DRY BULB ONION STORAGE IN STERILIZED PLASTIC CRATES COMPARED TO STORAGE IN OLD WOODEN BOXES

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Abstract

As part of the Food Safety Modernization Act (FSMA), the U. S. Food and Drug Administration, (FDA) has mandated that produce storage containers be sanitary for their intended purpose. This mandate could potentially lead to efforts to replace wooden storage bins with plastic totes under the assumption that plastic containers would be more sanitary. Therefore, we conducted a replicated study to compare the roles of wooden and plastic storage containers in contamination of onion bulbs with *Escherichia coli*. Onions from five replicated furrow-irrigated plots using water containing 62.4 to greater than 20,957 MPN (most probable number) *E. coli* /100 ml were harvested into 10 old wooden boxes and 10 sterilized plastic crates. Onions from five replicated drip-irrigated plots using water with 0 MPN *E. coli* /100 ml were harvested into an additional 10 old wooden boxes and 10 sterilized plastic crates. Onions were stored for 6 weeks and then prepared for shipment by removal of loose skin, roots, and soil. None of these packed-out onions had detectable *E. coli* on the bulb exteriors or interiors, regardless of storage container type or irrigation water source. Plastic containers did not provide added food safety value compared with wooden boxes for the storage of dry bulb onions.

Background

The Food Safety Modernization Act, signed into law in January 2011, is the first major federal reevaluation of food safety 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. One of the new regulations the FDA included in the final rules for the production of vegetables and fruits is that storage bins for produce must be adequately sanitary for their intended purpose. It is unknown whether onion storage in wooden crates or boxes contributes to *E. coli* counts on onion bulbs prepared for shipment after postharvest storage. Any requirement to convert from wooden crates to plastic totes would be very costly and might provide no human health benefits.

Previous studies conducted in 2013 and 2014 by Shock et al. (2013, 2015) at the Oregon State University Malheur Experiment Station showed no apparent benefit of storing onions in sterilized plastic crates over old wooden boxes. Neither sterilized plastic crates nor old wooden boxes seemed to be conducive to *E. coli* on stored onions, regardless of the amount of *E. coli* present in the irrigation water with which the onions were grown. In the preliminary study, traces of *E. coli* were found on 1 of 10 samples of drip-irrigated onions stored in a plastic crate and 1 in 10 samples of drip-irrigated onions stored in a wooden box, but no *E. coli* were found on the onions from the furrow-irrigated onions stored in either container type. In this study, we sought to further determine whether or not onion storage in sterilized plastic crates would be more effective in minimizing *E. coli* contamination on the exterior of packed-out onion bulbs than those stored in old, unwashed wooden boxes.

Materials and Methods

Cultural practices

Onions were grown in 2015 on an Owyhee silt loam at the Oregon State University Malheur Experiment Station, Ontario, Oregon. Cultural and pest management practices were similar to those for commercial dry bulb onion production in the region.

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

Seed was planted on March 13 in double rows spaced 3 inches apart at 150,000 seeds/acre. Each double row was planted on beds spaced 22 inches apart. Planting was done with customized John Deere Flexi Planter units equipped with disc openers. Immediately after planting, the onions received a narrow band of Lorsban® 15G at 3.7 oz/1,000 ft of row (0.82 lb ai/acre) over the planted rows, and the soil surface was rolled. Onion emergence started on March 30.

The field had drip tape laid at 4-inch depth between 2 onion beds during planting. The drip tape had emitters spaced 12 inches apart and emitter flow rate of 0.22 gal/min/100 ft (Toro Aqua-Traxx, Toro Co., El Cajon, CA). The distance between the tape and the center of each double row of onions was 11 inches. Until the experiment commenced, all onions were irrigated automatically with well water to maintain the soil water tension (SWT) in the onion root zone below 20 cb (Shock et al. 2000). Soil water tension was measured with four granular matrix sensors (GMS, Watermark Soil Moisture Sensors Model 200SS, Irrometer Co., Riverside, CA) installed at 8-inch depth in the center of the double row. Sensors had been calibrated to SWT (Shock et al. 1998).

