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Foodborne outbreaks involving fresh produce have been on the rise since the late 1990's. Pathogens such as *Salmonella* spp. and *Escherichia coli* are prevalent in agricultural environments and commonly travel between farms via irrigation water. The Food Safety Modernization Act (FSMA) has placed an increased emphasis on microbiological standards of irrigation water and fresh produce. The Produce Rule is a defined portion of FSMA that labels all ready-to-eat produce as covered under the rule and requires producers to comply with FDA's proposed water standards for a relative level of public safety. If producers can demonstrate a post-irrigation mitigation strategy to reduce microbial concentrations and meet FDA water standards, they will be able use of their water source even if it initially does not meet the microbiological criteria. The purpose of this study was to determine if the conventional finishing process for dry bulb onions is able to effectively reduce generic *E. coli* and *Salmonella* spp. to the FDA's standards. A secondary objective was to evaluate the contribution of soil type on the hypothetical irrigation-to-harvest intervals for root crops.

The first study investigated the effectiveness of the conventional curing process to reduce microbial levels on dry bulb onions grown with contaminated irrigation water. Spanish yellow dry bulb onions were grown in Owyhee silt loam and Semiahmoo muck soils in containers in two greenhouses and irrigated with contaminated water containing a cocktail of rifampicin-resistant generic *E. coli* and *Salmonella* spp. (4.80 log CFU/ml) every 2-3 days from day 40 to day 111. At maturity, irrigation was stopped for 12 days, lifted, and then followed by 16 days of curing. Onion and soil samples were collected, rinsed, and massaged with 0.1% peptone water (1:1). Serial dilutions of the rinsate were plated onto Hektoen Enteric (HE) Agar and selectively enumerated following incubation (37°C, 24 hours). As microbial levels decreased, a most-probable-number (MPN) was used in lieu of plating. The irrigation period resulted in a final contamination level of 3.69±0.34 log CFU/g onion of both *Salmonella* spp. and generic *E. coli*. 12 days after ceasing irrigation, generic *E. coli* and *Salmonella* spp. were reduced to <10 CFU/g. *E. coli* and *Salmonella* spp. levels were stable throughout the remainder of the curing process.

The second study analyzed differences in the survival of generic *E. coli* and *Salmonella* spp. in different agricultural soils around Oregon. Six soil types, Quatama loam, Latourell loam, Willamette loam, Adkins loam, Madras loam, and Cullius loam, were transported from OSU experiment stations to OSU campus greenhouses. Soils were irrigated with well water containing generic *E. coli* and *Salmonella* spp. (4.76 log CFU/ml) in the greenhouses used for the previous study. Sample collection and analysis matched those of the previous study. Generic *E. coli* and *Salmonella* spp. had the weakest survival and persistence in Quatama, Latourell, and Willamette loam. Madras, Adkins, and Cullius loams harbored generic *E. coli* and *Salmonella* spp. for extended periods (>80 d).

The first study verified that conventional curing practices mitigate the risk posed by contaminated irrigation water during onion production. 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. Based on similar survival curves, use of generic *E. coli* in future field trials would serve as a suitable predictor for the behavior of *Salmonella* spp. in these systems. The second study found a significant difference between the six soils in the microbial die-off of generic *E. coli* and *Salmonella* spp. We also observed that the rates of die-off for generic *E. coli* and *Salmonella* spp. were quite similar; however, the persistence between the two was significantly different in Quatama and Willamette loam soils.

Mitigation of Microbiological Water Quality by Last Irrigation-to-Harvest Intervals

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Alexander W. Emch, Author

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1. INTRODUCTION

Irrigation of produce can be a complicated issue in regards to availability, cost, and microbiological cleanliness. Many producers rely on irrigation systems that transport water from high moister areas to regions that have scarce amounts of agricultural-use water. The application of this water onto produce is usually through a crude design of open canals that lead to and from agricultural sectors. One of the most common vehicles for pathogens to reach produce is irrigation water. Direct or indirect application of contaminated irrigation water can lead to contaminated produce, which is a significant issue in crops that are irrigated right up to harvest. One of the most prominent produce industries in Oregon that make use of long-distanced irrigation canals is the dry bulb onions industry.

Dry bulb onions are a type of leafy vegetable that are either eaten raw or cooked by consumers. The Pacific North West supplies the majority of onions grown in the U.S. due to prime environmental conditions for onion growth, mostly in the Treasure Valley region shared between Oregon and Idaho. Under the Food Safety Modernization Act (FMSA) they are classified as covered produce, eaten raw. Onions have never been associated with a foodborne illness outbreak in literature, but under the new FSMA law onions must cater to strict irrigation water standards to ensure a minimally contaminated product.

Treasure Valley became an agricultural powerhouse through a multistate effort to create an irrigation system that supplied water to the arid high desert where produce is cultivated. The irrigation system that was installed makes use of a reclaim and reuse method by allowing the water between farms to be recycled and sent further down the canals. This reclamation of the irrigation water leads to high levels of fecal coliforms, such as generic *E. coli,* in the water. Conventional water testing typically uses generic *E. coli* and other coliforms as indicators for the possible presence of human pathogens. Under FSMA, the current concentration of fecal coliforms in the irrigation water used by many onion producers is far too high. There is currently no recognized critical control point that onion producers use to mitigate microbe growth/persistence, leaving the industry in jeopardy of losing their water source.

Many producers are appealing the state of their irrigation water, arguing that the curing methods used before harvest of produce should greatly reduce the microbial loads. Testing whether this curing method will reduce a microbial population inoculated on dry bulb onions would give reason to allow Treasure Valley growers to continue using their irrigation system without continuous testing and treatment of it. This research will attempt to validate whether the harvesting methods in dry bulb onion production are able to reduce a high microbial load to levels that the FSMA produce rule dictates for proper food safety. This research will demonstrate whether generic *E. coli* can be used as a viable surrogate for *Salmonella* to be used in future field trails. Results from the studies will also

demonstrate whether different soil types play an important role in microbial mitigation strategies.

2. LITERATURE REVIEW

2.1 Major bacterial pathogens involved in the production of produce with irrigation water

2.1.1 *Escherichia coli* O157:H7

Escherichia coli is a gram negative bacillus bacterium that is a generic resident of human and animal gastrointestinal tracts. There are multiple types of pathogenic *E. coli* that are listed as Enterohemorrhagic *E. coli* (EHEC), Enteroaggregative *E. coli* (EAEC), Enterotoxigenic *E. coli* (ETEC), and Enteropathogenic *E. coli* (EPEC) (CDC 2014). Some strains of pathogenic *E. coli* have to ability to produce Shiga toxin and are labeled as Shiga-toxin producing *E. coli* (STEC). One of the most conserved abilities of the pathogenic *E. coli* strains is their ability to colonize the intestinal mucosal surface despite peristalsis and competition for nutrients by the indigenous flora of the gut (Nataro & Kaper, 1998). *E. coli* is also quite hardy in the outside environment. Many studies have evaluated the survival of *E. coli* in a number of different environments including freshwater, soils, and stockpiled and field manures (Korhonen & Martikainen, 1991; Lau & Ingham, 2001; Maule, 2000; Mubiru, Coyne, & Grove, 2000; Zhai, Coyne, & Barnhisel, 1995). *E. coli* does not typically thrive once it is shed from the digestive tract, but it can remain viable for months in manure, can grow in soils under the right conditions, and can be sequestered and proliferate in soil protozoa (Barker, Humphrey, & Brown, 1999; Gagliardi & Karns, 2000; Jones, 1999; Kudva, Blanch, & Hovde, 1998; Solo-Gabriele, Wolfert, Desmarais, & Palmer, 2000). Outbreaks involving Shiga toxin-producing *E. coli* O157:H7 have been linked to beef more than any other

product but many severe outbreaks have also been traced back to contaminated sprouts and pre-packaged spinach (Berger et al., 2010).

2.1.2 *Salmonella*

Salmonella is a gram-negative bacillus that is commonly found in the gastrointestinal tract of humans and animals (Centers for Disease Control and Prevention, n.d.). *Salmonella* itself is a very hardy organism, able to survive in the environment for a significant amount of time before locating a proper host. *Salmonella* spp. are skilled at adapting to extracellular pH levels down to 3.99 and up to 9.5, salt concentrations up to 4% w v−1 NaCl, and temperatures as high as 54 °C or low as 2 °C (Spector & Kenyon, 2012). *Salmonella* strains can persist in the environment for years, enduring phases of stress and nutrient depletion (Parker, 2010). Previous studies have shown that *Salmonella* are able to survive drying when placed on a surface through droplets (La Ragione, Coles, Jørgensen, Humphrey, & Woodward, 2001).

Salmonella enterica, one of the most prevalent species, is the most common reported cause of bacterial food-borne illness in the United States (Centers for Disease Control and Prevention, 2010; Scallan et al., 2011). *Salmonella* can persist in the farm environment for prolonged periods due to circulation within the farm between different reservoirs such as animals, excrement, soil, and plants (Jacobsen & Bech, 2012). There are many outbreaks associated with the consumption of animal products, but many outbreaks are traced back to contaminated produce (Berger et al., 2010). In a review of outbreaks of foodborne illness

in the U.S., *Salmonella* was the most commonly reported bacterial pathogen, accounting for approximately half of the outbreaks due to bacteria (Sivapalasingam, Friedman, Cohen, & Tauxe, 2004).

2.2 Food Safety Modernization Act

The Food Safety and Modernization Act is a fairly new act that was signed into law January 4, 2011. Its purpose is to ensure the U.S. food supply is safe for consumption by shifting the focus on outbreak response to outbreak prevention. There are seven rules that the FDA has proposed under FSMA that address food safety issues. One of the most affected food commodities by one of the rules, the produce rule, is raw produce grown with irrigation water, defined as "covered produce" (Center for Food Safety and Applied Nutrition, 2015). When agricultural water is used for covered produce (other than sprouts) using a direct water application method, the microbial quality of the water must be tested using an appropriate analytical method. Generic *E. coli* is the microbial target for testing due to it being ubiquitous in the intestines of livestock and humans; it is a coliform that is typically used as an indicator for fecal contamination (FSIS, 2015). The proposed rules state that if the water is more than 235 colony forming units (CFU) (or most probable number (MPN)) generic *E. coli* per 100 ml for any single sample or a rolling geometric mean (n=5) of more than 126 CFU (or MPN) per 100 ml of water, the water is immediately discontinued for use from the original source and/or its distribution system. The FDA advises follow-up actions

to poor water quality including making changes to the system and re-testing, or treating the water (Center for Food Safety and Applied Nutrition, 2015).

Recently FDA has revised its proposed rule based on evidence found in field trials involving contaminated produce. The FDA review found that a minimum decay rate of 0.5 $\log_{10}$ CFU/g per day had been met by the majority of the studies involving covered produce (Snellman, Marianne, Ravaliya, & Assar, 2014). The FDA is now allowing a standard reduction due to microbial die-off in the field before harvest and consideration of additional die-off from activities such as storage or commercial washing. Producers concerned about water quality can now determine a time interval between the last irrigation and harvest to decrease the initial microbial load to a statistical threshold value (STV) of 410 or less CFU of generic *E. coli* per 100 mL of water or a geometric mean (GM) of 126 or less CFU of generic *E. coli* per 100 mL of water.

2.3 Dry Bulb Onions

2.3.1 General Overview

Approximately 150,000 acres of onions are produced each year in the U.S. for a sales value of approximately 1 billion dollars (United States Department of Agriculture, 2014). The United States is responsible for producing approximately 4% of the world's annual onion supply. Around half (47%) of onions grown in the U.S. are produced in the Pacific Northwest (PNW; Washington, Oregon, and Idaho) (National Onion Association, 2011). Oregon was able to produce 10,900 acres of summer storage onion in Malheur county in

2013 (United States Department of Agriculture, 2014). The primary onion production area in Oregon is within a radius of 30 miles (50 km) of Ontario on the Snake River plain and along the tributaries of the Snake River, a region frequently referred to as the Treasure Valley. In general, onion bulb yield in the Treasure Valley is greater than in any other onion-producing area. The majority of onions in the Treasure Valley region of Oregon and Idaho are furrow irrigated with approximately 22,000 acres of Sweet Spanish onions produced annually (Shock, Feibert, & Saunders, 2000). The United States bulb onion production areas consist of fall planted (spring harvested) and spring planted (summer harvested) regions (Shock, Ishida, Eldredge, & Seddigh, 2000). Two types of onions, dry bulb (storage) onions and non-storage (mostly Walla Walla variety) are produced in the PNW, the majority (98%) being storage onions in 2011 (Schwartz, 2013). Most 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 (DeFrancesco, 2004). The onions grown in the Pacific Northwest are mostly long-day cultivars and are marketed from August to April from storage (Shock, Ishida, et al., 2000).

2.3.2 Growth and Production

Onions are leafy monocots that can be grown from seed or small bulb starters in a variety of agricultural soils. After germination of the seed, the onion grows to produce upright leaves/stalks. Onions have a long period of slow growth for their leaves, continuously

growing for 50 to 70 days after planting (Sullivan et al., 2001). Onions have shallow, sparsely branched roots with the majority of the roots in the top foot of soil (Sullivan et al., 2001). Shallow-rooted crops such as onions are generally more difficult to irrigate and are typically furrow irrigated to mediate lower irrigation efficiency values (Al-Jamal, Ball, & Sammis, 2001).

Each layer of an onion is called a bulb scale in botanical terminology and is the base of a leaf (Sullivan et al., 2001). The bulb of the onion will form once there is an appropriate amount of daylight (Marianne, 2013). The bulb itself consists of fleshy leaves that form approximately 70-90 days after planting and continues to grow in size throughout the growing season (Sullivan et al., 2001). Dry bulb onions take about three to four months to fully mature when started from starter sets (Marianne, 2013). Onions are seen as fully mature and ready to harvest once their stalks have yellowed and begin to fall over (Peaceful Valley Farm and Garden Supply, 2014). In conventional harvesting practices, irrigation is stopped and the onions remain in the field without water for 10-14 days while the stalks dry out. Onion roots are then undercut and the bulb is lifted to the soil surface to cure on top of the soil for an additional 10-14 days. During this period, the external scale of the onion dries out and forms a hardened shell. Following curing, the dried stalks are cut from the bulb and the onions are stored in a circulated shed for about 6 months before being distributed to the market.

