CPS CENTER for PRODUCE SAFETY

CPS-WCFS 2013 RFP
FINAL PROJECT REPORT

Project Title

Survival of generic *E. coli* and *Salmonella* during the growth, curing, and storage of dry bulb onions produced with contaminated irrigation water

Project Period

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Objectives

1.) Quantify generic E. coli and Salmonella transfer from late season contaminated irrigation water to dry bulb onions through the soil

2.) Determine the impact of water cessation, curing, and storage on the survival of generic E. coli and Salmonella associated with dry bulb onions and soil

3.) Compare survival of generic E. coli and Salmonella standard water cessation and curing practices with abbreviated practices for early season "greentop" dry bulb onions

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Joy Waite-Cusic, Oregon State University
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Abstract

The Treasure Valley area of eastern Oregon and western Idaho is famous for the highest yield of dry bulb onions in the country; however, due to its high desert climate, water is a scarce resource. Water is supplied through irrigation canal systems that were originally constructed in the late 1920s and assisted in transforming the area into the agricultural powerhouse of today. This network of canals distributes and reclaims water multiple times throughout the valley to efficiently utilize the minimal water that is available. This continual reuse leads to high microbiological loads in the water, specifically generic *E. coli* . The recently proposed produce safety rules have indicated a maximum generic *E. coli* level (235 MPN/100 ml) that cannot be consistently met throughout the growing season and places a huge financial burden on onion growers in the region. Due to the relatively large time frame between the last irrigation and harvest, storage, and distribution of onions, it is likely that survival of generic *E. coli* and foodborne pathogens, such as *Salmonella*, would be very minimal in this crop. The primary aim of this study was to quantify the survival of generic *E. coli* and *Salmonella* spp. associated with dry bulb onions through the late stages of growth, water cessation, curing and storage when inoculated through contaminated irrigation water. It is possible for growing season water samples to contain 5,000-10,000 CFU/100 ml. To provide an additional safety factor (100x), irrigation water was applied with generic *E. coli* and *Salmonella* spp. at approximately 300,000 CFU/100 ml. Onions were irrigated with the contaminated water every 2-3 days for the final 9 weeks of growth resulting in a final contamination level of 3.5 log CFU/g of onion. Onions were then finished and cured following conventional or greentop practices. After 1 week of water cessation, generic *E. coli* and *Salmonella* spp. were reduced between 1.3 and 2.3 log CFU/g. An additional week of water cessation (conventional or greentop) achieved an additional reduction of 1.3-2.2 log CFU/g (total reductions of 3.2-3.8 log CFU/g). Remaining low levels of generic *E. coli* and *Salmonella* spp. (<1 CFU/g) were stable throughout the conventional 2-week curing process and an additional 2 weeks of storage.

Background

The majority of onions are produced in the Pacific Northwest (PNW; Washington, Oregon, and Idaho). Over 53,000 acres were planted in these states in 2010, yielding an estimated 3.5 million lbs of onions with a farmgate value of approximately $377 million (USDA ERS 2011b, 2011c, 2011d). Two types of onions, dry bulb (storage) onions and non-storage (mostly Walla Walla variety) are produced in the PNW. Dry bulb onion production occurs in two waves: spring-planted (summer/fall harvested) and fall-planted (spring harvested). The majority of the crop in the PNW is spring-planted with long-day varieties in the predominant production areas being the Treasure Valley (eastern Oregon and western Idaho), eastern Washington, the Columbia Basin (Umatilla and Morrow counties), the Willamette Valley, and the Klamath Basin. The majority of the storage onions produced in the PNW are grown in Treasure Valley (62% based on acreage) and are yellow sweet Spanish varieties (90%), with the remainder being red (5-6%) and white (3-4%) varieties (Kay Riley, personal communication; DeFrancesco 2004). These onions go to market from August through October and are then sold out of storage from October to April (Shock, Ishida, Eldredge, & Seddigh, 2000). There is also limited production of the fall-planted onions in the PNW, mostly within the Willamette Valley and Klamath Basin. The fall-planted crop will be marketed from April to August (Shock et al. 2000).

Onions are shallow rooted monocots that are typically grown from seed, but may also be grown from small bulb sets. Following germination, the onion plant will grow vegetatively, producing upright leaves (stalks). The length of daylight will induce the onion plant to form the bulb and the temperature will determine the growth rate of the bulb. The bulb is made of fleshy leaves (scales) that form from about 8-10 weeks after germination. The bulbs will continue to increase in size through the remainder of the growing season. For spring-planted onions, irrigation will be stopped sometime in August or early September in preparation for finishing and harvest. There are two primary approaches to finishing out onions: conventional and greentop. Following conventional practices, irrigation will be stopped in early September and the onions will remain in the field without water for 10-14 days for the stalks to dry out. The onion roots will then be undercut and the bulb will be lifted to the surface to cure on top of the soil for an additional 10-14 days. A properly field-cured bulb, ready for storage, should have a well-dried neck and have at least one, and preferably two, complete dry scales. Following curing, the dried stalks will be cut from the top of the bulb and the onions will be harvested and transferred to an onion storage shed and held for up to 6 months before distribution to market. During this time, final drying of the onion neck occurs and wounds caused during harvest will dry and heal. The storage shed is managed to optimize air circulation to promote additional curing and minimize the potential for mold and other diseases and root sprouting. Approximately 95% of dry bulb onions will be harvesting following conventional finishing (DeFrancesco 2004). The first 5% of the onion crop is finished by greentop practices, which is a more rapid curing process to fill market needs in August and early September as the fall-planted crop is depleted. High temperatures in August and early September prohibit conventional curing practices because of severe damage to the onion bulb if left on the soil surface to cure. Therefore, irrigation water is stopped for approximately 7 days and the onions are left in the field to dry. The onion stalks will be mowed and left in the ground for an additional 3-7 days for further drying. The bulbs will then be undercut, lifted, harvested, and transferred to the storage sheds on the same day (Kay Riley and Clint Shock, personal communications).