The onions were managed to minimize yield reductions from weeds, pests, diseases, water stress, and nutrient deficiencies. For weed control, the following herbicides were applied: on March 26, Roundup PowerMax® at 24 oz/acre was broadcast; on April 28, GoalTender® at 0.09 lb ai/acre (4 oz/acre), Buctril® at 0.25 lb ai/acre (16 oz/acre), and Poast® at 0.25 lb ai/acre (16 oz/acre) were broadcast; on May 4, Prowl® H_2O at 0.83 lb ai/acre (2 pt/acre) was broadcast; on June 4, GoalTender at 0.14 lb ai/acre (6 oz/acre), Buctril at 0.25 lb ai/acre (16 oz/acre), and Poast at 0.25 lb ai/acre (16 oz/acre) were broadcast.

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

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

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

Irrigation treatments

Starting on August 3 separate irrigation systems and water sources were employed as a means to compare the 'Vaquero' onions drip-irrigated with clean well water with Vaquero onions furrow-irrigated with ditch water containing enhanced levels of *E. coli*. The ditch water was enhanced with *E. coli* by intentionally pumping water above an actively used pasture and capturing the pasture runoff water in the ditch before the furrow irrigation outlet. Onions were grown in a replicated irrigation experiment where drip irrigation with well water and furrow irrigation with ditch water were two of the treatments. All plots were in the same field and other than irrigation system and water sources, all management of the onions was identical. Irrigation water sources were evaluated for *E. coli* several times during the growing season. The final irrigation occurred on August 24 and 25. Because of logistical constraints, the plots irrigated with the enhanced *E. coli* water were irrigated the day after the other plots. The onion bulbs used in this storage trial were lifted manually on September 22 and placed in containers.

Comparison of onion storage in plastic crates and old wooden boxes

Twenty 25-year-old wooden boxes in good repair were taken from storage, left unwashed, and labeled 1-20. They were hauled to the onion field in the bed of a pickup truck that had been sterilized with bleach and washed out with water. Twenty plastic crates (purchased in 2013 or 2015) were washed with water and allowed to dry in the sun. They were also labeled 1-20 and taken to the field in the same manner as the wooden boxes.

In the field prior to filling, the plastic crates were sterilized with a bleach solution and rinsed with distilled water, and allowed to dry in the sun. The wooden boxes were not rinsed or cleaned except for minor brushing off of any loose material such as leaves, spider webs, and old onion peels. Harvesting of the onions started on September 22. All the onions from the drip-irrigated plots were sampled first, then the onions from the furrow-irrigated plots. Sampling was done using sterile rubber gloves, a wire basket, and knife. Baskets and knives were sterilized and gloves were changed between treatments. Two wooden boxes and two plastic crates were filled from each irrigation treatment in each of the 5 replicates, for a total of 20 wooden boxes and 20 plastic crates. Onions were randomly chosen, topped, and placed into the wire basket, and then transferred to the storage boxes. The randomization of the onion selection was achieved by pulling every sixth onion, then going back and pulling every fifth onion, and so on until the boxes were full.

The sampled onions remained in the field for 8 days until September 30, when storage containers were transferred into a cold air storage unit. The 10 wooden boxes from each irrigation type were placed onto pallets. The storage conditions were monitored and maintained similar to those used for commercial onion storage.

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

Packout involved the removal of loose skins and roots that are ordinarily removed from onion bulbs through mechanical movement in conventional harvesting and packout operations. Packed-out bulbs were placed into double-bagged 30-gal plastic bags labeled accordingly. Following packout, the bulb exteriors and interiors were analyzed for *E. coli*, as described below.

External analysis of onion bulbs for *E. coli*

In the laboratory the roots, small remnants of soil, skin, and outer peel of the onions were removed from the bulbs and weighed. The skins, peels, roots, and soil were then thoroughly washed in 1 L of water. A 100-ml sample of the wash water was used to estimate a Most Probable Number (MPN) of generic *E. coli* present on the exteriors of the onions, using IDEXX *Colilert® +Quanti-Tray/2000®* (IDEXX Laboratories, Westbrook, ME) (Edberg and Edberg 1988; Edberg et al. 1990). The *E. coli* MPN per onion bulb exterior was then calculated based on the number of onions in each sample.

Internal analysis of onion bulbs for generic *E. coli* and *E. coli* O157:H7

The outer skins and scales were peeled from all the onions in each sample set and 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 ziplock food grade bag and mixed. The wedges were then 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 90 ml of Universal Pre-enrichment Broth (UPB, Accumedia, Nedgen, MI) and sealed. The UPB broth was placed in an incubator for 48 hours at 35^{o}C (95°F).

Along with every batch of samples, an additional positively 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 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 hours at 35^{o}C. After 24 hours the Colisure was tested with UV light for fluorescence indicating the presence of generic *E. coli* (Edberg and Edberg 1988; Edberg et al. 1990).