2.3.3 Microbial issues with irrigation water

The concern over onion producers meeting the FSMA standards for irrigation water comes from the high microbial loads found in the irrigation water used in the Treasure Valley region. Microbial levels typically grow higher as the growing season ends and harvest begins, giving cause for concern for human pathogens being present in produce. The irrigation network that the Treasure Valley growers depend on utilizes a system of reclamation and reuse of irrigation water. As water is distributed down the valley, the water's coliform levels begin to rise (Shock, Pinto, Laubacher, Ryan, & Mahony, 2013). Studies have found that the most common sources for contamination in farm-to-table production stem from irrigation water, runoff water from livestock farms, manure, wash water, animal fertilizers, and wildlife (Beuchat & Ryu, 1997; Tauxe et al., 1997). There have been many studies testing the duration of time that foodborne pathogens are able to survive in different agricultural environments. This field of research is quite important in understanding how pathogens such as *E. coli* O157:H7 and *Salmonella* spp. cope with stresses in the environment. The research involving pathogenic durability in the environment stems from the spread of foodborne outbreaks that are assumed preventable. One study that looked at Centers for Disease Control (CDC) outbreak data from 1998-2008 concluded that *Salmonella* spp. infections were transmitted primarily by tomatoes, juice, mangoes, sprouts, and peppers (Painter et al., 2013). The majority of these products fall under the covered ready to eat produce defined by FSMA. The largest outbreak of *Salmonella* in the U.S. in 2008 found that the food most commonly associated with illness were raw tomatoes (Barton Behravesh et al., 2011). The authors reporting on the outbreak

concluded that rapid detection is difficult because many *Salmonella* illnesses are not confirmed by culture and it is likely that many more occurred than were identified.

2.4 Pathogen persistence in irrigation water

Many studies have produced data demonstrating how different microbial loads in irrigation systems are able to survive in the system and then contact and persist on produce during the final stages of irrigation. A review of irrigation systems found positive associations between *Salmonella* frequencies and rainfall events observed in many studies, indicating that surface runoff plays a main role in introducing *Salmonella* into reservoirs of water used in agriculture (Levantesi et al., 2012). The range of contaminants found in fresh produce varies across samples collected from irrigation sites from different irrigation systems. It is vital to understand the role of reservoirs that harbor pathogens which end up in irrigation systems. Analysis of isolated serovars of *Salmonella* consistently showed a mixed human and animal origin of *Salmonella* in surface water environments (Levantesi et al., 2012). This analysis links the role of wild life animals in water contamination along with the more accepted livestock and human sources (Levantesi et al., 2012).

One study analyzed the origins of *Salmonella* isolates in California and found that most of the strains in the study were isolated from water sources, most being from streams, rivers, and creeks in the region (Gorski et al., 2011). A survey of surface waters in a region of Georgia associated with a high incidence of salmonellosis cases had a corresponding high incidence of *Salmonella* in samples collected from the surrounding watershed—79% of the

samples collected (Haley, Cole, & Lipp, 2009). Another survey in North Carolina found that 54.7 % of samples collected from surface waters were positive for *Salmonella* (Patchanee, Molla, White, Line, & Gebreyes, 2010). Wildlife and water reservoirs often share isolates for *Salmonella* such as *S.* Give, *S.* Infantis, and *S.* Typhimurium indicating that these strains survive and transport between these two environments (Gorski et al., 2011).

The availability of water plays a key role in the survival of pathogens in a food production system. Shock et al. found that *E. coli* on irrigated onion bulbs decreased from an MPN of 4033 to 611 by the end of water cessation and onion drying/curing, from August 27 to October 1 (Shock et al., 2013). A number of studies have also spent time observing the role that irrigation plays in distributing bacteria to areas other than just directly on plant surface. Irrigation water contaminated with *Salmonella* spp. not only acts as a vehicle for transferring the pathogen to the surface of produce, but the irrigation water will most likely contaminate soil and root vegetables (Fatica & Schneider, 2011). This implies that indirect application of contaminated irrigation water reaching produce through soil and their roots could lead to contamination of the crop.

The type of canal system used to distribute irrigation water also has an impact on the quality of water. Duffy et al found that water samples collected from cemented irrigation canals in Texas were 6% positive for *E. coli* while sample collected from dirt irrigation canals were 50% *E. coli* positive (Duffy et al., 2005). The study also found that one hundred percent of irrigation water samples collected from wells were contaminated with *E. coli* (Duffy et al., 2005). Benjamin et al. found that mean generic *E. coli* concentrations in

sediments in canal beds were much higher than in the overlying water source in canals and streams (Benjamin et al., 2013).

2.5 Pathogen persistence on plant phyllosphere

Many studies are finding that although enteropathogenic bacteria are not as durable as normal plant flora, they have the ability to persist and possibly grow on crop plants (Barak & Liang, 2008; Brandl & Sundin, 2013; M C Erickson et al., 2013). A plant's phyllosphere can actually harbor and increase the environmental durability of microorganisms. It is suggested that if a pathogen can persist in a product's phyllosphere, then there is a greater chance for a higher microbial load on the produce once it reaches the consumer (Heaton & Jones, 2008). A study in Uppsala, Sweden found that *Salmonella* Weltevreden travelled from contaminated soil to the phyllosphere of spinach plants and persisted for 21 days with minimal reductions in cell numbers after being inoculated at a level of 10^6 CFU/g soil (Arthurson, Sessitsch, & Jäderlund, 2011). Another study found that *S.* enterica was able to attach to and contaminate the phyllosphere and rhizosphere of tomato plants through soil following contaminated irrigation (Barak & Liang, 2008).

Several other studies have demonstrated the effect of plant phyllospheres on survivability rates of bacteria. In a study involving direct application to spinach, leaves *E. coli* O157:H7 strains with initial concentrations at 5 log CFU/200 g soil declined over time but persisted through the 28-day study (Patel, Millner, Nou, & Sharma, 2010). Alternately, there have

been instances of rapid death rates of pathogens on produce. A UK study found that *E. coli* O157:H7 and *Salmonella* Enteritidis had fallen below the detection limit on lettuce and spinach after 1 week in low inoculum (log 3) and high inoculum (log 5) samples (M. L. Hutchison, Avery, & Monaghan, 2008). *Salmonella* was able to survive for a longer period of time in soil samples—three weeks after the irrigation of the crops (M. L. Hutchison et al., 2008). Many of the authors in these studies hypothesize that bacterial survivors on plant phyllosphere were able to adapt to the hazardous conditions and facilitate persistence. Kroupitski et al, 2013 discovered that under stress, *S. enterica* is able to adapt and express genes which facilitate its survival on plant surfaces. The misL gene was one of several *S. enterica* genes induced during cold storage of lettuce which expressed key factors supporting attachment to lettuce leaf tissue (Kroupitski et al., 2013). One study found that moisture content is a critical factor in the survival of *E. coli* O157:H7 and *Salmonella* spp. on butterhead lettuce by comparing growth in a high stable humidity growth chamber vs a fluctuating greenhouse (Van der Linden et al., 2013). In regards to mature dry bulb onions, bulb chemistry has been found to inhibit most human pathogenic organisms (Block, 1985; Elnima, Ahmed, Mekkawi, & Mossa, 1983; Griffiths, Trueman, Crowther, Thomas, & Smith, 2002; Indu, Hatha, Abirosh, Harsha, & Vivekanandan, 2006; Islam, Doyle, Phatak, Millner, & Jiang, 2005; Johnson & Vaughn, 1969; Kyung & Lee, 2001; Srinivasan, Nathan, Suresh, & Lakshmana Perumalsamy, 2001; Ye, Dai, & Hu, 2013; Yin & Tsao, 1999; Zohri, Abdel-Gawad, & Saber, 1995).

There may be instances where a pathogen will create a symbiotic relationship with normal plant flora to increase survivability on the plant. One experiment found that *Salmonella*

enterica has the ability to create a biofilm when it grows in close proximity to another bacterium, *Pantoea agglomerans*, on the leaf surface of cilantro (Brandl & Mandrell, 2002). Another study found that survival of *S. enterica* on lettuce and cilantro leaves appears to be strongly dependent on the presence of common epiphytic bacteria which protect such incoming bacteria from stresses such as desiccation on leaf surfaces (Poza-carrion, Suslow, & Lindow, 2013). After observing bacterial survival in soil, Erickson et al. speculated that even *Salmonella* and *E. coli* O157:H7 may have overlapping stress resistances and through this they may exhibit enhanced survival (M C Erickson et al., 2013).

Pathogens have the ability to colonize the internal layers of produce, creating more difficulty in sanitizing produce for human consumption. High numbers of bacteria in the rhizosphere of produce have been found to increase the risk of internal plant contamination via roots (Klerks, Franz, van Gent-Pelzer, Zijlstra, & van Bruggen, 2007). One study by Ge et al. found that green onions had an internalization level 3.49 logs higher under drought conditions than optimal irrigation conditions when initially contaminated with 7 log CFU/g of *Salmonella*. The study demonstrated that the internalization of *Salmonella* is more likely to occur in the roots of green onions rather than the leafy sections (Ge, Lee, & Lee, 2012). One study observed that *E. coli* O157:H7 inoculated onto the leaves of spinach plants were able internalize themselves within the tissue and survive for up to 14 days (Mitra et al., 2009). *E. coli* inoculated onto green onion leaf tissue in the fall in a Georgian study at 1,000,000 CFU/100ml was recovered up to 74 days after inoculation (Islam, Doyle, Phatak, Millner, & Jiang, 2004). Depending on the amount of bacteria

internalized into the tissue, this internalization could cause problems in regards to sanitizers and curing methods for produce. Another study speculated that colonization of spinach and lettuce roots by *E. coli* occurs due to the pathogen exploiting natural openings such as growing lateral roots (Wright et al., 2013). However, the study also found that only a small percentage (~0.5%) of the starting bacteria were internalized within the produce roots.

Root samples of green onions have been shown to be more susceptible to internal colonization by *Salmonella* Typhimurium than green onion leaflets (Wright et al., 2013). This observation would most likely be linked with the type of irrigation (drip, furrow, or overhead) that is applied to a crop and which one would facilitate greater internalization to the phyllosphere or rhizosphere. Pathogens have also shown the ability to spread throughout a plant once they have been internalized. A study growing *Salmonella* contaminated tomatoes in field found that *Salmonella* Typhimurium had been internalized into directly inoculated leaflets of the plants as well as the adjacent uninoculated leaflets (Gu et al., 2011). *E. coli* O157:H7 has also demonstrated its ability to colonize internal root and leaflet layers of produce including spinach, lettuce, and parsley by travelling through contaminated soil (Marilyn C Erickson et al., 2010).

2.6 Pathogen persistence in soil

Initially, once pathogens are applied to the field, they bind very quickly to soil particles and are found near the surface (Chandler & Craven, 1978; Fenlon, Ogden, Vinten, & Svoboda, 2000). The type of soil may play a role in the survival of pathogens or generic *E. coli* in onion production. Danyluk et al found that there were no significant differences in counts of *Salmonella* Enteritidis between clay loam and sandy loam soils held at the same environmental conditions (Danyluk et al., 2008). However, another study found that *E. coli* O157:H7 was able to persist longer in clay soil than sandy loam soil (Ibekwe, Watt, Shouse, & Grieve, 2004). One study looked at different climates and the soils effect on *E. coli* O157:H7 survival in the arid Summerland, British Columbia and maritime Kentville, Nova Scotia and found no differences in survival rates between the two plots (Bezanson et al., 2012). A study in Dublin, Ireland found that verocytotoxigenic *E. coli* survived for long durations in farm soil, from 31 to 48 days in clay loam and from 50 to 75 days in sandy loam soils (Bolton et al., 2011). Topp et al performed a study in Ontario, Canada and found variations of survival in different soils between strains of *E. coli* by observing one strain persisting and growing in manure amended loam and sandy soil while another strain did not. Notably, both strains did not persist very well in clay soil (Topp et al., 2003).

Experimental field studies testing the survival of *Salmonella* spp., *E. coli* O157:H7 and *E. coli* O26 in soils have ranged from 147 to 231 days (Fremaux et al., 2008; Islam et al., 2005; Islam, Doyle, Phatak, Millner, & Jiang, 2004; Islam, Morgan, et al., 2004a, 2004b; Natvig, Ingham, Ingham, Cooperband, & Roper, 2002). Other scientific studies applying similar initial loads recorded shorter survival times for these enteric pathogens with 64 days on

fescue grass plots (Monaghan & Hutchison, 2012) and 54 to 105 days in Dutch soil plots (Franz & van Bruggen, 2008). Studies have also found that generic *E. coli* has similar survival curves to those of S. Typhimurium in agricultural soils (Natvig et al., 2002; Plachá, Venglovský, Sasáková, & Svoboda, 2001). The concentration of bacteria entering the soil also plays a major role in a pathogens initial die-off. Arthurson, Sessitsch, & Jäderlund found that the survival times of *Salmonella* Weltevreden in soil correlated with bacterial inoculation levels with larger numbers of initial bacteria resulting in higher bacteria densities through the study (Arthurson et al., 2011).

There may be differences in the microbial decay rate between *E. coli* and *Salmonella* depending on the type of soil. One study performed in Denmark observed that *E. coli* applied to clay soil at 3 $\log_{10}$/g was detectable for 21 days while *Salmonella* applied at 4 $\log_{10}$/g only lasted for 10 days at a detectable level (Boes et al., 2005). However, Natvig et al found that *S.* enterica serovar Typhimurium and *E. coli* death rates did not differ significantly between sandy loam and silt clay soils (Natvig et al., 2002). There are significant differences between studies in regards to the survival of *E. coli* and *Salmonella* on different soil types and this is mostly likely due to the type of crop grown in the soil, the environment used to conduct the study (growth chamber, greenhouse, or field trial), and the inoculum level used at the start of each study.

Islam et al 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., 2005). Some studies demonstrated soils used in vegetable production have harbored pathogens for prolonged periods of time. In moist soil plots containing tomatoes at 20^{o} C, *Salmonella* spp. inoculated at 8 log CFU/g remained at constant levels for 14 days and only decreased by 1 log after 45 days (Guo, Chen, Brackett, & Beuchat, 2002). The use of manure on a plot of soil significantly increases chances for survival or even growth of pathogens. Even though pathogens have been found to survive for prolonged periods of time in soil, manure and manure amended soil has still shown to harbor bacteria for longer durations (M L Hutchison, Walters, Moore, Crookes, & Avery, 2004).