Irrigation

100% of the PNW onion crop is irrigated (USDA ERS 2011e). Irrigation may be accomplished by a variety of approaches and varies by state. Oregon and Idaho irrigate a majority of their crops by furrow irrigation (84%), with the remaining crop being irrigated by drip systems. Washington state predominantly irrigates with overhead sprinklers (60%), with the remainder split evenly between furrow and drip (DeFrancesco 2004). In the Treasure Valley growing region, approximately 90% of the irrigation water is surface water with a small amount of well water available to specific growers (Kay Riley, personal communication).

The Treasure Valley area of eastern Oregon and western Idaho is an agricultural mecca on the Snake River plain in the midst of the high desert. This area was transformed following the construction of a network of irrigation canals and reservoirs constructed in the 1920s. Approximately 150 growers farm over 20,000 acres of the Treasure Valley for high yield dry bulb onion production (740-760 cwt/acre) (Shock et al. 2000; USDA ERS 2011b, 2011d). Due to the scarcity of water in the area, these growers rely on the irrigation canal system along with reclamation and reuse of the water multiple times throughout the valley. The combination of open irrigation canals and ditches along with the recycling of the water after passing through fields leads to unpredictably high levels of generic *E. coli*.

Regulatory Changes

Following the passage of the Food Safety Modernization Act (FSMA) by Congress in early 2011, the Food and Drug Administration (FDA) published proposed rules for the Standards for the Growing, Harvesting, Packing and Holding of Produce for Human Consumption that drastically impact the

agricultural production around the country. A major portion of the proposed rules focuses on the microbiological quality of irrigation water used for produce that is consumed raw ("covered produce"). The proposed water standards require weekly testing of surface water used for irrigation throughout the growing season. The proposed microbiological standards mimic the Environmental Protection Agency (EPA) requirements for recreational water, which require a rolling geometric mean of no more than 126 CFU or MPN per 100 ml sample with no single sample greater than 235 CFU or MPN per 100 ml. If these levels are exceeded, the proposed rule states that the grower should stop using this water for irrigation, investigate the source of the contamination, and remedy the problem before resuming the use of the water source.

Many growing areas do not have the luxury of multiple sources of irrigation water and have limited control over the quality of the water that is delivered to their farm. The proposed water testing requirements place a significant financial burden on growers in the Treasure Valley area where there are approximately 1900 distinct fields of onions planted annually. These fields are irrigated for an average of 15 weeks during the growing season. At $30 per generic *E. coli* test, the testing cost for Treasure Valley growers would be $855,000. Rough estimates predict the economic impact to be triple this figure to >$2.5 million with the inclusion of labor and transportation costs associated with testing requirements.

Previous knowledge on onion safety

Dry bulb onions have not been previously associated with a foodborne outbreak; however, there is limited evidence in the primary literature to support this food safety record. A single field study in Georgia on the potential for contamination of onions with *E. coli* O157:H7 via manure compost and irrigation water was published in 2005. This study investigated the impact of an early season, single application of contaminated irrigation water (5 log CFU/ml *E. coli* O157:H7) at 3 weeks after seeds were planted. *E. coli* O157:H7 was detected at low levels in onion samples for 6 weeks following the contamination event. This data suggests the potential for later season irrigation to be a potential source for contamination of the harvested product. The literature is void of information related to late season contamination as well as information related to the survival of bacterial contaminants during finishing and storage of onions.

Research Methods and Results

Greenhouse setup

Two greenhouses (West 6-5 – 700 sq. ft; West 7-6 – 340 sq. ft) at Oregon State University were used for onion production. Both greenhouses have solid concrete floors with steel mesh grid tables to accommodate a wide variety of greenhouse research needs. Greenhouse temperatures were maintained by thermostat control (High: 24C; Low: 10-12C). Greenhouse temperatures were monitored and recorded throughout the study using a weather station (Easyweather Proweather station, Tycon Power Systems, Bluffdale, UT). Large grow trays (4' x 4'; Botanicare, Chandler, AZ) were placed on each table to contain any contaminated runoff and could accommodate up to 25 pots each. PVC cages with mosquito netting were constructed to eliminate access of flying insects after contamination was begun. The greenhouse facilities are actively managed and maintained 24 hours per day, 7 days per week, year-round by on-site university staff and students. Greenhouse staff monitored the complex for pertinent pests and recommended and applied pesticides as needed throughout the course of the study. To satisfy university requirements to work with BSL-2 organisms in the greenhouse, additional precautions were required to ensure that pathogens would be contained. Greenhouse doors were required to be locked at all times with biohazard signs displayed. When entering the greenhouses, disposable polypropylene booties were placed over footwear. Additional personal protective equipment (face shields, scrub pants etc) were required