E. coli O157:H7 was tested with PCR. One ml from the positive Colisure was taken and DNA was extracted from this 1 ml. The DNA extract was tested for the presence of O157 with O157:H7 specific primers.

Analysis of the soil for *E. coli*

Soil from the top 2 inches (5 cm) of each plot was sampled September 22 for *E. coli*, immediately following onion sampling. A composite soil sample was taken from four random spots where the rows of drip-irrigated onions were harvested and a composite soil sample was taken from four random spots where the furrow-irrigated onions were harvested. This was repeated for each replicate. Soil samples were refrigerated until analyzed. 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 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, ME) (Edberg and Edberg 1988).

E. coli in the irrigation water

The irrigation water was sampled for each system and water source every hour for the first 8 hours of irrigation, including when the water was first turned on, for a total of 9 samples per treatment. 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 1-inch hoses that carried the water to the drip tapes. For the furrow-irrigated treatment with enhanced *E. coli*, irrigation runoff water from a cattle pasture was intentionally captured and added to the ditch water to assure high *E. coli* counts for the enhanced ditch water treatment.

All water samples were collected from the top 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. Water samples were run with Idexx-Colilert (Adams et al. 1990).

Results

The irrigation water was tested several times during the season and ranged from 62.4 to over 20,957 MPN of *E. coli* per 100 ml for the enhanced ditch water, but the well water consistently had no detectable *E. coli* (Table 1). The upper 5 cm of soil was tested for *E. coli* when onions were collected in storage containers, which was 4 weeks after the last irrigation. *E. coli* was not detected in the soil that had been irrigated with well water; low levels of *E. coli* were detected in soil that had been irrigated with enhanced ditch water (Table 2).

Drip-irrigated onion packed out from plastic crates vs. old wooden boxes

No *E. coli* were present on the exterior of the packed-out onions grown under drip irrigation with clean well water (Table 3). This coincides with the absence of *E. coli* in the irrigation water during the season and the absence of *E. coli* in the soil water at the time of harvest (Table 2). Analyses of onion interiors for the presence of *E. coli* were negative for all samples (Table 4). Because *E. coli* was not found on the exteriors or in the interiors of any of the packed-out onions, storage in old wooden boxes did not contribute any additional *E. coli* contamination to the onion bulbs compared to storage in sterilized plastic crates.

Furrow-irrigated onion packed out from plastic crates vs. old wooden boxes

Despite the high amounts of *E. coli* present in the *E. coli*-enhanced irrigation water (Table 1), no generic *E. coli* was detected on any of the packed-out onions grown under furrow irrigation with ditch water. Further, there were only very modest amounts of *E. coli* in the soil at harvest, which was 4 weeks after the last irrigation (Table 2). Bulbs from only two of the sampled plastic crates and one of the sampled wooden boxes tested positive for traces of *E. coli*. Additionally, no generic *E. coli* or pathogenic *E. coli* O157:H7 were found internally in any of the samples. Because low numbers of *E. coli* were found on onions stored in both plastic crates and wooden boxes, and internalization of *E. coli* did not occur, storage in new sterilized plastic crates did not provide any advantage in preventing *E. coli* contamination over storage in old wooden boxes.

Discussion

Very few *E. coli* were detected on the exterior of onions at packout. Neither new sterilized plastic crates nor old wooden boxes seemed to be conducive to *E. coli* on stored onions. No *E. coli* was found to be internalized in any of the onion samples, which suggests that the storage container is not necessarily a determining factor for onion contamination. At the very least, neither storage container type held any practical advantage over the other with respect to inhibiting bacterial contamination.

The wooden boxes and plastic crates used in these replicated trials were much smaller than commercial storage bins and totes. Commercial wooden onion bins with external dimensions of 6 ft by 4 ft by 2.5 ft have an internal volume of 1.25 m^3 while the wooden boxes used in this trial had an internal volume of 0.063 m^3. Commercial plastic totes used for onion storage with external dimensions of 44.75 inches by 48.25 inches by 34.62 inches have an internal volume of 0.907 m^3 (MacroBin 34-FV, MacroPlastics, Yakima, WA) while the plastic crates used in these trials had an internal volume of 0.031 m^3. If the wood in the boxes was to have negative interactions with the onion bulbs, the risk should have been accentuated in the current trial due to greater contact of the bulbs with the wooden surface than in commercial wooden bins.

