certain conditions may have allowed the bacteria to begin to repopulate for a time, as seen in both experiments, before ultimately dying off, as seen in Experiment 2. There are likely more complications to *E. coli* survival on onion bulbs that warrants further study.

The FDA has revised the Food Safety Modernization Act (FSMA) rules regarding agricultural water quality. As part of the revisions, the FDA has proposed to allow a 0.5-log per day-die off rate between the last irrigation and harvest (when onions are taken out of fields). The die-off rates that were observed were less than 0.5 log but still were of a comparable magnitude. Given the normal length of time that onions cure in the field, onions would be within FSMA standards with the observed or proposed die-off rates (Fig. 4).

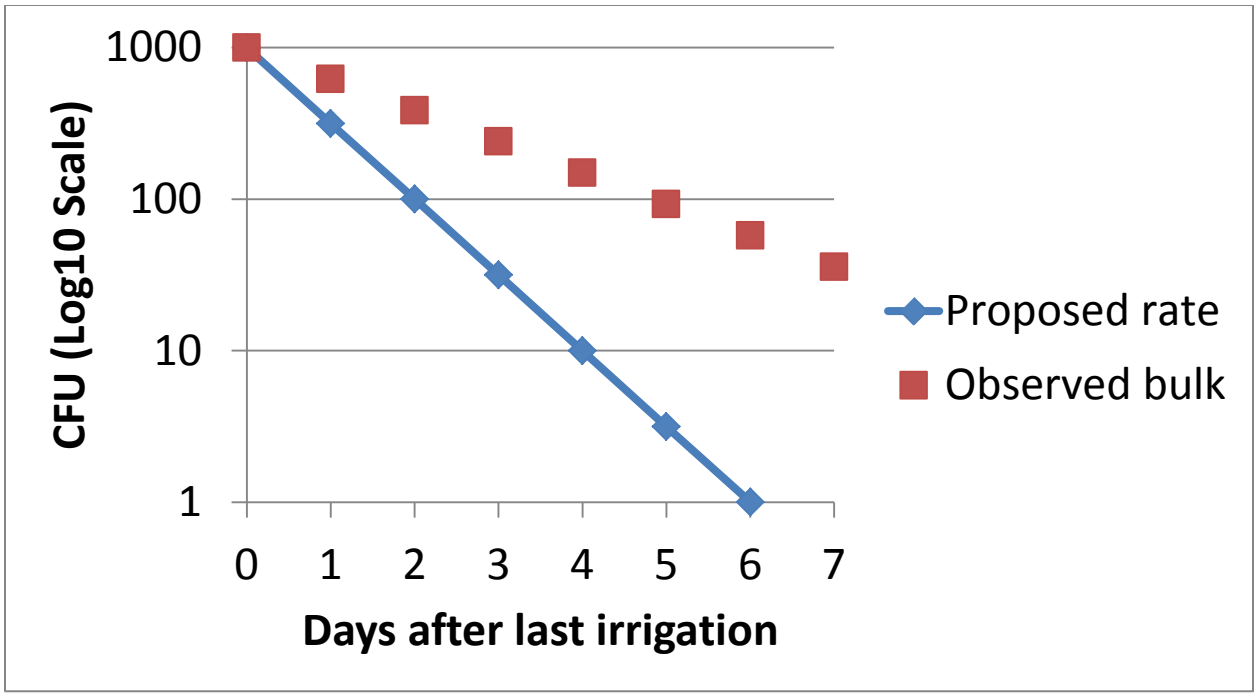

Figure 4. Hypothetical comparison of the proposed FDA E. coli *die-off rate and observed die off. Even with the lower observed die-off rate, if the water quality at the last irrigation was 1,000 colony-forming units (CFU)/100 ml, it would take less than 5 days for levels to be below the FDA standard of 126 CFU/100 ml.*

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