2.7 Summary

There is an apparent increase in the number of foodborne outbreaks involving fresh produce. Bacterial pathogens such as *Salmonella* spp. are able to reach ready to eat produce through contaminated irrigation water, bringing up safety concerns for produce irrigated just before harvest. With the new FSMA water standards being put in place, fresh produce commodities are being grouped together as covered produce for standardized food safety practices under the produce rule. Water testing required by the produce rule places a huge financial burden on dry bulb onion producers and may not represent the true microbiological safety of the produce.

There have been no trials found in literature testing the viability of post irrigation mitigation strategies on dry bulb onions to reduce microbial loads brought on by contaminated irrigation water. One study has demonstrated the real world application of Treasure Valley irrigation water on dry bulb onions to enumerate and observe survival of generic *E. coli* on dry bulb onions. However, there have been no studies that have placed a known inoculum of generic *E. coli* and *Salmonella* spp. in irrigation water used for onion production. This research was designed to validate whether the dry bulb onion finishing process effectively mitigates a known level of generic *E. coli* and a target pathogen, *Salmonella* spp.

There also have not been many studies demonstrating whether soil type has an adverse effect on microbial decay rate. Results from the examination of microbial decay rates in different soils may provide an understanding of the chemical and physical characteristics of soil types that determine the survival of generic *E. coli* or *Salmonella* spp.

3. CONVENTIONAL CURING PRACTICES REDUCE GENERIC *E. COLI* AND *SALMONELLA* SPP. ON DRY BULB ONIONS PRODUCED WITH CONTAMINATED IRRIGATION WATER

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3.1 Abstract

Food Safety Modernization Act (FSMA) has emphasized microbial risks associated with irrigation water. Treasure Valley (eastern Oregon/western Idaho) has the highest yield of dry bulb onions in the country; however, their irrigation water is often of poor microbiological quality. Conventional curing practices may provide a mechanism to mitigate irrigation water quality to comply with FSMA regulations. Dry bulb onions were grown in Owyhee silt loam and Semiahmoo muck soils in greenhouses and irrigated with water containing a cocktail of rifampicin-resistant generic *E. coli* and *Salmonella* spp. (4.80 log CFU/ml). To mimic conventional practices, mature onions remained undisturbed in soil without irrigation for 12 days prior to being lifted and cured for 16 additional days. Surviving generic *E. coli* and *Salmonella* spp. were selectively enumerated on Hektoen Enteric Agar with rifampicin using standard plating or most probable number methods. Generic *E. coli* and *Salmonella* spp. on onions decreased 0.19-0.26 log CFU/g·d during the initial 12 days of finishing. At lifting, generic *E. coli* and *Salmonella* spp. had been reduced to <1 CFU/g and persisted through the end of curing. This study demonstrates conventional curing practices as an effective mitigation strategy for dry bulb onions produced with water of poor microbiological quality.

Keywords: onion; generic *E. coli*; *Salmonella*; greenhouse; curing; soils

3.2 Introduction

Fresh produce is increasingly recognized as a source for foodborne outbreaks due to microbial contamination close to harvest. CDC outbreak data indicates that, from 1998-2008, 46% of foodborne illnesses have been attributed to single item raw produce (Painter et al., 2013). Studies have found that the most common sources for microbial contamination in farm-to-table production stem from irrigation water, runoff water from livestock farms, manure, wash water, animal fertilizers, and wildlife (Beuchat & Ryu, 1997; Tauxe et al., 1997). Reviews of outbreaks of foodborne illness in the U.S. found that *Salmonella* was the most commonly reported bacterial pathogen, accounting for approximately half of the reported outbreaks due to bacteria (Centers for Disease Control and Prevention, 2010; Scanlan et al., 2011; Sivapalasingam et al., 2004).

The Food Safety Modernization Act (FSMA) was signed into law by Congress in early 2011 to address a myriad of food safety issues. Following the passage of the FSMA, the Food and Drug Administration (FDA) published proposed rules for the Standards for the Growing, Harvesting, Packing and Holding of Produce for Human Consumption (The Produce Rule) that would drastically impact agricultural production around the country (Food and Drug Administration, 2013). A major portion of the produce rule focuses on the microbiological quality of irrigation water used for produce that is consumed raw ("covered produce"). The proposed rule mandates testing agricultural water for generic *E. coli* levels. Generic *E. coli* is a common indicator of fecal contamination and potential presence of pathogens. A supplemental notice of proposed rulemaking for the Produce Rule was published in September 29, 2014 (Food and Drug Administration, 2014). The supplemental notice

proposed to require producers to test and evaluate the microbiological quality of their agricultural water by creating a water quality profile (WQP) that provides a characterization of risk based on two calculated values: the geometric mean (GM) and the statistical threshold value (STV) (Food and Drug Administration, 2014b). Producers must monitor the microbiological quality of their water annually to ensure that their WQP accurately describes their water supply. If the water source does not meet the GM or STV criteria, producers must discontinue use of that water source or apply a mitigation strategy to reduce the microbial load before continuing use of the water.

The Treasure Valley growing region of eastern Oregon and western Idaho is an agricultural production area with limited water resources that relies on a complex irrigation canal system coupled with reclamation and reuse. The combination of open irrigation canals and ditches along with the reclamation of water after passing through fields leads to unpredictably high levels of generic *E. coli*, occasionally >2500 MPN/100 ml (Shock et al., 2013). This region includes approximately 150 growers that farm over 20,000 acres of high yield dry bulb onions (740-760 cwt/acre) (Shock et al., 2000; USDA Economic Research Service, 2011a and 2011b). Dry bulb onions fall under the covered produce definition; therefore, growers must comply with the water quality standards described above. Producers may mitigate the microbial risk of their water by determining a time interval between the last irrigation and harvest that would achieve a calculated reduction of the initial microbial load to below the mandated GM and STV criteria. This mitigation strategy is allowed due to recent evidence of a common microbial decay rate of 0.5 $\log_{10}$ CFU/g·day on produce during field trials (Snellman et al., 2014). Conventional finishing practices for dry

bulb onion production included an extended period of time between last irrigation and harvest. The primary objective of this study was to evaluate conventional onion curing methods as an effective mitigation strategy to reduce generic *E. coli* and *Salmonella* spp. applied via contaminated irrigation water. A secondary objective was to compare the survival of generic *E. coli* and/or *Salmonella* spp. on onions produced in two soil types used from commercial onion production.

3.3 Materials and Methods

3.3.1 Greenhouse setup

Two OSU greenhouses (West 6-5 – 700 sq. ft.; West 7-6 – 340 sq. ft.) were used for the onion production studies. Both greenhouses were constructed of solid concrete floors with steel mesh grid tables (5' x 15'). Greenhouse temperatures were maintained by thermostatic control (High: 24^{o}C; Low: 10^{o}C) and temperature values throughout the study were recorded 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. Polyvinyl chloride pipe cages with mosquito netting were constructed around each tray to prevent flying insects from accessing contaminated plants and soil.

3.3.2 Soil preparation

Soils (~110 kg/soil type) were transported from a commercial onion field in the Willamette Valley (Semiahmoo muck soil) and from the Malheur County Agricultural Experiment Station in the Treasure Valley (Owyhee silt loam) into the Oregon State University greenhouses. Soil was prepared by hand grinding through wire mesh grid boxes (approximately 1.2 cm grid). Two-gallon injection molded planting pots (Gro Pro, Sunlight Supply, Inc., Vancouver, WA) were lined with synthetic cheesecloth (Dairy Connection, Madison, WI) to prevent soil erosion and filled with approximately 3500 g of soil. Prepared pots were distributed into the trays (17-19 pots/tray) throughout both 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 analysis.

3.3.3 Onion production and finishing

Spanish yellow dry bulb onion sets (Ovation variety; Nunhems USA, Parma, ID) were planted directly into pots. The onion sets had been previously grown from seed (Sakata Seed Company, Morgan Hill, CA) in Buckeye, AZ. Each tray of onion plants (17-19 plants per tray) was treated as a block for a given treatment (inoculated/uninoculated, soil type) and trays (A-X) were randomized across both greenhouses. Each onion 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 manufacturer's instructions during week 4 and week 5 after planting. After 5 weeks of

growth, irrigation was transitioned to well water (private residence, Philomath, OR) for the remainder of the growing period. Irrigation was ceased when onions were determined to be fully mature based on browning and dropping of the onion leaf stalks. To finish the onions, plants remained undisturbed in soil (water cessation) for 12 days and then lifted and set on soil surface to cure for an additional 16 days.

3.3.4 Bacterial strain, culture conditions, and preparation of inocula

Generic *E. coli* strains (LJH-1247, LJH-1612, LJH-1613) and *Salmonella* spp. (Montevideo LJH-614, Michigan LJH-615, Saintpaul LJH-1262) were used in the inoculation cocktail. Generic *E. coli* strains had been previously isolated by Trevor Suslow's laboratory (University of California-Davis) from lettuce, irrigation water, and soil from the Salinas Valley and were adapted to be rifampicin-resistant by the Linda Harris laboratory (University of California-Davis). *Salmonella* strains were originally 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^{o}C in Tryptic Soy Broth (TSB; Neogen, Lansing, MI) with 40% glycerol. Frozen cultures of each strain 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 each of three separate Tryptic Soy Agar plates (TSA, Neogen) containing rifampicin (50 mg/L; Alfa Aesar, Ward Hill, MA; TSA+rif) 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 15 ml sterile conical tubes. The cocktail was prepared by mixing 1 ml of each of

the harvested lawns of the six strains into a 15 ml conical tube 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). The cocktail solution (6 ml) was then added to well water (19 L) in a polypropylene carboy (U-Line, Pleasant Prairie, WI). Irrigation water samples were analyzed for microbial concentration after each irrigation event.

3.3.5 Inoculation of irrigation water

Plants were watered with contaminated irrigation water by hand with 1-liter polypropylene measuring cups at a rate of approximately 200 ml/sample (10^6 CFU/sample) every three days from day 40 after planting through maturity (day 110) for a total of 24 contaminated irrigation events. Control samples continued to receive well water that had not been inoculated.

3.3.6 Sample analysis – onions

Onion samples were collected immediately after watering by lifting the onions by the stalk and aseptically separating the bulb into a 1.5 liter sterile Whirl-Pak bag (Nasco, Salida, CA). Five replicates per day were sampled for enumeration of bacterial density on onion bulbs. Onions were washed with 0.1% peptone water by vigorously mixing by hand for approximately 20-30 seconds. 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.

3.3.7 Sample analysis – soil

The entire soil content of a single pot was transferred to a 1.5 liter sterile Whirl-Pak bag (Nasco). Five replicates per day were sampled for enumeration of bacterial density in each soil. Bulk soil samples were homogenized by shaking and turning bags end over end until visibly mixed. A 100 g subsample of soil was transferred to a 710 ml sterile Whirl-Pak filter bag (Nasco) 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 spread plated onto HE+rif. Plates were incubated as described previously.

3.3.8 Sample analysis – Most Probable Number Method

When microbial counts from onion or soil samples fell below the detection limit of 1 CFU/g for standard plating methods, a 96-well Most Probable Number (MPN) method was employed. Following rinsing or homogenization, the entirety of the 0.1% peptone water rinsate was transferred in 1 ml aliquots into 96 well deep well plates (VWR International, Radnor, PA). An additional 1 ml of lactose broth containing rifampicin (50 mg/L; L+rif) was added to each well and plates were incubated at 37°C for 24 hours. Following incubation,

each well was spotted onto HE+rif plates using a 96 well tip comb and incubated at 37°C for 24 hours.

3.3.9 Statistical analysis

The experimental design randomized onion/soil samples across both greenhouses with blocking by tray. Each data point represents a mean of five replicates. Qualitative positive results for *E. coli* and *Salmonella* were calculated as MPN/ml using Poisson Distribution where d = -2.303/v log(s/n), n = total # of well, s = # of negative wells, and v = volume per well (IDEXX, 2015). Data collected from onion bulb size and bacterial survival data on onions and in soil were analyzed using the interpolation function (second order, third order, or sigmoidal) within the GraphPad Prism 6 software package (GraphPad Software Inc, La Jolla, CA).

3.4 Results

3.4.1 Onion production in greenhouses

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). 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

(1.1 – 196, Figure 1). On average, onions grown in Semiahmoo muck soil were larger in size at 82 g (ranged 9.5-196) compared to those grown in the Owyhee silt loam soil with an average of 57 g (ranged 1.1-157). 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. Temperature values in the large greenhouse ranged from lows at ~15°C to highs of 38°C during the water cessation and curing period (days 111 – 139) while values in the smaller greenhouse ranged from 13°C to 32°C during the same period. *E. coli* and *Salmonella* spp. concentrations in the irrigation water averaged 4.57 ± 0.34 log CFU/ml and 4.58 ± 0.34 log CFU/ml, respectively. Application of *E. coli* and *Salmonella* led to direct contact of the irrigation water with the onion bulb, and as expected, relatively high averages of generic *E. coli*, 3.21 ± 0.46 log CFU/g, and *Salmonella* spp., 3.36 ± 0.48 log CFU/g, in soil and onion samples (Figure 1). The soil analysis revealed that Owyhee silt loam has a higher pH at 8.1 with Semiahmoo muck being at 6.2. Semiahmoo muck had higher concentrations of basic minerals such as free nitrogen, calcium, copper, and iron.