during irrigation. Inocula were transported to the greenhouses in a 3-barrier container system. Likewise, contaminated samples were transported from the greenhouses to the laboratory in a 3-barrier container system. Remaining contaminated debris (plant waste, soil) and pots were autoclaved prior to disposal. Trays and greenhouse surfaces were disinfected with appropriate sanitizers (bleach or quaternary ammonia compounds).

Soil preparation

Soil (~3000 lbs/soil type) was transferred from a commercial onion field in the Willamette Valley (muck soil) and from the Malheur Country Agricultural Experiment Station in the treasure Treasure Valley (silt loam) into the Oregon State University greenhouses. Samples of both soil types (100 g x 3 replicates) were submitted to the Central Analytical Laboratory at Oregon State University for pH and mineral composition. Results of soil analyses are shown in Table 1. Soil was prepared by hand grinding through wire mesh grid boxes (approximately 0.5" grid). Two-gallon planting pots (Gro Pro, Sunlight Supply, Inc, Vancouver, WA) were lined with synthetic cheesecloth (12" x 12"; Dairy Connection, Madison, WI) to prevent soil erosion and filled with approximately 3500 g of soil. Prepared pots were distributed into the trays throughout both greenhouses.

Table 1. Silt Loam and Muck soil analysis results (n = 3).

Soil Type	pH	Bray-P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Na (ppm)	B (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)	Fe (ppm)	NO_3-N (ppm)	NH_4-N (ppm)	%OM LOI
Sandy Loam	8.1 ± 0.1	63 ± 2	648 ± 16	2586 ± 96	627 ± 19	94 ± 3	0.1 ± 0.1	1.2 ± 0.1	122 ± 9	4.1 ± 0.2	102 ± 5	2.3 ± 1.3	1.8 ± 0.4	2.32 ± 0.00
Muck	6.2 ± 0.0	114 ± 3	449 ± 18	5282 ± 400	727 ± 36	101 ± 2	0.5 ± 0.1	52.8 ± 0.7	318 ± 35	44.1 ± 1.4	1945 ± 55	35.9 ± 5.4	22.0 ± 5.0	12.7 ± 0.4

Onion production and finishing

Spanish yellow dry bulb onion sets (Ovation variety; 1 plant per pot; Nunhems USA, Parma, ID) were planted on March 22nd. Sets were previously grown from seed (Sakata Seed Company, Morgan Hill, CA) in Buckeye, AZ from November to March prior to shipment to Nunhems. Each tray of onion plants (17-19 plants per tray) was treated as a block for a given treatment (inoculated/uninoculated, soil type, finishing regiment) with the treatments being randomized across both greenhouses. Each plant was watered with 200 ml of municipal water in the morning every 2-3 days, as needed. Onion plants were fertilized with OmegaGrow 5-1-1 Organic Liquid Fertilizer (Dixondale Farms, Carrizo Springs, TX) per manufacturers instructions on April 22nd and April 29th. After 5 weeks of growth (May 1st), irrigation was transitioned to well water for contamination stage which was continued through maturity (July 10th). Onions were determined to be fully mature based on browning and dropping of the stalks.

Two finishing methods were evaluated: conventional and greentop (Table 2). Conventional onions were left in the soil undisturbed for two weeks following water cessation, onions were then lifted to the surface of the soil and cured for two additional weeks, followed by harvesting by removing the stalks and transferring to mesh onion bags for storage. Greentop onions were left in the soil undisturbed for one week, the stalks were cut and the onion bulb remained in the soil for an additional week, followed by harvesting by transferring to mesh onion bags for storage.

Table 2. Conventional and Greentop Finishing Practices

Finishing	Contaminated	Water Cessation	Curing	Storage

Practice	**Irrigation**			
Conventional	9 weeks	2 weeks	2 weeks	Remainder of study**
Greentop		1 week/1 week*	N/A	N/A

**For greentop finishing, the stalks were aseptically removed from the bulbs and remained undisturbed in the soil for the second week of water cessation. Greentop onions are sold directly to fresh market; therefore, the impact of storage was not evaluated for the greentop finishing practice.*
***There were a small number of storage samples and were scheduled for analysis after the completion deadline for this project. Therefore, the results from the storage samples have not been included in this report.*

Bacterial strain, culture conditions, and preparation of inocula

Generic *E. coli* strains (LJH-1247, LJH-1612, LJH-1613) and *Salmonella* spp. (LJH-614 - Montevideo, LJH-615 - Michigan, LJH-1262 - Saintpaul) were used in the inoculation cocktail (Table 3). The generic *E. coli* strains had been previously isolated by Trevor Suslow from lettuce, irrigation water, and soil from the Salinas Valley and were adapted to be rifampicin resistant by Linda Harris's laboratory at UC-Davis. *Salmonella* strains had been isolated from samples associated with foodborne outbreaks and had also been adapted to be resistant to rifampicin by the Harris laboratory.