Onions furrow-irrigated with ditch water intentionally enhanced with pasture runoff did have an increase in *E. coli* contamination compared to drip-irrigated onions. None of the samples from either the furrow-irrigated plots or the well water drip-irrigated plots had any *E. coli* detected, a difference from previous results (Shock et al. 2013, 2015) where trace amounts of *E. coli* were detected in some samples, but did not appear to be related to irrigation water sources (Table 4).

Table 1. *E. coli* counts in the ditch water intentionally enhanced with pasture runoff water used for furrow-irrigated onions. Values of *E. coli* are in most probable number (MPN)/100 mL water, Oregon State University, Malheur Experiment Station, Ontario, OR, 2015.

Time after initiation of irrigation (hour)	Aug 6	Aug 11	Aug 18	Aug 28
1	1,413.60	156.50	20,957.14	275.50
2	770.10	2,610.00	5,000.00	186.00
3	727.00	2,419.60	2,510.00	111.20
4	613.10	1,203.30	2,420.00	62.40
5	517.20	980.40	1,986.30	109.20
6	613.10	980.40	1,119.90	1,046.20
7	-		1,299.70	307.60

Table 2: Results of the soil water analysis performed at onion harvest on September 22, 2015. Values of *E. coli* are in most probable number (MPN)/100 mL soil water.

	Drip-irrigated with well water	Furrow-irrigated with water with enhanced *E. coli*
Replicate 1	nd*	nd
Replicate 2	nd	5.10
Replicate 3	nd	9.80
Replicate 4	nd	9.70
Replicate 5	nd	7.50

*nd, not detected

Table 3. *E. coli* on the exterior of onions drip-irrigated with clean well water, packed out of storage from sterilized plastic crates and old unsanitized wooden boxes, Oregon State University, Malheur Experiment Station, Ontario, OR, 2015.

	Enhanced *E. coli* furrow irrigation		Well water drip irrigation	
Sample	Average weight of skins, peel, roots, and soil on 16,17 November 2015 (g/bulb)	Average MPN *E. coli* per onion bulb exterior	Average weight of skins, peel, roots, and soil on 16,17 November 2015 (g/bulb)	Average MPN *E. coli* per onion bulb exterior on 16,17 November 2015
Sterilized plastic crates				
1	9.83	nd*	4.95	nd
2	9.26	nd	8.93	nd
3	5.38	nd	9.78	nd
4	8.45	nd	5.78	nd
5	9.42	nd	6.01	nd
6	8.75	nd	7.57	nd
7	8.10	nd	5.99	nd
8	7.64	nd	5.17	nd
9	6.70	nd	9.60	nd
10	10.26	nd	5.10	nd
Old wooden boxes				
11	9.07	nd	9.22	nd
12	9.22	nd	5.80	nd
13	8.53	nd	10.62	nd
14	7.93	nd	6.83	nd
15	11.11	nd	14.14	nd
16	10.69	nd	12.98	nd
17	7.04	nd	13.93	nd
18	9.01	nd	11.47	nd
19	10.22	nd	15.16	nd
20	7.35	nd	13.84	nd

*nd, not detected

Table 4. Summary of the effects of irrigation system and storage container on the *E. coli* on and in onions packed out of storage. Sterilized plastic crates were compared with old unsanitized wooden boxes, Oregon State University, Malheur Experiment Station, Ontario, Oregon, 2015.

Irrigation system and water source	Storage container	*E. coli* on onion exterior, MPN per bulb	*E. coli* in onion interiors	*E. coli* O157:H7 in onion interior
Drip with well water	Old wooden boxes	nd	nd*	nd
	Sterilized plastic crates	nd	nd	nd
Furrow with enhanced ditch water	Old wooden boxes	nd	nd	nd
	Sterilized plastic crates	nd	nd	nd
Average	Old wooden boxes	nd	nd	nd
	Sterilized plastic crates	nd	nd	nd
LSD (0.05) $_{\text{container}}$		ns**	------	-------
LSD (0.05) $_{\text{water source}}$		ns	------	-------
LSD (0.05) $_{\text{container x water source}}$		ns	------	-------

*nd, not detected
**ns, not significantly different

Conclusions

The use of contaminated ditch water for furrow irrigation did not contribute to the *E. coli* load on onions packed out after storage. The bacterial content of the water used for furrow irrigation had been intentionally enriched by pumping water through an actively used cattle pasture and capturing the water for onion furrow irrigation.

The use of sterilized plastic crates provided no advantage over old wooden boxes in avoiding *E. coli* contamination on or in onions.

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