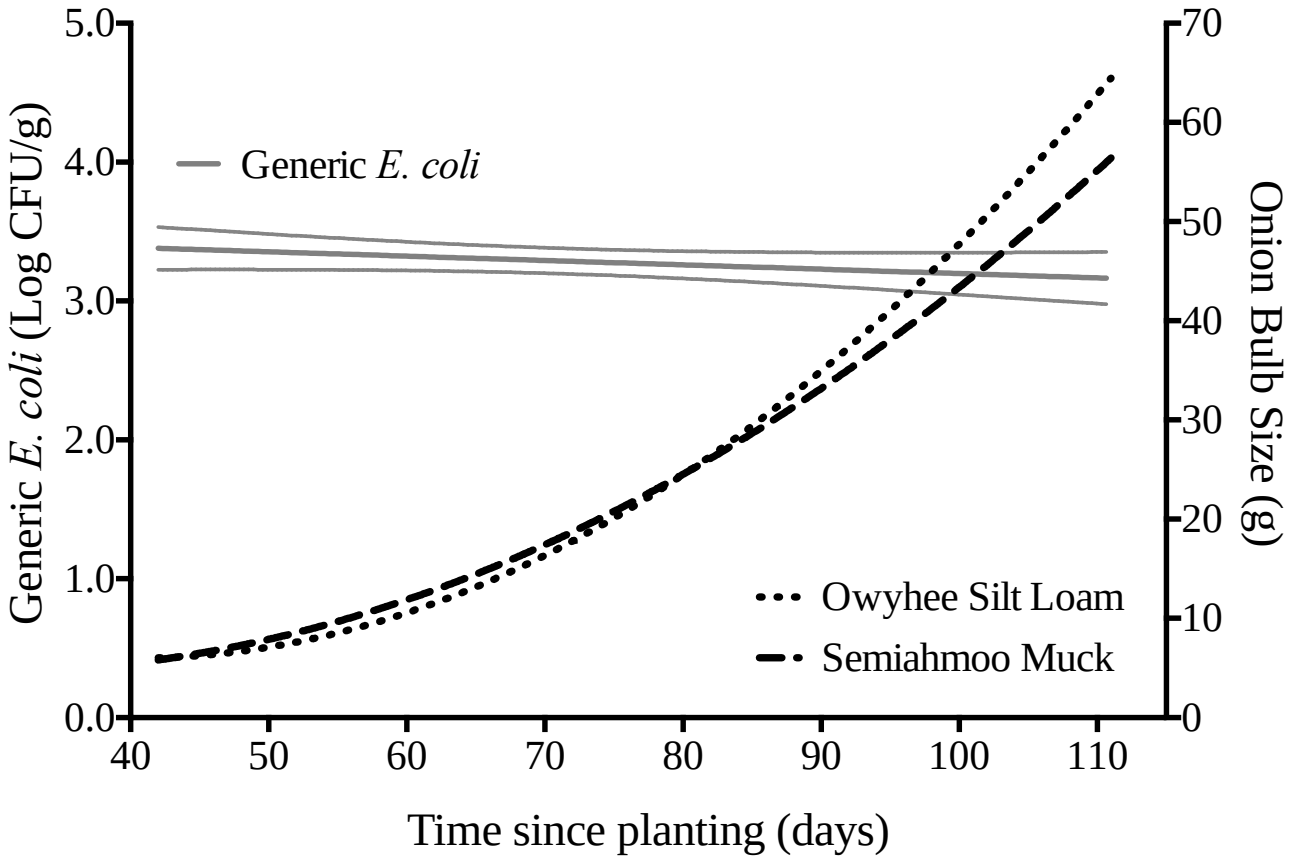
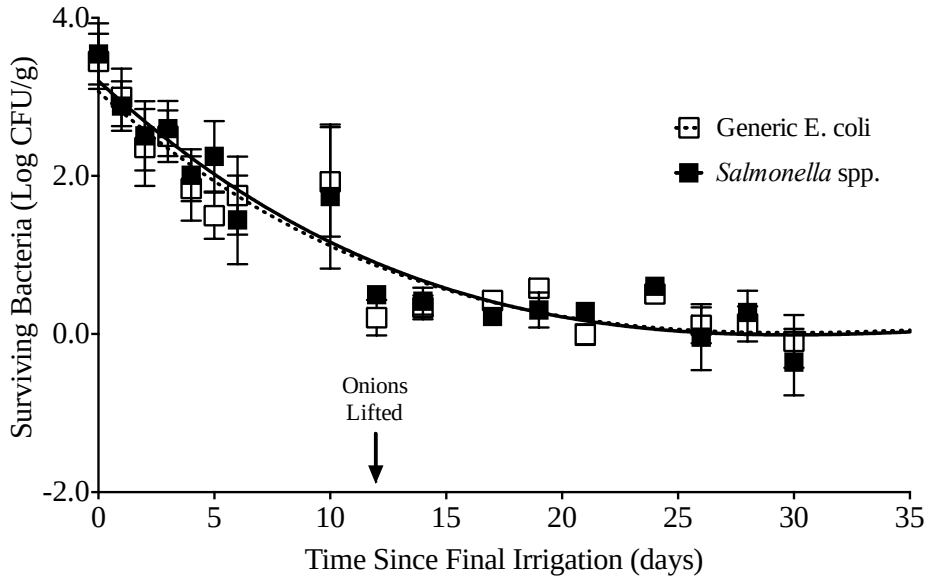


Figure 1. Linear regression with 95% confidence interval of generic *E. coli* levels on onions (log CFU/g) throughout the contaminated irrigation period (days 42-111 after planting) with reference to average bulb size (g) of onions grown in Owyhee silt loam or Semiahmoo muck soils (second order polynomial interpolation; n = 5).

3.4.2 Survival of generic E. coli and Salmonella spp. on onions during curing

Survival of generic *E. coli* and *Salmonella* spp. on onions grown in Semiahmoo muck and Owyhee silt loam soil are shown in Figure 2. At the last days of irrigation, average generic *E. coli* and *Salmonella* spp. levels were 3.49 and 3.39 log CFU/g for all onion samples respectively. During the first six days of water cessation, survivors were reduced to averages of 1.77 and 1.83 log CFU/g of onion for generic *E. coli* and *Salmonella* spp. respectively. After 12 days post irrigation, all samples averaged <10 CFU/g. During curing (2 weeks), generic *E. coli* and *Salmonella* spp. levels remained relatively stable at low levels on onions (~1 MPN/g). The bacterial decay rates on onions after water cessation were determined after irrigation to the start of curing on day 13 (Table 1). Generic *E. coli* and *Salmonella* had similar decay rates on onions grown in Owyhee silt loam (0.26-0.27 log CFU/g·day) Semiahmoo muck (0.30-0.35 log CFU/g·day).

a) Owhyee Silt Loam



b) Semiahmoo Muck

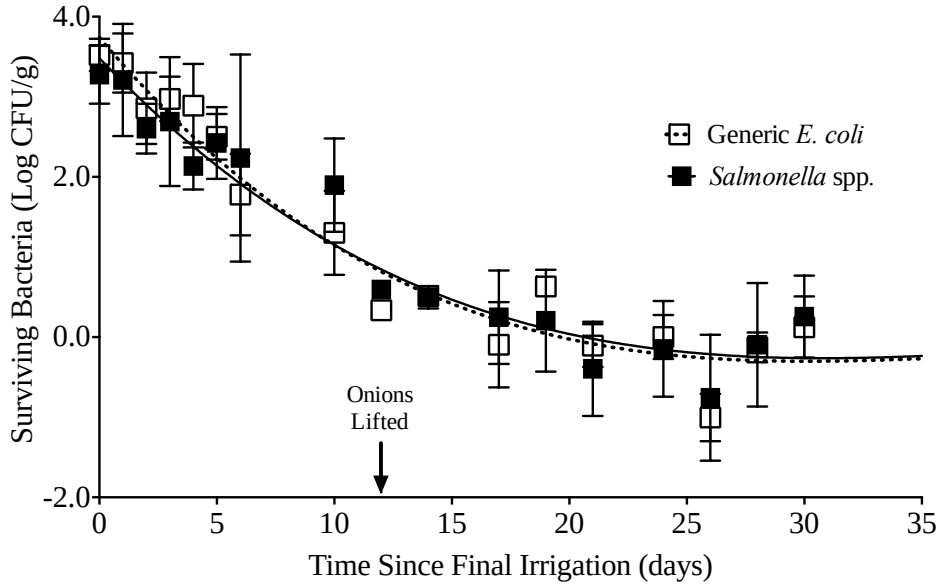


Figure 2.

Reduction and persistence of generic *E. coli* and *Salmonella* spp. on dry bulb onions grown in a) Owyhee silt loam or b) Semiahmoo muck and finished with conventional practices (12 days undisturbed in soil, lifted, 16 days undisturbed on soil surface). Data points represent

the mean (n=5) with error bars indicating the standard error of the mean. Lines represent the third order polynomial interpolation of each data set.

Table 1. Microbial decay rate (log CFU/g·d) of generic *E. coli* and *Salmonella* spp. on dry bulb onions and in Owyhee silt loam and Semiahmoo muck soils during linear decay periods after irrigation was ceased.

Soil Type	Microbial Decay Rates (log CFU/g·d[a])			
	Onion[b]		Soil[c]	
	Generic *E. coli*	*Salmonella* spp.	Generic *E. coli*	*Salmonella* spp.
Owyhee Silt Loam	-0.26 ± 0.07[d]	-0.27 ± 0.07	-0.25 ± 0.15	-0.23 ± 0.11
Semiahmoo Muck	-0.35 ± 0.07	-0.31 ± 0.06	-0.23 ± 0.10	-0.20 ± 0.07

[a] Logarithmic transformation of colony forming units per gram per day

[b] Decay rates for onion samples were calculated using third order polynomial interpolation function in GraphPad Prism. Rates displayed are the best-fit value represented as B1 in the software.

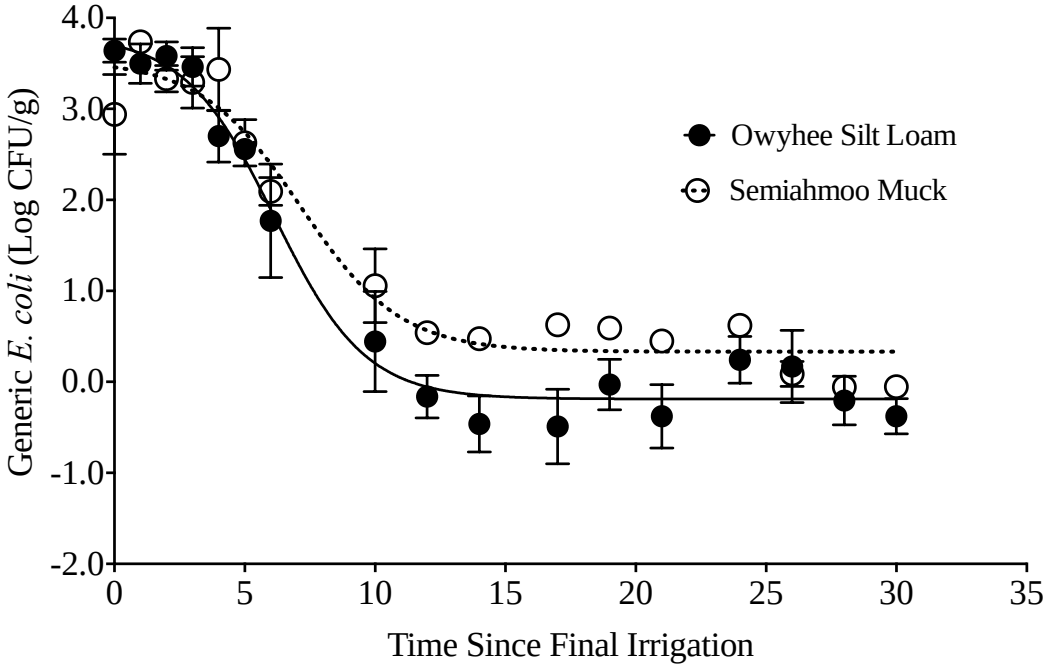
[c] Decay rates for soil samples were calculated using the sigmoidal interpolation function in GraphPad Prism. Rates displayed are the best-fit value represented as HillSlope in the software.

[d] Decay rates are presented as the mean rate calculated by the respective interpolation function with the variability indicating the 95% confidence interval of the rate.

3.4.3 Survival of generic E. coli and Salmonella spp. in soils

Survival of generic *E. coli* and *Salmonella* spp. in soil samples are shown in Figure 3. At the last irrigation time point, average generic *E. coli* and *Salmonella* levels in soil were 3.25 and 3.75 log CFU/g respectively. After 6 days of water cessation, generic *E. coli* and *Salmonella* spp. survivors were reduced to an average of 1.93 and 1.99 log CFU/g respectively. After 12 days of water cessation, all samples averaged below 10 CFU/g. Generic *E. coli* and *Salmonella* averages were approximately 0.65 log CFU/g lower in Owyhee silt loam soil samples than in Semiahmoo muck samples after 12 days. However, generic *E. coli* and *Salmonella* levels were similar in both soil types near the end of curing by day 26. Negative control onion and soil samples revealed no instances of rifampicin-resistant generic *E. coli* or *Salmonella.*

a) Generic *E. coli*



b) *Salmonella* spp.

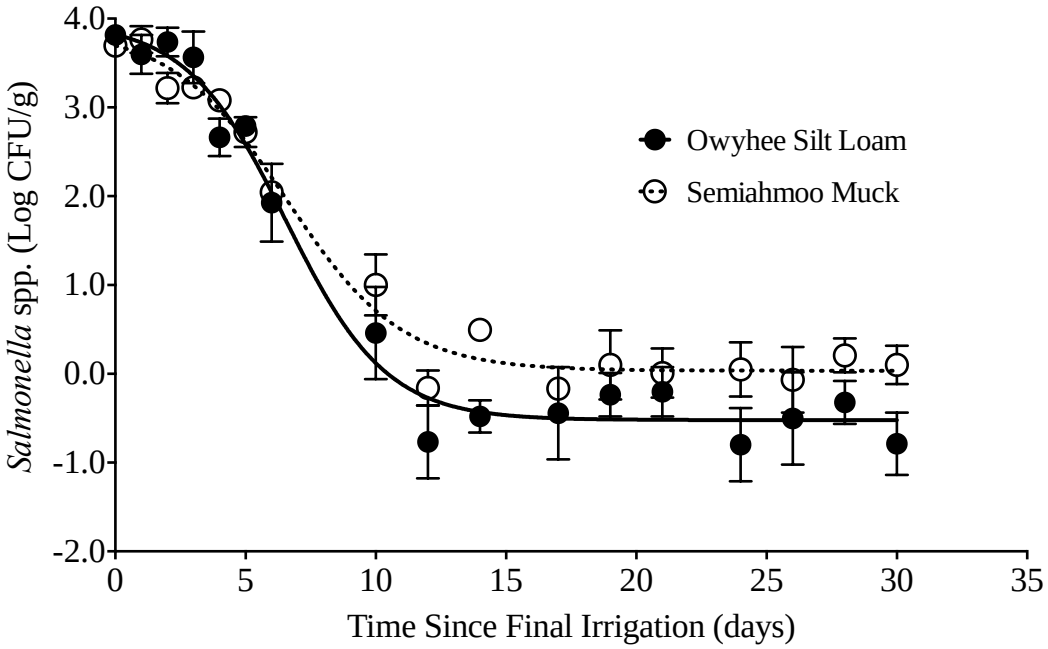


Figure 3.
Reduction and persistence of a) generic *E. coli* and b) *Salmonella* spp. in Owyhee silt loam and Semiahmoo muck soils following the final irrigation. Data points represent the mean (n=5) with error bars indicating the standard error of the mean. Lines represent the sigmoidal interpolation of each data set.