Stock cultures were stored at -80°C in Tryptic Soy Broth (TSB; Neogen, Lansing, MI) with 40% glycerol. Frozen cultures of each stain were activated by transferring to TSB with incubation at 37°C for 24 hours. For each strain, 0.1 ml of overnight culture was spread onto three tryptic soy agar plates containing rifampicin (50 mg/L; Alfa Aesar, Ward Hill, MA; TSA+rif) plate and incubated at 37°C for 22-26 hours. Bacterial lawns were harvested by adding 3 ml of 0.1% peptone water and scraping with a disposable cell spreader. Cell suspensions for each strain were collected separately and transferred to individual sterile tubes. The cocktail was prepared by mixing 1 ml of each of the six strains and held at 4°C for up to 2 weeks. The stock cocktail solution was enumerated using standard serial dilution and spread plating techniques on Hektoen Enteric agar (Neogen) plates containing rifampicin (50 mg/L; HE+rif).

Table 3. Strains used in soil survival study.

Species	Strain Designation	Original Isolate Source*
Generic *E. coli*	2204R (LJH-1612)	Irrigation water from Salinas Valley
Generic *E. coli*	2205R (LJH-1247)	Lettuce from Salinas Valley
Generic *E. coli*	2206R (LJH-1613)	Soil sample from Salinas Valley
Salmonella Montevideo	1118R (LJH-614)	Human Isolate from Tomato Outbreak
Salmonella Michigan	1119R (LJH-615)	Cantaloupe
Salmonella Saintpaul	1120R (LJH-1262)	Human Isolate from Jalapeno Outbreak

*Rifampicin-resistant strains used in this study were developed from the original isolates by exposing cultures to increasing concentration of rifampicin. The Harris Laboratory (UC-Davis) performed this work and the resulting strains were kindly shared with the Waite-Cusic Laboratory for this project.

Inoculation of irrigation water

Well water from a private well in Philomath, OR was collected into 50-gallon drums and transported to Oregon State University. On irrigation days, water was pumped into 5-gallon carboys for transportation to the greenhouses. Contaminated irrigation water (10^4 CFU/ml) was prepared in the greenhouse by adding 10 ml of diluted stock cocktail solution (10^8 CFU/ml) into 5 gallons of well water. Water samples were collected and enumerated at each irrigation point using standard plating methods on HE+rif with incubation at 37°C for 24 hours.

Inoculation of soil and onions
Plants were watered with contaminated irrigation water at a rate of 200 ml/plant (10^6 CFU/sample) every three days from five weeks after planting (May 1st) through maturity (July 10th). Onion samples were collected immediately after watering by lifting the onions by the stalk and aseptically separating the bulb into a sterile Whirl-Pak bag (Nasco, Salida, CA). The entire soil contents of a single pot were transferred to a large sterile Whirl-Pak bag (Whirlpak). Onion and soil samples were transported back to the laboratory for enumeration. Five random samples from each treatment were analyzed at each timepoint.

Sample analysis – onions
Upon returning to the laboratory, an equivalent volume of 0.1% peptone water was added to the onion samples. Onions were washed by vigorously mixing by hand for approximately 20-30 seconds by massaging the surface of the onion with one hand and shaking the bag with the other. Serial dilutions of rinsate were prepared in 0.1% peptone water and plated onto HE+rif. Plates were enumerated following incubation at 37°C for 24 hours.

Sample analysis – soil
Upon returning to the laboratory, bulk soil samples were homogenized by shaking and turning bags end over end until visibly mixed. A 100 g subsample was transferred to a sterile filter sample bag and mixed with 100 ml of 0.1% peptone water and mixed by hand for 20-30 seconds. Serial dilutions were prepared in 0.1% peptone water and plated onto HE+rif. Plates were incubated as described previously.

When microbial counts from onion or soil samples fell below the detection limit for standard plating methods, a 96-well Most Probable Number (MPN) method was employed. Following rinsing or homogenization, the entirety of the liquid was transferred in 1 ml aliquots to 96 well deep well plates. An additional 1 ml of lactose broth containing rifampicin (50 mg/L; L+rif) was added to each well and the plate was incubated at 37°C for 24 hours. Following incubation, each well was spotted onto HE+rif plates using a 96 well tip comb. HE+rif plates were incubated at 37°C for 24 hours. Qualitative positive results for *E. coli* and *Salmonella* were treated as 1 MPN/ml of the rinsate and summed to create a total MPN/sample which was converted to an overall MPN/g.

