CONTROLLING *E. COLI* WITH IN-FIELD APPLICATION OF COPPER FUNGICIDES

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Introduction

The Food Safety Modernization Act (FSMA) seeks to protect humans from food-borne pathogens. The U.S. Food and Drug Administration (FDA) has proposed rules to implement the FSMA that include limits on the microbial content in agricultural irrigation water. Under the final rules, if water quality standards are exceeded, produce could still be considered safe for human consumption if appropriate remedial actions are taken. One potential remediation measure could be the use of currently registered copper fungicides that also have general bactericidal effects. Therefore, we conducted a field trial to compare generic *Escherichia coli* (*E. coli*) levels on onion bulbs from plots that had been treated with a copper fungicide just before lifting.

Materials and Methods

Cultural practices

The experiment was conducted at the Malheur Experiment Station, on a field of 'Vaquero' onions that was dedicated for *E. coli* research.

Onions were grown in 2015 on an Owyhee silt loam. 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 to 5.5% lime, 1.66% organic matter, 101% base saturation, 20 ppm nitrate, 7 ppm ammonium, 34 ppm phosphorus (P), 252 ppm 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 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. 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.

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 an 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.

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®, a copper-based fungicide, was applied aerially at 0.28 lb ai/acre (1 pt/acre) on June 4. The presence of *E. coli* on bulb exteriors just before the application of Kocide® suggests that Badge did not completely inhibit *E. coli* from June through August.

Fungicide treatment

There were four irrigation treatments with each treatment replicated five times. The irrigation treatments were: 1) drip irrigated with well water; 2) drip irrigated with ditch water; 3) furrow irrigated with ditch water; 4) furrow irrigated with ditch water with enhanced *E. coli* levels. Each of these plots was subdivided into two plots for the fungicide trial. Subplots were 33 ft long and 3 beds wide. One subplot of each main plot was left untreated and the other subplot was treated once on August 28 with Kocide 2000 DF (53.8% copper hydroxide) at 1.5 lb/acre. The application was made with a CO_2-powered backpack sprayer, with a 4-nozzle boom and 11004 nozzles, set at 30 psi and 35 gal/acre.

Data collection and analysis

Onions were lifted on August 31, 2015, 3 days after the fungicide application and 7 days after the last irrigation. Onions were sampled for *E. coli* four times: 1) August 27 the day before the fungicide application was made and 3 days after the last irrigation; 2) September 2, 3; 3) September 9, 10; and 4) September 16, 17 to determine changes before and after application.

On each date, three samples of 20 onions were collected from each plot. No onions were discarded. Onions were topped and placed in wire baskets and then placed in plastic storage bags. To minimize potential inadvertent cross contamination, workers wore gloves, and used sterilized knives and baskets in the field. Knives and baskets were sterilized with bleach and workers exchanged gloves as they moved from plot to plot. Because we anticipated that irrigation treatments would contain different amounts of *E. coli*, we sampled them in order of expected *E. coli* levels, going from low to high (i.e., drip, well water; drip, ditch water; furrow;

furrow with *E. coli*-enhanced water). Within irrigation treatments, we expected treated onions to have lower *E. coli* levels than untreated onions. Therefore, we sampled the treated plots first.

Onions were transported to Western Laboratories, Inc. (Parma, ID) for *E. coli* analysis. In the laboratory, the roots, small remnants of soil, skins, 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). The *E. coli* MPN per onion bulb exterior was then calculated based on the number of onions in each sample.

Bulb interiors were tested. The outer skins and scales were peeled from all the onions in the 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 and Discussion

E. coli was detected on onions sampled before the copper fungicide treatment. Most samples had no *E. coli,* some had minor levels (<126 MPN), and a few had high levels. These results occurred across all four irrigation treatments (Table 1). For example, the high mean in the well-water treatment was from a single sample with 13,950 MPN per bulb. This variation suggests there can be random, variable amounts of *E. coli* that can be found on onion exteriors. None of the onion interiors from this sampling were contaminated with *E. coli.*

In the first samples collected after lifting (September 2, 3), *E. coli* levels on onion exteriors were reduced considerably in all irrigation treatments, regardless of fungicide treatment. *E. coli* levels approached 0 in the furrow, drip (well water) and drip (ditch water) irrigation treatments. In the enhanced furrow-irrigation treatment *E. coli* levels remained high. Levels were higher in onions that did not receive the fungicide treatment than in onions that did receive the fungicide treatment. Although this suggests the fungicide treatment helped reduce *E. coli* levels, the difference was not statistically significant because of the high amount of variation among samples. Most samples had no detectable *E. coli,* but a few had high amounts.

In the following sample collections, most samples had no detectable *E. coli* on bulb exteriors. A few samples did have some present, again indicating the potential for random deposition of *E.*

coli. However, the levels on these samples collected after lifting were less than those found on bulbs during irrigation.

All bulb interiors, regardless of irrigation or fungicide treatment, were negative for all microbial contaminants.

Given that most samples did not have detectable levels of *E. coli*, it was not possible to gauge the effectiveness of copper-based fungicides in controlling *E. coli* on onions. The general lack of *E. coli* on most untreated onions and declines in levels after lifting are still significant findings.

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Table 1. *E. coli* levels on onion exteriors before and following treatment with copper sprays (Kocide). Values are the most probable number (MPN) of *E. coli* per onion bulb.

Irrigation	Collection time	Untreated Mean	Untreated standard error	Fungicide treated Mean	Fungicide treated standard error
Furrow	Before treatment	963.25	570.82	963.25*	570.82*
Furrow	1 Week after treatment	0.13	0.13	3.73	3.17
Furrow	2 Weeks after treatment	2.76	2.76	0.00	0.00
Furrow	3 Weeks after treatment	20.61	19.33	0.13	0.13
Enhanced	Before treatment	279.80	141.17	279.80*	141.17*
Enhanced	1 Week after treatment	132.62	132.41	2.37	1.37
Enhanced	2 Weeks after treatment	1.07	1.07	21.70	21.70
Enhanced	3 Weeks after treatment	0.20	0.15	1.65	1.44
Drip well	Before treatment	931.99	929.86	931.99*	929.86*
Drip well	1 Week after treatment	0.00	0.00	0.00	0.00
Drip well	2 Weeks after treatment	0.00	0.00	0.00	0.00
Drip well	3 Weeks after treatment	24.49	24.35	0.89	0.89
Drip ditch	Before Treatment	9.31	5.89	9.31*	5.89*
Drip ditch	1 Week after treatment	0.00	0.00	0.00	0.00
Drip ditch	2 Weeks after treatment	0.00	0.00	0.35	0.35
Drip ditch	3 Weeks after treatment	0.97	0.90	0.00	0.00

* Before treatment values were obtained from onion collected from across the entire plot.