3.5 Discussion

In this study, results revealed that conventional finishing practices effectively reduce generic *E. coli* and *Salmonella* spp. on onion. The bacterial decay rates observed in this study (Table 2) were significantly lower than the rates observed in fresh produce in the field trials summarized in the FDA report (Snellman et al., 2014). The supplemental to the proposed Produce Rule provides a conservative decay rate of -0.5 log CFU/g·d based on seven field studies using lettuce, spinach, and parsley as the model produce (Bezanson et al., 2012; Fonseca et al., 2011; Hamilton et al., 2006; Hutchison et al., 2008; Kisluk and Yaron, 2012; Moyne et al., 2011; Wood et al., 2010). The decay rate of -0.5 log CFU/g·d was determined from the lowest decay rate of the studies reviewed involving the survival of *E. coli* O157:H7 on lettuce (Fonseca et al., 2011). This decay rate is higher than our own for generic *E. coli* most likely due to the lettuce crop being further distanced from soil than dry bulb onions, the lettuce plant receiving more UV exposure, and the plant losing moisture at a faster rate immediately after irrigation due to being above ground.

The practical application of FDA's proposed decay rate (-0.5 log CFU/g·d) would require a minimum irrigation-to-harvest interval of 9 days with the grossly contaminated water (3,700,000 CFU/100 ml) used in this study (Table 2). Using experimentally calculated decay rates, generic *E. coli* would be reduced to the required level (≤125 CFU/100 ml) with an irrigation-to-harvest interval of at least 13 days for onions grown in Semiahmoo muck and 18 days for onions grown in Owyhee silt loam. Despite the significant differences in these decay rates, conventional curing practices for dry bulb onions dictate a typical 30-day

irrigation-to-harvest interval that exceeds all calculated limits. Our experimental initial generic *E. coli* concentration represents an extreme excess of generic *E. coli* in irrigation water compared to "real world" concentrations which can approximate 2400 CFU/100 ml. Calculated intervals presented in Table 2 demonstrate that even under an extreme excess of generic *E. coli* conventional curing practices would reduce microbial levels well before harvest.

Very little work has been performed on survival of bacteria on dry bulb onions. Islam *et al*. conducted a field trial in Georgia to measure the survival of *E. coli* O157:H7 with an early season (3 weeks after planting), single application of contaminated irrigation water (5 log CFU/ml) (Islam et al., 2005). At an initial level of 2.0 to 2.4 log CFU/g of onion, *E. coli* O157:H7 was able to persist at their detection limit of ≥1 CFU/g for 74 days after contamination throughout the growing season. This data suggests the potential for later season irrigation to be a potential source for contamination of the harvested product. While Islam et al. focused on contamination during early growing season, both studies demonstrate the relatively long-term persistence of *E. coli* on dry bulb onions.

Table 2: Comparison of proposed decay rates[a] to experimentally calculated decay rates of generic *E. coli* on dry bulb onions and their associated irrigation-to-harvest interval as a mitigation strategy to comply with proposed water quality standards for irrigation water.

Soil Type	Generic *E. coli* concentration in irrigation water	Supplemental to the Proposed Produce Rule		Experimental (Current Study)	
		Decay Rate (log CFU/g·d)	Irrigation-to-harvest interval (d[b])	Decay Rate (log CFU/g·d)	Irrigation-to-harvest interval (d)
Owyhee Silt Loam	3,370,000[c] CFU/100 ml	-0.5	9	-0.26	18
Semiahmoo Muck				-0.35	13
Owyhee Silt Loam	2400[d] CFU/100 ml		3	-0.26	5
Semiahmoo Muck				-0.35	4

[a] Decay rate of 0.5 log CFU/g·d provided in the Supplemental Notice of Proposed Rulemaking for the Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption (79 FR 58433, September 29, 2014).

[b] Calculated number of days with associated decay rate to reduce generic *E. coli* levels of less than or equal to 125 CFU/100 ml.

[c] Generic *E. coli* levels used in the current study. These levels are in extreme excess of generic *E. coli* levels measured in commercial agricultural water. High levels were necessary to evaluate quantifiable reductions throughout finishing of dry bulb onions.

[d] Maximum quantifiable generic *E. coli* levels using typical water analysis methods (ColiSure, IDEXX). These maximum levels are not uncommon in agricultural water testing performed by producers in Treasure Valley (personal communication).

The current study demonstrates that *Salmonella* spp. behaves similarly to generic *E. coli* on dry bulb onions and soil during production and finishing. Survival of generic *E. coli* and *Salmonella* spp. were comparable at all time points in all samples, indicating that these generic *E. coli* strains would provide an accurate prediction, as a surrogate, for the survival of *Salmonella* spp. in soils and on onions for future field trials. This observation agrees with findings of generic *E. coli* having similar survival curves to those of *Salmonella* Typhimurium in agricultural soils (Natvig et al., 2002; Plachá et al., 2001). The survival characteristics of *E. coli* and *Salmonella* in soil are important to predict the risks associated with the use of furrow and drip irrigation. Studies have found that once pathogens are applied to a crop via irrigation, they bind rapidly to soil and are found near the surface in close proximity to the produce (Chandler and Craven, 1978; Fenlon, et al., 2000). Many experimental field studies have evaluated the survival of *Salmonella* spp., *E. coli* O157:H7 and *E. coli* O26 in soil with survival persisting from 147 to 231 days (Fremaux et al., 2008; Islam et al., 2005; Islam et al 2004a, 2004b, 2004c, 2005; Natvig et al., 2002).

Limitations of this study included greenhouse conditions with limited UV, wind, et cetera and plant health and crop yield were not representative of field conditions. Irrigation was accomplished by ease of application that did not simulate industry practices. However, the irrigation water was in direct contact with the onion bulb rather than the indirect methods of furrow or drip commonly used by industry, creating a higher microbial load on the bulb. While levels of generic *E. coli* and *Salmonella* spp. were not completely eliminated through curing, the post irrigation microbial 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. A recent study by Shock *et al.* performed in Treasure Valley found that onion bulbs irrigated with ditch water containing 218 to >2400 MPN *E. coli* per 100 ml of water decreased from an MPN of 4033 to 611 by the end of field curing, from August 27 to October 1 (Shock et al., 2013). The information gained on the microbial reduction from curing methods and the demonstrated similarities between generic *E. coli* and *Salmonella* on dry bulb onions prove useful in our understanding of enteric microorganisms on dry bulb onions.

3.6 Conclusion

Conventional curing practices mitigate the risk posed by contaminated irrigation water during onion production. The results of this study also provide 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. 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.

3.7 Acknowledgments

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Oslund, Sam Mertz, and Whitney Nielsen. Amy Emch, Joey Cusic, Robin Frojen deserve a huge thank you for supporting their spouses and friends.

4. SURVIVAL OF GENERIC *E. COLI* AND *SALMONELLA* SPP. IN OREGON'S AGRICULTURAL SOILS

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4.1 Abstract

Irrigation-to-harvest intervals may reduce the risk of foodborne illnesses associated with contaminated irrigation water used for fruit and vegetable production, including root crops. Survival and persistence of generic *E. coli* and *Salmonella* spp. were evaluated in six Oregon agricultural soils. *E. coli* and *Salmonella* had the weakest survival and persistence in Quatama, Latourell, and Willamette loam. Madras, Adkins, and Cullius loams harbored generic *E. coli* and *Salmonella* spp. for extended periods (>80 d). Two inoculum concentrations of generic *E. coli* and *Salmonella* spp. were used to determine any difference in persistence based on starting inoculum density.

4.2 Introduction

The number of foodborne outbreaks involving fresh produce in the U.S. has increased in recent years (Gould et al., 2013). Bacterial pathogens such as *Salmonella* spp. are able to contaminate produce through poor quality irrigation water, raising significant safety concerns for produce irrigated just before harvest. Root crops are at an increased risk for microbial contamination due to direct contact with soil throughout the growing season. Both soil and root crops may become contaminated with fecal microorganisms, including foodborne pathogens such as *Salmonella* spp. via contaminated irrigation water (Islam et al., 2005). Once contaminated irrigation water is applied to the field, pathogens bind very quickly to soil particles and remain near the surface and can easily access the root crop surface (Chandler and Craven, 1978; Fenlon, et al., 2000). Previous studies indicated that

foodborne pathogens, such as *E. coli* O157:H7 and *Salmonella* spp., survive and persist at different levels in different soil types (Emch and Waite-Cusic, 2015, Ibekwe et al., 2004; Natvig et al., 2002). Differences in pathogen survival between agricultural soils may impact the risk analysis for individual produce production areas, particularly for those farms involved in root crop production. The primary objective of this study was to compare the survival and persistence of generic *E. coli* and *Salmonella* spp. in a variety of Oregon's agricultural soils when irrigated with contaminated water.

4.3 Materials and Methods

Greenhouse setup may be referenced in our previous study (Emch & Waite-Cusic, 2015). Six soils (~226 kg/soil type) were transported from Oregon State University Experiment Stations to the Oregon State University greenhouses (Table 1). Soil samples (500 g) were submitted to Edge Analytical Laboratories (Corvallis, OR) for pH and mineral composition analysis (Table 1). Soil was prepared by hand grinding through wire mesh grid boxes (approximately 1.27 cm grid). Sample cups (500 ml; Party City Corporation, Rockaway, NJ) were filled with approximately 400 g of soil and distributed into trays (100 cups/tray). Each tray was treated as a block for a given treatment (high or low inoculation and soil type) with the treatments randomized across both greenhouses. Rifampicin-resistant generic *E. coli* strains (LJH-1247, LJH-1612, LJH-1613) and *Salmonella* spp. (Montevideo LJH-614, Michigan LJH-615, Saintpaul LJH-1262) were used in the inoculation cocktail. Culture conditions, and preparation of inocula may be referenced from our previous study (Emch and Waite-Cusic, 2015).

Soil samples were inoculated by applying 25 ml of contaminated well water to each soil sample (400 g). Soil samples were inoculated on two occasions with two days between the two applications to normalize inoculum levels. Three replicates of irrigation water were analyzed after each irrigation event. Soils samples were maintained in the greenhouse for 86 days. Greenhouse temperature conditions were set at 24°C during the day and 10°C during the night; however, temperatures ranged from 6°C to 45°C in both greenhouses. Greenhouses were covered with polyethylene fabric shade cloth. All greenhouse operations were managed by OSU greenhouse personnel. Soil samples were collected by aseptically pouring the entire soil content from a single cup into a sterile 710 ml Whirl-Pak filter bag (Nasco, Salida, CA). The 400 g sample was mixed with 400 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 Hektoen Enteric Agar (Neogen, Lansing, MI) with rifampicin (50 mg/L; Alfa Aesar, Ward Hill, MA; HE+rif;). Plates were enumerated following incubation at 37°C for 24 h. Five replicates were used to enumerate the bacterial density every 2 days in the first 20 days after the final contamination event and then every 3 days thereafter.

When microbial counts from soil samples fell below the detection limit of 1 CFU/g for standard plating methods, a 96-well Most Probable Number (MPN) method was employed. The soil:peptone water mixture was aliquoted (1 ml) to 96 well deep well plates (VWR International, Radnor, PA). An additional 1 ml of Lactose Broth (Neogen) containing rifampicin (50 mg/L; L+rif) was added to each well and incubated at 37°C for 24 h. Following incubation, each well was spotted onto HE+rif plates using a 96 well tip comb. HE+rif plates were incubated 25°C for 48 hours prior to evaluation. Five MPN samples

were evaluated every three days. Qualitative positive results for generic *E. coli* and *Salmonella* spp. were calculated as MPN/ml using Poisson Distribution where d = -2.303/v log(s/n), n= total # of well, s= # of negative wells, and v= volume per well (IDEXX, 2015).

Table 1: Mineral compositional analysis[a] and pH of Oregon agricultural soils.

Soil	Quatama loam	Latourell loam	Willamette loam	Madras loam	Adkins loam	Cullius loam
Sample Source	NWREC[b]	NWREC	NWREC	COARC[c]	HAREC[d]	COARC
pH	5	5.4	5.1	6.5	6.1	6.6
Organic Matter (%)	4.3	4.3	3.9	2.6	1.8	2.6
NO_3-N (ppm)	18	26	30	21	126	9
P Weak Bray (ppm)	78	89	91	35	69	32
P NaHCO3-P (ppm)	93	130	125	44	55	38
K (ppm)	205	228	289	485	560	491
Mg (ppm)	162	129	92	587	212	595
Ca (ppm)	1157	1140	1071	1507	1050	1509
Na (ppm)	4	3	2	37	33	39
SO_4-S (ppm)	10	6	5	4	17	3
Zn (ppm)	2.9	0.8	0.9	0.8	2	0.8
Mn (ppm)	3	2	5	8	5	14
Fe (ppm)	84	75	117	49	29	49
Cu (ppm)	1	0.7	0.4	2	0.8	2.1
B (ppm)	0.1	0.2	0.2	0.3	0.7	0.3
Silt %[g]	37.6	41.9	66.6	60	36.3	33.8
Sand %[g]	39.3	44.5	8.8	60	39.4	27.1
Clay %[g]	23.1	13.5	24.6	6	24.3	39.1

[a] All soil analysis performed by Edge Analytical Laboratories, Corvallis, OR.
[b] North Willamette Research and Extension Center, Aurora, OR.
[c] Central Oregon Agricultural Research Center, Madras, OR.
[d] Hermiston Agricultural Research & Extension Center, Hermiston, OR.
[g] Physical composition obtained through NRCS (National Resources Conservation Service, 2015)

Once microbial counts fell below the detection limit of 1 MPN/96 g for the MPN method for two consecutive days, a qualitative total sample enrichment was employed. Ten soil samples were collected weekly and enriched with 800 ml of L+rif and incubated at 37°C for 24 h. Following incubation, enrichments were streaked onto HE+rif plates and incubated at 37°C for 24 h. Generic *E. coli* presence was confirmed on Eosin Methylene Blue (EMB; Neogen) agar following incubation at 37°C for 24 h. Soil analyses were discontinued after generic *E. coli* or *Salmonella* spp. were not detected in any of the 10 enrichment samples.