Results:

Onion production
Onion sets were planted in late March and produced healthy leaves and flourished early in the greenhouse. The plants produced typical, healthy stalks with approximately 7 leaves per stalk before beginning to bulb (around day 60 after planting). Pictures of the growth of one of the onions (X-14) in muck soil up to the initial bulb development through lifting and curing are shown in Figure 1. Upon completion of the study, X-14 was one of the largest onions in the greenhouse. Onion X-14 finished out at 154 g after conventional finishing. Despite healthy stalk production and initial bulb development in the vast majority of the onions, the mature bulbs were very small (average of 75 g) at the time of full maturity and were highly variable in size. The variability of onion bulb size throughout the course of the study is shown in Figure 2. On average, onions grown in muck soil were larger in size compared to those grown in the silt loam soil. Regardless of the variability in size, the overwhelming majority of the onions did produce robust healthy stalks, enlarged bulbs, drooped tops at maturity, and displayed characteristic outer skin development during curing. The thermostats in both greenhouses were set at identical conditions; however, average high temperatures in the large greenhouse (93.5°F) were consistently higher than the small

greenhouse (78.8°F)(Figure 3). At the point of initial contamination (Day 40), the large greenhouse also experienced one day of exceedingly high temperatures (128°F) due to vandalism of the cooling system. It is likely that this significant temperature shock along with the consistently higher temperatures caused some damage to the plants and could explain the decreased bulb size for plants in the large greenhouse.

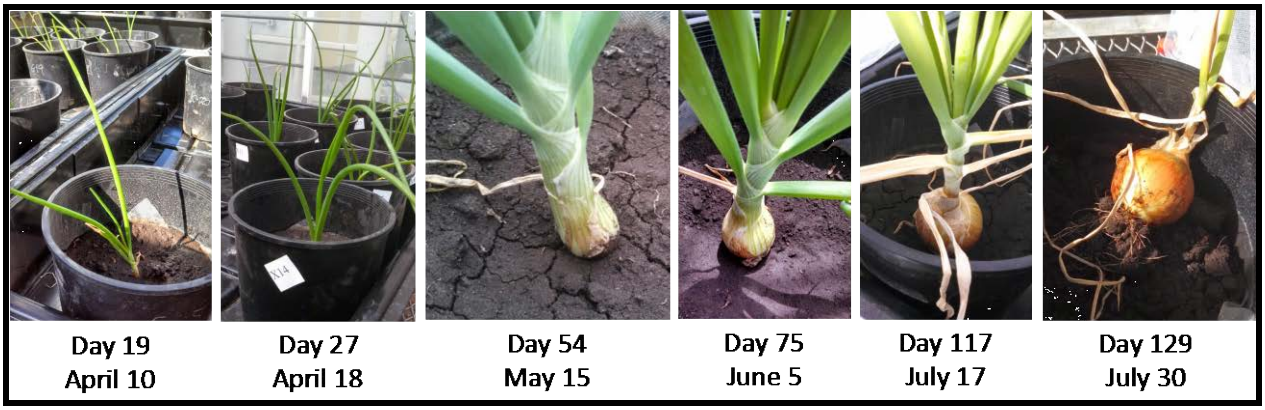

Figure 1. Pictures of the growth of a yellow dry bulb onion (X-14) in muck soil through stalk growth, bulb development, water cessation, and curing.