4.4 Results

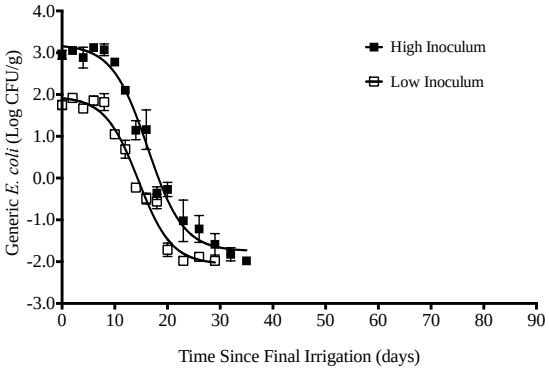
Generic *E. coli* and *Salmonella* spp. concentrations in the high inoculation irrigation water were 4.59 ± 0.06 log CFU/ml and 4.93 ± 0.04 log CFU/ml, respectively. Generic *E. coli* and *Salmonella* spp. concentrations in the low inoculation irrigation water were 2.70 ± 0.09 log CFU/ml and 2.76 ± 0.12 log CFU/ml, respectively. Immediately following irrigation (day 0), average generic *E. coli* and *Salmonella* spp. levels in high inoculum soil samples were 3.36 ± 0.31 and 3.60 ± 0.21 log CFU/g, respectively. Generic *E. coli* and *Salmonella* spp. levels in low inoculum soil samples were 2.09 ± 0.45 and 2.39 ± 0.45 log CFU/g, respectively.

Survival of high inoculum and low inoculum generic *E. coli*. in each soil are shown in Figure 1. After 47 days, generic *E. coli* levels in high inoculum samples in all soils dropped below 1 CFU/g with microbial loads in Quatama dropping below this level first within the first 18 days. Microbial loads in Cullius loam were the most persistent. This pattern was similar for low inoculum soil samples. Generic *E. coli* levels in Quatama loam fell below 1 CFU/g

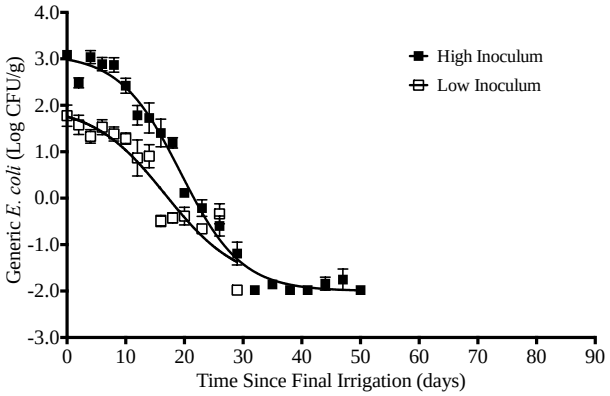
wihtin 16 days and microbial loads in Cullius loam were the most persistent. General reduction rates of generic *E. coli* and *Salmonella* spp. were comparable throughout the study (data not shown).

Persistence of generic *E. coli* and *Salmonella* spp. in each soil type is show in Table 2. In low inoculum samples, the last detection of generic *E. coli* and *Salmonella* spp. in Quatama loam was 26 and 42 days after contamination, respectively. Generic *E. coli* and *Salmonella* spp. persisted in Willamette loam for 32 and 41 days, respectively, while in Latourell soil, both generic *E. coli* and *Salmonella* spp. persisted for 42 days. Generic *E. coli* and *Salmonella* spp. initially inoculated at low levels remained detectable in Madras, Adkins, and Cullius loams for the remainder of the study (>79 days). In high inoculum samples, generic *E. coli* and *Salmonella* spp. were undetectable in Quatama soil after 62 days while still persisting in all other soils for the remainder of the study (>81 days).

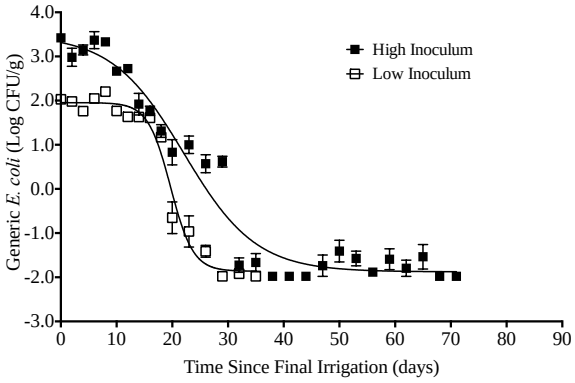
a) Quatama Loam



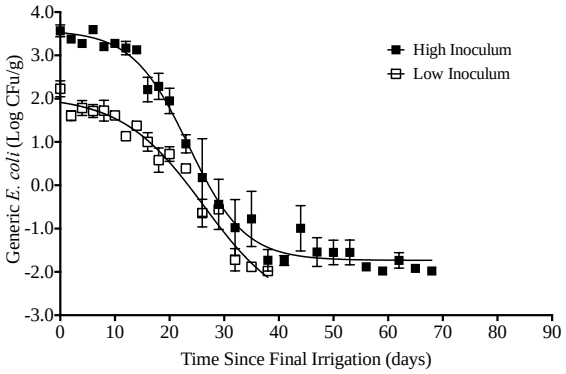
b) Latourell Loam



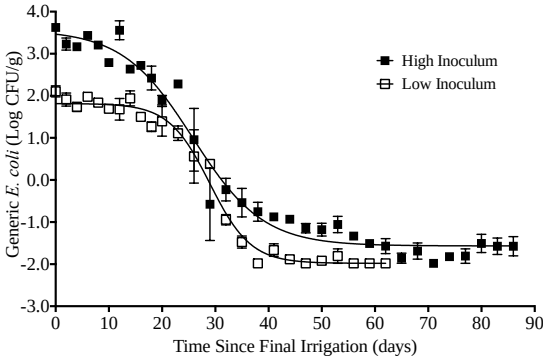
c) Willamette Loam



d) Madras Loam



e) Adkins Loam



f) Cullius Loam

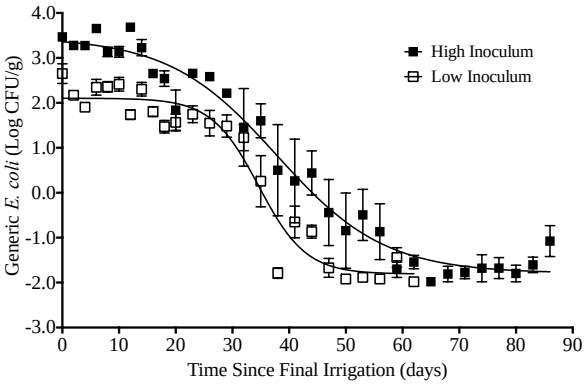


Figure 1. Reduction and persistence of generic *E. coli* in a) Quatama loam, b) Latourell loam, c) Willamette loam, d) Madras loam, e) Adkins loam, f) Cullius loam soils when inoculated at high (3.5 log CFU/g) and low (2.0 log CFU/g) levels. Data points represent the mean (n = 5) with error bars indicating the standard error of the mean. Lines represent the sigmoidal interpolation of each data set.

Table 2. Qualitative persistence of generic *E. coli* and *Salmonella* spp. in various Oregon agricultural soils following applications of irrigation water with low (2.0 log CFU/g) and high (3.5 log CFU/g) levels of contamination.

Low Initial Inoculum																	
Quatama Loam			**Latourell Loam**			**Willamette Loam**			**Madras Loam**			**Adkins Loam**			**Cullius Loam**		
day	**E**	**S**	**day**	**E**	**S**	**day**	**E**	**S**	**day**	**E**	**S**	**day**	**E**	**S**	**day**	**E**	**S**
26	1/5[a]	0/5	32	0/5	0/5	32	1/5	2/5	58	1/10	1/10	65	0/5	0/5	65	0/5	0/5
29	0/5	0/5	35	0/10	2/10	35	0/5	0/5	65	1/10	2/10	68	1/10	8/10	68	1/10	1/10
32	0/5	0/5	42	1/10	1/10	38	0/5	0/5	72	2/10	1/10	75	2/10	8/10	75	3/10	3/10
35	0/10[b]	1/10	49	0/10	0/10	41	0/10	1/10	79	3/10	1/10	82	2/10	10/10	82	4/10	1/10
42	0/10	1/10				48	0/10	0/10									
49	0/10	0/10															
High initial Inoculum																	
Quatama Loam			**Latourell Loam**			**Willamette Loam**			**Madras Loam**			**Adkins Loam**			**Cullius Loam**		
day	**E**	**S**	**day**	**E**	**S**	**day**	**E**	**S**	**day**	**E**	**S**	**day**	**E**	**S**	**day**	**E**	**S**
48	4/10	3/10	63	1/10	3/10	71	0/5	0/5	68	0/5	0/5	77	2/5	5/5	77	2/5	2/5
55	0/10	3/10	70	1/10	3/10	74	0/5	0/5	71	0/5	0/5	80	3/5	5/5	80	1/5	1/5
62	2/10	2/10	78	0/10	2/10	77	0/10	4/10	74	10/10	4/10	83	4/5	5/5	83	2/5	1/5
69	0/10	0/10	84	2/10	2/10	84	3/10	5/10	81	5/10	8/10	86	3/5	5/5	86	5/5	1/5

[a] Results with a denominator of 5 indicate samples on respective day were analyzed using Most Probable Number methodology. Numerator indicates number of samples analyzed that were positive for either generic E. coli or Salmonella spp.

[b] Results with a denominator of 10 indicate samples on respective day were analyzed for the qualitative presence or absence of generic E. coli or Salmonella spp. by enrichment methodology. Numerator indicates the number of samples analyzed that were positive for either generic E. coli or Salmonella spp.

4.5 Discussion

This study is the first to demonstrate a distinct difference in microbial survival and persistence of generic *E. coli* and *Salmonella* spp. in Oregon's agricultural soils. Our results are consistent with another study in eastern China that found significant differences in survival times of *E. coli* O157:H7 in 14 different soils with longer survival times in neutral or alkaline soils than those in acidic soils (Wang et al., 2014). There have also been differences in *E. coli* survival in soils that have different physical characteristics, one study showing that *E. coli* survives for longer period in sandy soil than in loam soil (Cools, Merckx, Vlassak, & Verhaegen, 2001). Another previous study found that *E. coli* O157:H7 was able to persist longer in clay compared to sandy loam soil (Ibekwe et al., 2004). Lau and Ingham (2001) also found *E. coli* to have a slower rate of die-off in silty clay loam soil than in sandy loam soil.

We also observed that generic *E. coli* and *Salmonella* spp. had comparable survival patterns before reaching low concentrations in the soils. Other studies have observed that generic *E. coli* has similar survival curves to those of *Salmonella* Typhimurium in agricultural soils (Natvig et al., 2002; Plachá, Venglovský, Sasáková, & Svoboda, 2001). The current study demonstrates differences in the persistence of generic *E. coli* and *Salmonella* spp. at low levels (< 1 CFU/g) with *Salmonella* spp. persisting longer than generic *E. coli* in Willamette loam. A previous study in Denmark observed that *E. coli* applied to clay soil at 3 log CFU/g was detectable for 21 days while *Salmonella* spp. applied at 4 log CFU/g only lasted for 10 days at a detectable level (Boes et al., 2005). Generic *E. coli* and *Salmonella* spp. are able to persist for significant periods of time without water application in a wide variety of soils.

Bolton et al. (2011) also demonstrated that verocytotoxigenic *E. coli* could survive for long periods in various agricultural soils: 31-48 days in clay and 50-75 days in sandy loam.

It should be noted that environmental conditions in soil have been shown to change once specific crops are planted. Islam et al. (2005) demonstrated that the persistence of *E. coli* O157:H7 in soil is dependent on the type of vegetable grown in the soil, with microbial decay more rapid in soil in which onions are grown than in soil in which carrots are grown. In this current study, there was a slow microbial die-off during the first few days after irrigation before microbial levels began to rapidly decline, creating a shoulder in the survival curve. Our previous study involving survival of generic *E. coli* and *Salmonella* spp. on dry bulb onions and in soil showed a similar shoulder for survival in soils; however, the shoulder was not as extensive (Emch and Waite-Cusic, 2015).

In summary**,** this study found a significant difference in the survival and persistence of generic *E. coli* and *Salmonella* spp. in various agricultural soils from Oregon. This study demonstrated similar rates of die-off for generic *E. coli* and *Salmonella* spp; however, the persistence may differ. Mitigation strategies for reducing pathogens in soil and associated root crops may need to be adjusted based on the soil type of growing region/farm.

4.6 Acknowledgments

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5. OVERALL SUMMARY AND FUTURE WORK

Determining the effectiveness of post-irrigation to harvest microbiological mitigation strategies will help ensure the safety of dry bulb onions and soils used for the production of leafy greens. The first study demonstrated that generic *E. coli* and *Salmonella* spp. inoculated at high concentrations are significantly reduced during the post-irrigation period on dry bulb onions and in soil samples. Results from the first study confirmed that the dry bulb onion finishing process effectively reduces generic *E. coli* and *Salmonella* spp. to the FDA's GM and STV criteria. Both studies were performed in two greenhouses that did not have similar temperature ranges through both studies. Although both studies were performed in a greenhouse setting that did not fully mimic the field, the studies provided crucial preliminary data needed to justify field trials involving microbial decay on dry bulb onions in Treasure Valley.

The second study demonstrated that there is a significant difference in microbial die-off between agricultural soils. The rates of die-off for generic *E. coli* and *Salmonella* spp. were quite similar; however, the persistence between the two was significantly different in some

soils. This study demonstrates the increased microbiological risk of some soils. Post-irrigation strategies for reducing pathogens in soil and associated root crops may need to be adjusted based on the soil type of growing region/farm.

Future studies would most likely involve field trials further determining the efficacy of conventional curing as a mitigation process for dry bulb onions. Field trials would also allow for a better "real-world" approach for determining differences in microbial survival between soil types used in fresh produce development.

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APPENDIX

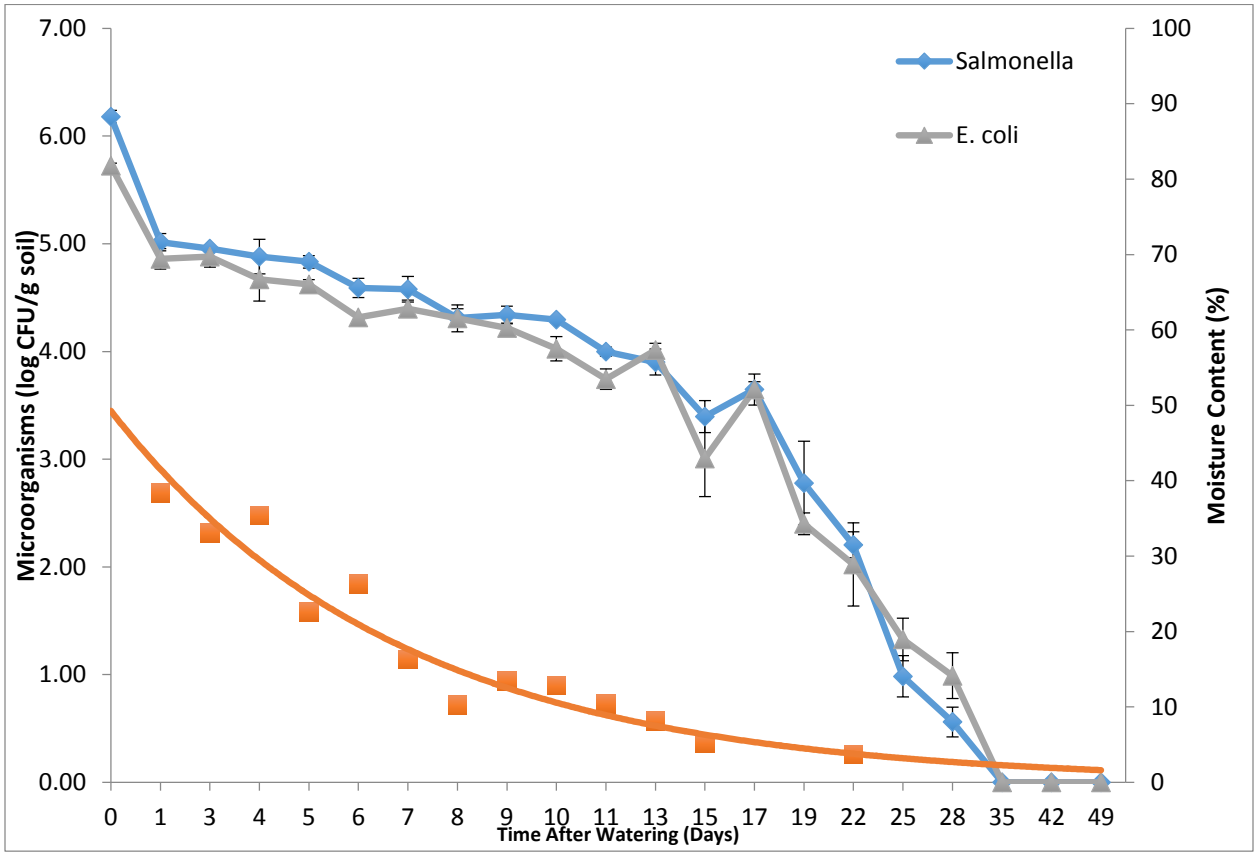
Appendix A

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

Soil analysis	Owyhee Silt Loam	Semiahmoo Muck
pH	8.1 ± 0.1	6.2 ± 0.0
Bray-P (ppm)	63 ± 2	114 ± 3
B (ppm)	0.1 ± 0.1	0.5 ± 0.1
Ca (ppm)	2586 ± 96	5282 ± 400
Cu (ppm)	1.2 ± 0.1	52.8 ± 0.7
Fe (ppm)	102 ± 5	1945 ± 55
K (ppm)	648 ± 16	449 ± 18
Mg (ppm)	627 ± 19	727 ± 36
Mn (ppm)	122 ± 9	318 ± 35
Na (ppm)	94 ± 3	101 ± 2
NH_4-N (ppm)	1.8 ± 0.4	22.0 ± 5.0
NO_3-N (ppm)	2.3 ± 1.3	35.9 ± 5.4
%OM LOI	2.32 ± 0.00	12.7 ± 0.4
Zn (ppm)	4.1 ± 0.2	44.1 ± 1.4
Sand %	14.2	15
Silt %	71.8	60
Clay %	14	25

*Analysis performed by Central Analytical Laboratory at OSU. n=3

Appendix B: Growth Chamber Study 2014



Survival of generic E. coli and Salmonella spp. on in Owyhee silt loam in a growth chamber. Data points represent the mean (n=3) with error bars indicating the standard error of the mean. Moisture content data was acquired after each sampling day (n=1).

Appendix C: CPS 2015 Poster

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

AGRICULTURAL WATER

Day 19 April 10 | Day 27 April 18 | Day 54 May 15 | Day 75 June 5 | Day 117 July 17 | Day 129 July 30

SUMMARY

The Food Safety Modernization Act (FSMA) has increased emphasis on microbial risks of irrigation water. The Treasure Valley of eastern Oregon and western Idaho has the highest yield of dry bulb onions in the country. Unfortunately, their irrigation water is often of poor microbiological quality. Dry bulb onions were grown in a greenhouse and continuously irrigated with water containing high levels (4.58±0.34 log CFU/ml) of generic *E. coli* and *Salmonella* spp. Upon maturity, irrigation was ceased and conventional curing practices were evaluated for their ability to reduce these bacteria on onion surfaces and soil. Within two weeks of ceasing irrigation, generic *E. coli* and *Salmonella* spp. levels were reduced from 3.45 log CFU/g to 0.41 log CFU/g on onions grown in both Semiahmoo muck and Owyhee silt loam soils. This data supports conventional curing practices as an effective strategy to mitigate risk associated with irrigation water of poor microbiological quality.

OBJECTIVES

- Evaluate conventional onion curing methods as an effective mitigation strategy to reduce generic *E. coli* and *Salmonella* applied via contaminated irrigation water.
- Compare survival and persistence of generic *E. coli* and *Salmonella* spp. on onions grown in two soil types.

METHODS

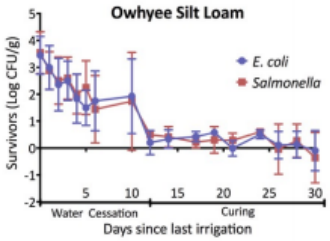
Spanish yellow dry bulb variety onions (Ovation variety) were grown in Owyhee silt loam and Semiahmoo muck soils in individual containers in two greenhouses and irrigated with contaminated water containing a cocktail of rifampicin resistant generic *E. coli* and *Salmonella* spp. (3.47 log CFU/ml) every 2-3 days throughout the growing period. At maturity, irrigation was ceased and onions were left undisturbed in the soil for two weeks. Onions were then lifted to the surface of the soil to cure for an additional two weeks. Onion and soil samples were collected, rinsed, and massaged with 0.1% peptone water (1:1). Serial dilutions of the rinsate were plated onto Hektoen Enteric Agar containing rifampicin (HE+rif) and selectively enumerated following incubation (37°C, 24-48 hours). As microbial levels decreased, a 96-well most-probable-number (MPN) assay was used in lieu of plating.

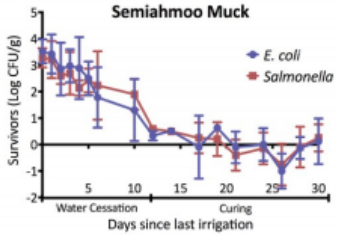
RESULTS TO DATE

The irrigation period resulted in a final contamination level of 3.69 ± 0.34 log CFU/g onion of both *Salmonella* and generic *E. coli*. During the first week of water cessation, survivors were reduced to 1.44 and 2.23 log CFU/g of onion and soil. After two weeks of sitting in soil undisturbed, generic *E. coli* and *Salmonella* spp. were reduced to a level of <1 CFU/g that persisted throughout the additional two weeks of curing. Generic *E. coli* and *Salmonella* spp. had similar decay rates (0.2 log CFU/g*d) on onions grown in both soil types.

BENEFITS TO THE INDUSTRY

This study demonstrates that conventional curing practices used in commercial onion production lead to a significant reduction of generic *E. coli* and *Salmonella* spp. Therefore, risks associated with the use of irrigation water of poor microbiological quality for onion production does not pose a substantial risk to end consumers. This data may be used by the onion industry to support their extensive irrigation-to-harvest interval as an effective mitigation strategy to comply with Produce Rule agricultural water quality requirements.





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CPS CENTER for PRODUCE SAFETY

Efficacy of conventional curing practices to reduce generic *E. coli* and *Salmonella* on dry bulb onions

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ABSTRACT

The Food Safety Modernization Act (FSMA) has placed increased emphasis on microbial risks from irrigation water. Treasure Valley (eastern Oregon and western Idaho) has the highest yield of dry bulb onions in the country. Unfortunately, their irrigation water is often of poor microbiological quality. Conventional curing practices may greatly reduce generic *E. coli* and *Salmonella* in dry bulb onions and provide a mechanism to comply with FSMA regulations. Spanish yellow dry bulb onions were grown in silt loam and muck soils in two greenhouses and irrigated with water containing a cocktail of rifampicin-resistant generic *E. coli* and *Salmonella* spp. (3.47 log CFU/ml) every 2-3 days during the final weeks of growth. At maturity, irrigation was ceased and onions remained in the soil for two weeks, lifted, and cured on the soil surface for an additional two. Onion and soil were collected, rinsed, and massaged with 0.1% peptone water (1:1). Serial dilutions of rinsate were plated onto Hektoen Enteric Agar with rifampicin and selectively enumerated following incubation (37°C, 24 hours). As microbial levels decreased, a most-probable-number (MPN) method was used in lieu of plating. The irrigation period resulted in a final contamination level of 3.69 ± 0.34 log CFU/g onion of *Salmonella* and generic *E. coli*. Thirteen days after ceasing irrigation, generic *E. coli* and *Salmonella* spp. were reduced to a consistent level of <1 CFU/g. Reduction of generic *E. coli* and *Salmonella* levels in onions during conventional curing demonstrates the low risk of contaminated irrigation water used in onion production.

OBJECTIVES

- Evaluate conventional onion curing methods as an effective mitigation strategy to reduce generic *E. coli* and *Salmonella* applied via contaminated irrigation water.
- Compare survival of generic *E. coli* and *Salmonella* spp. on onions and in different soils.
- Calculate rate of microbial decay in different soil types.

RESULTS

Conventional Curing Practices Reduce *E. coli* and *Salmonella* on Onions:

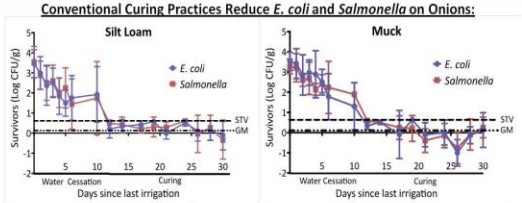


Figure 1. Reduction of generic *E. coli* and *Salmonella* spp. on onions grown in silt loam or muck soils and finished with conventional practices.
Water cessation = 12 days; Curing: 16 days. Error bars represent the standard error of each data set (n=5).

Table 1. Predicted values of Last Irrigation-to-Harvest Interval (days) to achieve the equivalent public health risk associated as proposed by the supplemental to the proposed Produce Rule.

Soil	Starting Generic *E. Coli* on Onions (log CFU/g)	Reduction Rates of generic *E. coli* (log reduction/day)		Last Irrigation-to-Harvest Interval (days) to Achieve Equivalent Public Health Risk	
		FDA Proposed Rate (Supplemental Produce Rule)	Experimental Generic *E. coli* Decay Rate	FDA Proposed Rate	Experimental Rate
Muck	3.64 ± 0.59	0.5	0.26 ± 0.02	GM: 8 days STV: 7 days	GM: 14 days STV: 12 days
Silt Loam	3.42 ± 0.43		0.20 ± 0.04	GM: 7 days STV: 6 days	GM: 17 days STV: 14 days

Microbial Reduction in Soils:

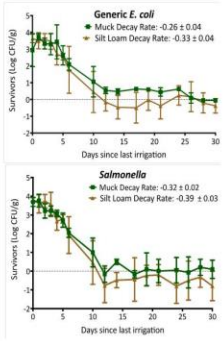
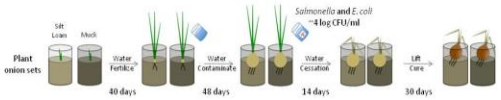


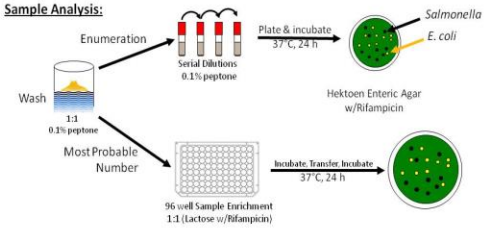
Figure 2. Reduction of generic *E. coli* and *Salmonella* spp. in muck and silt loam after ceasing irrigation.
Error bars represent the standard error of each data set (n=5).

METHODS

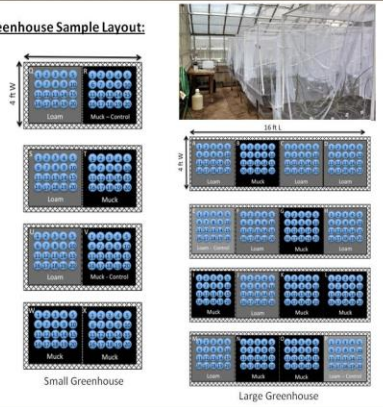
Onion Growing and Finishing Process:



Sample Analysis:



Greenhouse Sample Layout:



CONCLUSIONS

- Water cessation and curing effectively reduced the microbial load on dry bulb onions in soil.
- Reduction rates of generic *E. coli* and *Salmonella* on dry bulb onions were lower than the FDA-proposed reduction rates in the supplemental Produce Rule.
- Generic *E. coli* and *Salmonella* spp. exhibited a very similar response to water cessation and curing in both soil types and on the surface of the onion bulb, indicating generic *E. coli* would serve as a suitable predictor for the survival of *Salmonella* in future field trials.