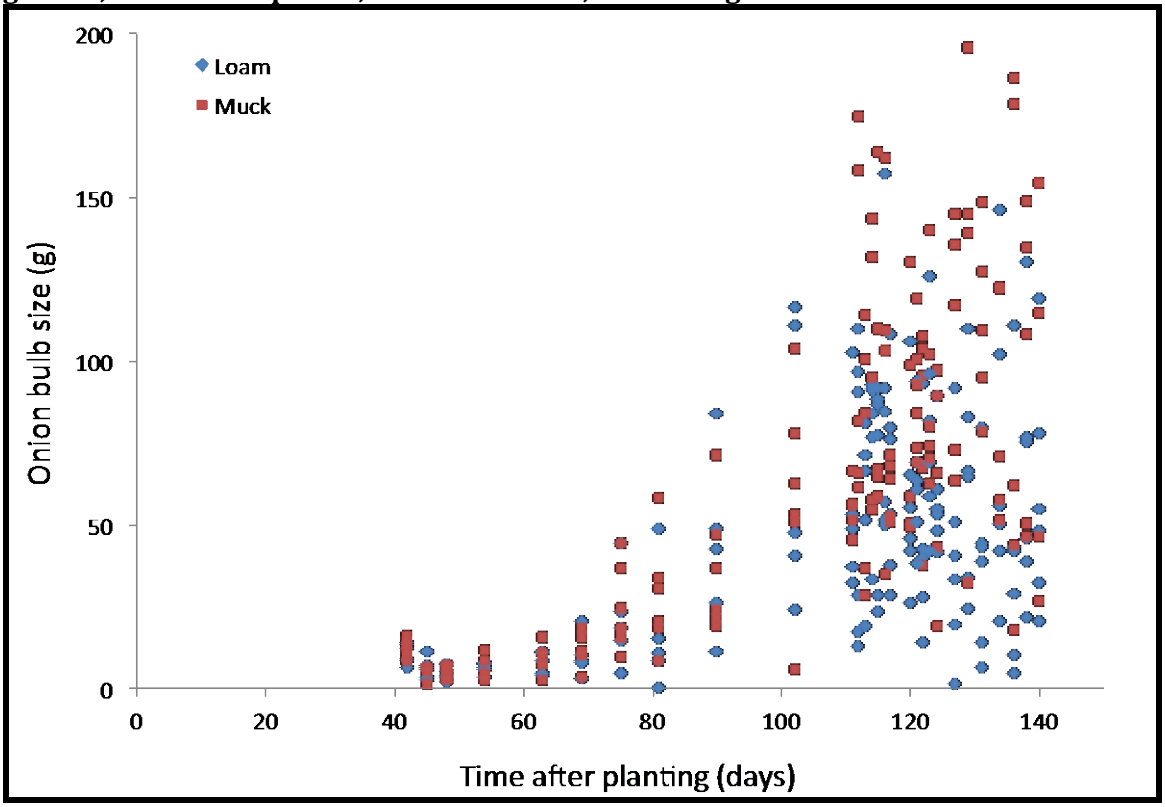

Figure 2. Variability in onion bulb size throughout growth and finishing.

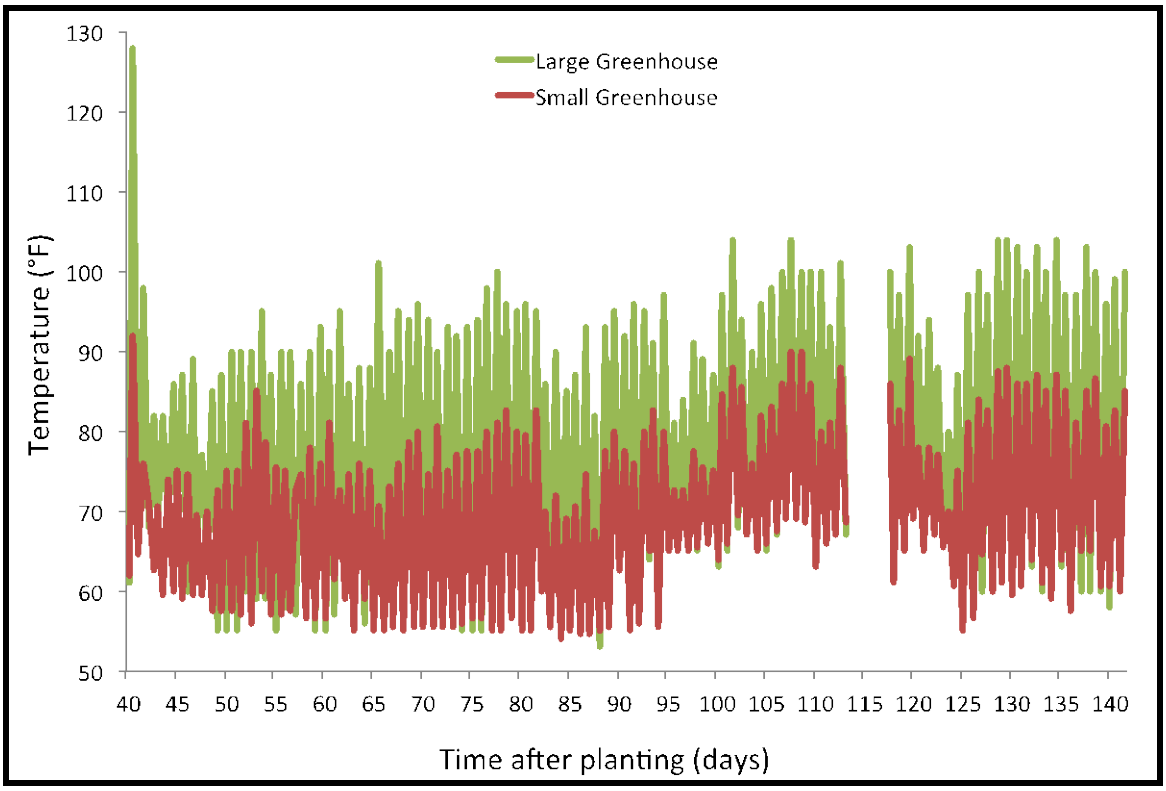

Figure 3. Temperature date for irrigation and finishing stages in the two greenhouses.

Onion and soil contamination following bulb production.
Onions and soil were repeatedly contaminated every 3 days with generic *E. coli* and *Salmonella* spp. via contaminated well water (10^6 CFU/ml). An aliquot of 200 ml of contaminated water was poured into each pot to moisten all soil around the plant. Application by this method led to direct contact of the irrigation water with the onion bulb, and as expected, relatively high levels of soil and onion contamination (Table 4). Typical production practices using furrow or drip irrigation systems would prevent or minimize this direct contact with potentially contaminated water. While this approach was not directly applicable to commercial irrigation systems, this method of inoculation did provide a very liberal estimate of contamination from irrigation water with exceedingly high levels of microorganisms and allowed for quantifiable reductions during water cessation and curing. It is likely that generic *E. coli* and *Salmonella* spp. levels were reduced during the period between each watering (2-3 days); however, this reduction was not quantified during the watering period. As expected, generic *E. coli* and *Salmonella* spp. did not grow in either soil type or on the onions throughout the course of the study.

Reduction of generic *E. coli* and *Salmonella* with conventional finishing
Onions were determined to have reached full maturity when approximately half of the onion stalked had fallen over. Full maturity for this study was 117 days post planting (July 11th). To mimic conventional finishing practices, the onions remained undisturbed in the ground without further irrigation for two weeks. After the initial two-week period, the onions were then lifted from the soil and allowed to cure on the surface of the soil for an additional two weeks to encourage outer skin development and stalk dehydration. After curing was completed, the stalks were aseptically removed and bulbs were transferred into individual onion bags for storage. Survival of generic *E. coli* and *Salmonella* spp. in soil and on onions grown and finishing in muck and loam soil are shown in Figure 4. At the last irrigation time point (day 111), average generic *E. coli* and

Salmonella levels were between 3.2 and 3.6 log CFU/g for all onion and soil samples. During the first week of water cessation, survivors were reduced to average of between 1.2 and 2.1 log CFU/g of onion and soil. After the second week of water cessation, all sample types averaged below 0.0 log CFU/g. On day 124, conventional onions were lifted to the surface of the soil for an additional two weeks of curing. During curing (2 weeks) and storage (2 weeks), generic *E. coli* and *Salmonella* spp. levels remained relatively stable at low levels in soil and on onions (<1 MPN/g; storage data not shown).

Survival of generic *E. coli* and *Salmonella* were comparable at all time points in all samples, indicating that these generic *E. coli* strains would provide an accurate prediction of the survival of *Salmonella* spp. in soils and on onions for future field trials.

Table 4. Microbial loads (log CFU/g and log CFU/sample; mean ± standard deviation) of water, soil, and onions immediately after irrigation with contaminated water.

Soil Type	Microbial Load in Irrigation Water		Microbial Load in Soil After Irrigation		Microbial Load on Onion After Irrigation		Units
	Generic *E. coli*	*Salmonella*	Generic *E. coli*	*Salmonella*	Generic *E. coli*	*Salmonella*	
Muck	4.57 ± 0.37 (n = 33)	4.54 ± 0.35 (n = 33)	3.58 ± 0.37 (n = 55)	3.72 ± 0.36 (n = 55)	3.64 ± 0.59 (n = 52)	3.75 ± 0.60 (n = 52)	Log CFU/g
Silt Loam			3.67 ± 0.26 (n = 52)	3.80 ± 0.39 (n = 52)	3.42 ± 0.43 (n = 54)	3.42 ± 0.34 (n = 53)	
Muck	6.87 ± 0.37 (n = 33)	6.85 ± 0.35 (n = 33)	7.23 ± 0.38 (n = 55)	7.08 ± 0.37 (n = 55)	4.74 ± 0.31 (n = 52)	4.82 ± 0.26 (n = 52)	Log CFU/sample*
Silt Loam			7.23 ± 0.26 (n = 52)	7.36 ± 0.39 (n = 52)	4.58 ± 0.35 (n = 54)	4.63 ± 0.36 (n = 53)	

**For water, sample indicates 200 ml/pot/irrigation. For soil, sample indicates 3500 g. For onion, sample indicates bulb.*

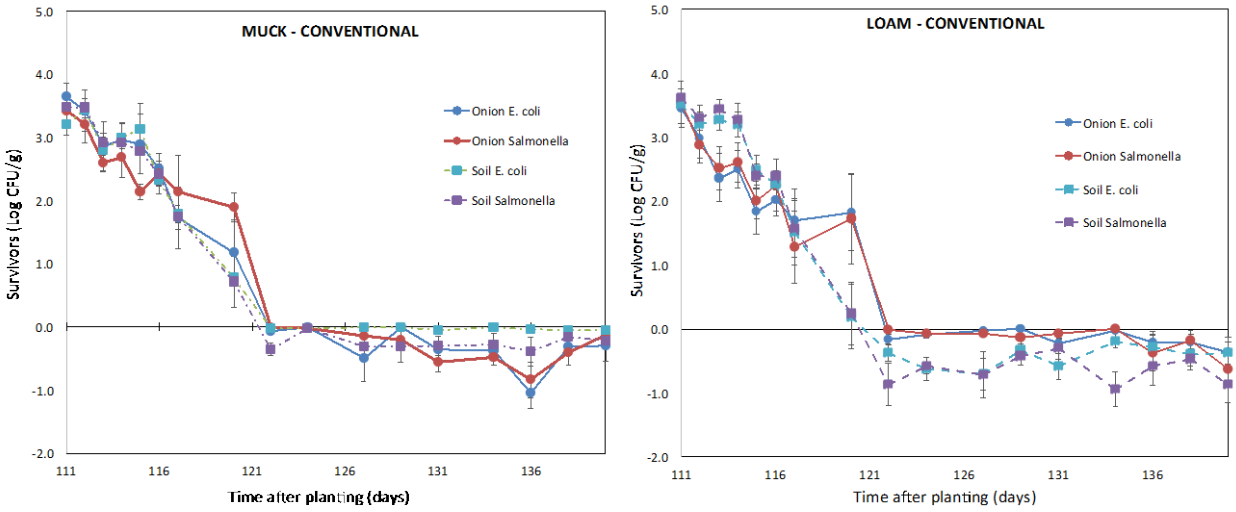

Figure 4. Survival of generic *E. coli* and *Salmonella* spp. in soil and on onions finished with conventional practices.

Comparison of greentop finishing practices to conventional finishing practices

Greentop onions were grown and irrigated in an identical manner to the conventional onions until after the first week of water cessation. After the first week of water cessation, the stalks were aseptically removed from the onions and the bulbs remained in the soil undisturbed for an additional week. After this week, the bulbs were harvested and transferred to individual onion bags for storage. The exploration of the differences in these finishing practices focused on potential differences during this second week of water cessation, specifically on days 80-85. Reduction of both generic *E. coli* and *Salmonella* was rapid during the first two weeks of water cessation. This was unaffected by the presence or absence of the onion stalks (data not shown). It is possible that abbreviated greentop practices could be a potential area to evaluate the potential risk for minimal finishing practices.

Acknowledgments
This research project was completed only because of a large number of dedicated researchers and through collective interaction with a variety of contacts in the onion industry. Realization of the project, including planning and acquisition of appropriate strains and determination of suitable inoculation levels, was only accomplished by having the opportunity to be mentored by Dr. Linda Harris at UC-Davis. Dr. Clint Shock and additional employees of OSU's Malheur County Experiment Station were gracious to supply us with ample volumes of silt loam soil from the station farms. Joe Waite was kind enough to transport the soil from Treasure Valley to main campus for experiments. Greg Bennett of NW Onion provided muck soil from his commercial onion farm in the Willamette Valley. Jim Ervin and Gloria O'Brien of OSU's Greenhouse Team assisted with everything related to the greenhouse, including advising on proper growth conditions and handling many problems that arose during the course of the study. The OSU Biosafety Committee, specifically Matt Philpott and Luis Bermudez, were instrumental in assisting us with developing suitable procedures for irrigation and decontamination procedures that would satisfy university requirements and provide suitable protection to laboratory personnel and other non-laboratory personnel and the environment. Many current and former members of the Waite-Cusic research group poured their sweat into this project to make it a success: Alex Emch, Chris Letchworth, Claire Oslund, Sam Mertz, Whitney Nielsen. Additional people assisted with the monumental task of soil preparation for the greenhouse studies; Amy Emch, Joey Cusic, Robin Frojen deserve a huge thank you for supporting their spouses and friends with sweat equity.

Outcomes and Accomplishments

This greenhouse study was designed to provide an opportunity to compare the survival of an indicator (generic *E. coli*) to the target pathogen (*Salmonella* spp.) from contaminated irrigation water to soil and dry bulb onions using industry-relevant finishing practices. Growth conditions and plant health/crop yield were not ideal. Similarly, irrigation was accomplished by vary crude means that did not simulate industry practices. Despite these drawbacks and limitations, this study demonstrated that generic *E. coli* and *Salmonella* spp. exhibited very similar response to water cessation, curing, and storage in two soil types and on the surface of the onion bulb. This information provides substantial evidence that the use of generic *E. coli* in future field trials would serve as a suitable predictor for the behavior of *Salmonella* in these systems.

Summary of Findings and Recommendations

Generic *E. coli* and *Salmonella* spp. levels on onions and in soil were significantly reduced by conventional finishing process used in this study. During the first two weeks of water cessation, generic *E. coli* and *Salmonella* spp. counts were reduced by 3.22 to 3.66 log CFU/g in muck soil and onion samples and by 3.69 to 4.19 log CFU/g in silt loam soil and onion samples. Generic *E. coli* and *Salmonella* spp. levels remained stable at low levels (<1 MPN/g) throughout the 2-week curing and storage periods. The greentop storage practices tested in this study did not demonstrate any differences in potential risk; however, abbreviated water cessation practices for greentop harvest should be evaluated. While levels of generic *E. coli* and *Salmonella* spp. were not completely eliminated through curing and storage, the >3.2 log CFU/g reduction in combination with the use of "indirect" irrigation practices (furrow or drip) should efficiently mitigate any risk associated with lower levels of natural *E. coli* in irrigation water.

Survival of generic *E. coli* and *Salmonella* were comparable at all time points in all samples, indicating that these generic *E. coli* strains would provide an accurate prediction of the survival of *Salmonella* spp. in soils and on onions for future field trials. There was no difference in the survival of these organisms in the two different soil types (muck and silt loam).

Literature Citations

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APPENDICES

Publications and Presentations (required)

No publications at the current time. One publication is in preparation and expected to be submitted by November 2014.

Budget Summary (required)

Brief narrative breakdown of how the grant funds were spent and comment on whether you had the necessary funds to fully implement this project.

Budget Category	Amount
Salaries	$17,976.63
OPE (Fringe & Tuition)	$15,932.62
Travel	$2,707.14
Materials and Supplies	$13,781.10
Greenhouse rental	$1,211.64
F&A (formerly indirect)	$16,450.98
Total	**$48,060.11**

Tables and Figures (optional)

Raw data, calculations, graphs, and other quantitative materials that were part of the research, but would be distracting in the report itself.

Suggestions to CPS (optional)