ACKNOWLEDGMENTS

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Appendix E: Conventional Curing PRISM data: Interpolation of Muck Onion CFU/g

	E. coli	Salmonella
Third order polynomial (cubic)		
Best-fit values		
B0	3.741	3.484
B1	-0.3479	-0.3078
B2	0.009734	0.008137
B3	-8.763e-005	-6.793e-005
95% Confidence Intervals		
B0	3.374 to 4.109	3.160 to 3.808
B1	-0.4171 to -0.2788	-0.3683 to -0.2474
B2	0.006592 to 0.01288	0.005428 to 0.01085
B3	-0.0001245 to -5.075e-005	-9.938e-005 to -3.648e-005
Goodness of Fit		
Degrees of Freedom	97	97
R square	0.7863	0.7934
Adjusted R square	0.7797	0.7871
Absolute Sum of Squares	56.71	44.21
Replicates test for lack of fit		
SD replicates	0.7517	0.6373
SD lack of fit	0.8229	0.8301
Discrepancy (F)	1.198	1.696
P value	0.2852	0.0606
Evidence of inadequate model?	No	No
Number of points		
Analyzed	101	101

Interpolation of Loam Onion CFU/g

	E. coli	Salmonella
Third order polynomial (cubic)		
Best-fit values		
B0	3.066	3.203
B1	-0.2609	-0.2711
B2	0.007211	0.007343
B3	-6.338e-005	-6.258e-005
95% Confidence Intervals		
B0	2.708 to 3.425	2.820 to 3.585
B1	-0.3277 to -0.1941	-0.3425 to -0.1996
B2	0.004210 to 0.01021	0.004133 to 0.01055
B3	-9.820e-005 to -2.855e-005	-9.980e-005 to -2.537e-005
Goodness of Fit		
Degrees of Freedom	95	94
R square	0.6906	0.6792
Adjusted R square	0.6808	0.6690
Absolute Sum of Squares	50.40	56.77
Replicates test for lack of fit		
SD replicates	0.7262	0.7996
SD lack of fit	0.7380	0.6659
Discrepancy (F)	1.033	0.6936
P value	0.4342	0.7997
Evidence of inadequate model?	No	No
Number of points		
Analyzed	99	98

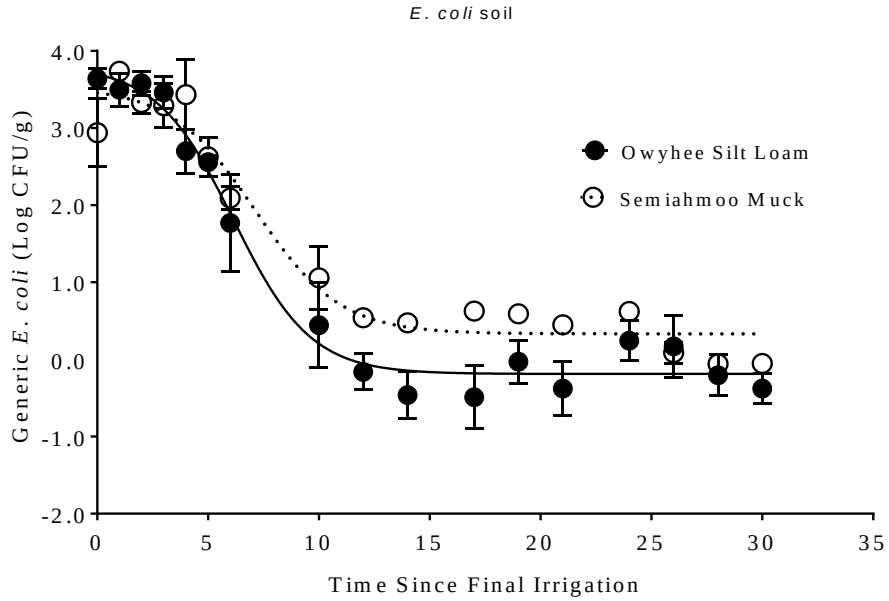
Interpolation of Muck cfu/g

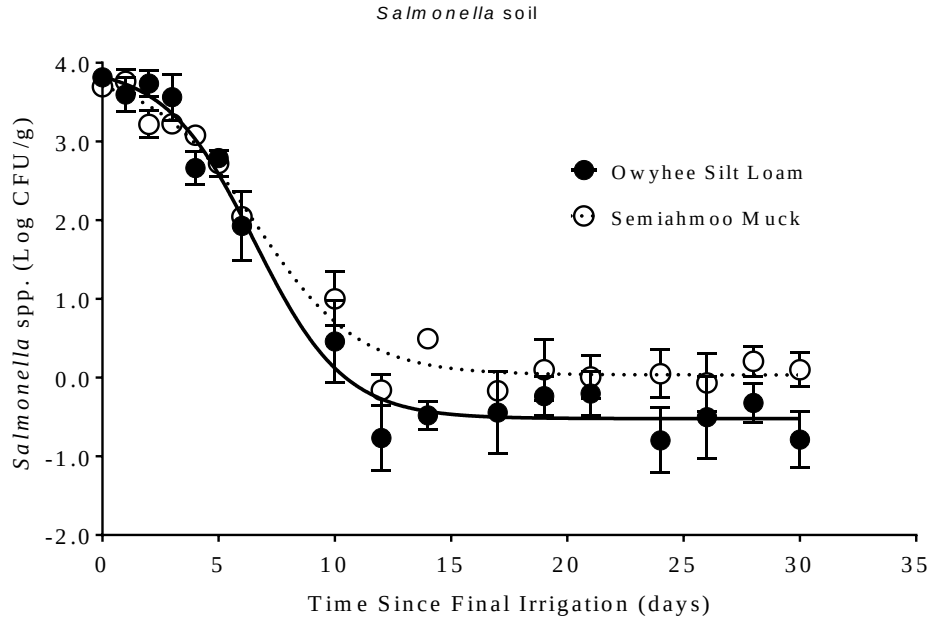
	Muck-Soil-E. coli	Muck-Soil-Salmonella
Sigmoidal, 4PL, X is log(concentration)		
Best-fit values		
Top	3.533	3.882
Bottom	0.3326	0.03499
LogIC50	7.130	6.585
HillSlope	-0.2270	-0.1967
IC50	1.348e+007	3.846e+006
Span	3.200	3.847
95% Confidence Intervals		
Top	3.128 to 3.938	3.367 to 4.397
Bottom	0.1584 to 0.5067	-0.1339 to 0.2039
LogIC50	6.021 to 8.238	5.574 to 7.596
HillSlope	-0.3281 to -0.1259	-0.2715 to -0.1219
IC50	1.050e+006 to 1.731e+008	375041 to 3.944e+007
Span	2.732 to 3.668	3.269 to 4.425
Goodness of Fit		
Degrees of Freedom	81	81
R square	0.8703	0.9051
Adjusted R square	0.8655	0.9016
Absolute Sum of Squares	22.49	20.11
Replicates test for lack of fit		
SD replicates	0.4859	0.4957
SD lack of fit	0.7032	0.5114
Discrepancy (F)	2.094	1.064
P value	0.0255	0.4041
Evidence of inadequate model?	Yes	No
Number of points		
Analyzed	85	85

Interpolation of LOAM cfu/g

	Loam-Soil-E. coli	Loam-Soil-Salmonella
Sigmoidal, 4PL, X is log(concentration)		
Best-fit values		
Top	3.809	3.965
Bottom	-0.1880	-0.5205
LogIC50	6.145	6.556
HillSlope	-0.2496	-0.2258
IC50	1.397e+006	3.601e+006
Span	3.997	4.485
95% Confidence Intervals		
Top	3.164 to 4.454	3.306 to 4.624
Bottom	-0.4100 to 0.03408	-0.7626 to -0.2785
LogIC50	5.139 to 7.151	5.491 to 7.622
HillSlope	-0.3974 to -0.1018	-0.3357 to -0.1160
IC50	137752 to 1.416e+007	309883 to 4.184e+007
Span	3.270 to 4.724	3.736 to 5.235
Goodness of Fit		
Degrees of Freedom	80	78
R square	0.8451	0.8685
Adjusted R square	0.8393	0.8634
Absolute Sum of Squares	39.28	40.32
Replicates test for lack of fit		
SD replicates	0.7245	0.7378
SD lack of fit	0.5623	0.6163
Discrepancy (F)	0.6024	0.6978
P value	0.8437	0.7585
Evidence of inadequate model?	No	No
Number of points		
Analyzed	84	82

Appendix F: Conventional Curing Soil Data

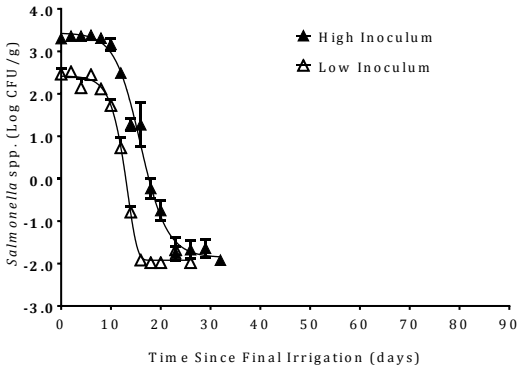




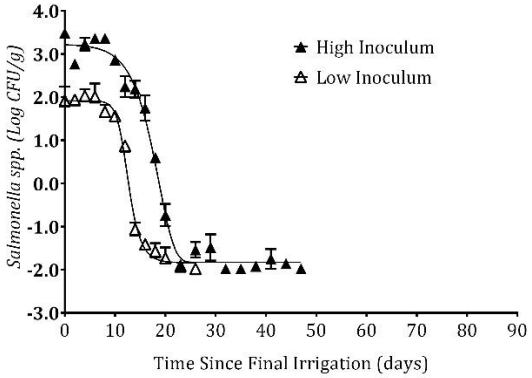
Reduction and persistence of generic *E. coli* and *Salmonella* spp. in Owyhee silt loam and Semiahmoo muck soil. Data points represent the mean (n=5) with error bars indicating the standard error of the mean. Lines represent the third order polynomial interpolation of each data set.

Appendix G: Reduction and persistence of *Salmonella* spp. in Oregon Soils

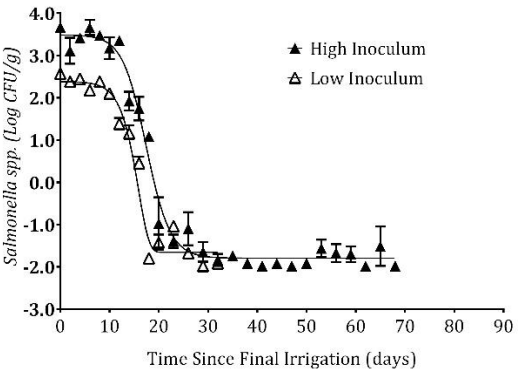
a) Quatama Loam



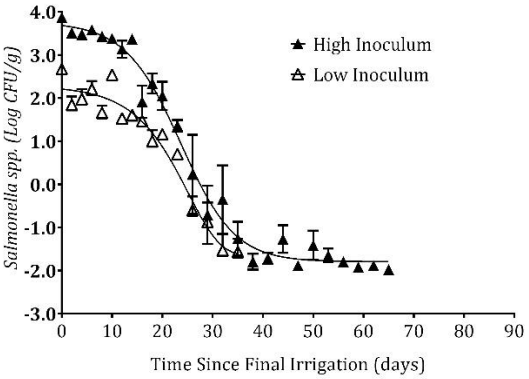
b) Latourell Loam



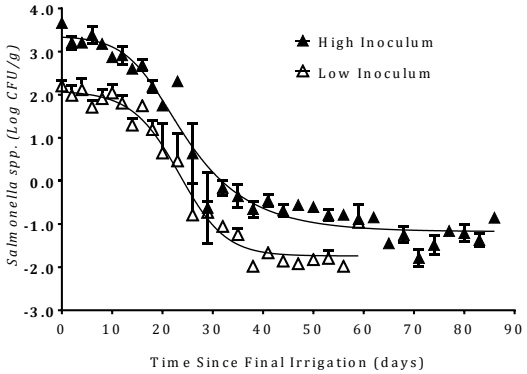
c) Willamette Loam



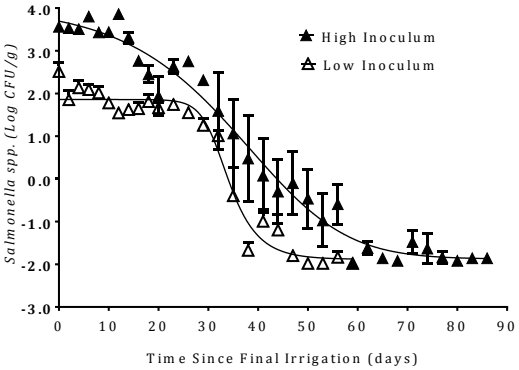
d) Madras Loam



e) Adkins Loam



f) Cullius Loam



Reduction and persistence of *Salmonella* spp. in a) Quatama loam, b) Latourell loam, c) Willamette loam, d) Madras loam, e) Adkins loam, f) Cullius loam soils when inoculated at high (3.5 log CFU/g) and low (2.0 log CFU/g) levels. Data points represent the mean (n = 5) with error bars indicating the standard error of the mean. Lines represent the sigmoidal interpolation of each data set.

Appendix H: Survival in Soils Prism Data: Survival in soils: Interpolation of High Inoculum

	Q-E. coli	Q-Salmonella	L-E. coli	L-Salmonella	W-E. coli	w-Salmonella	s-e.coli	s-salmonella	sc-E. coli	sc-Salmonella	c-E. coli	c-salmonella
Sigmoidal, 4PL, X is log(concentration)												
Best-fit values												
Top	3.196	3.44	3.106	3.165	3.486	3.49	3.565	3.52	3.579	3.742	3.457	3.831
Bottom	-1.746	-1.841	-1.998	-1.873	-1.876	-1.793	-1.566	-1.081	-1.732	-1.803	-1.792	-1.98
LogIC50	16.25	16.01	19.35	17.38	22.09	17.48	25.77	23.8	23.46	23.55	37.83	36.36
HillSlope	-0.1284	-0.1659	-0.08374	-0.1916	-0.06729	-0.1808	-0.066	-0.06961	-0.08433	-0.08037	-0.04571	-0.0404
IC50	1.786E+16	1.019E+16	2.216E+19	2.415E+17	1.227E+22	2.999E+17	5.949E+25	6.382E+23	2.866E+23	3.58E+23	6.826E+37	2.296E+36
Span	4.942	5.281	5.104	5.038	5.362	5.283	5.131	4.601	5.311	5.545	5.249	5.811
95% Confidence Intervals												
Top	2.903 to 3.488	3.252 to 3.627	2.818 to 3.393	2.998 to 3.333	3.122 to 3.849	3.275 to 3.704	3.232 to 3.898	3.175 to 3.865	3.223 to 3.935	3.368 to 4.11	3.013 to 3.900	3.259 to 4.403
Bottom	-2.027 to -1.46	-2.056 to -1.62	-2.176 to -1.81	-2.005 to -1.74	-2.037 to -1.71	-1.916 to -1.67	-1.718 to -1.41	-1.224 to -0.93	-1.946 to -1.51	-2.037 to -1.5	-2.157 to -1.42	-2.388 to -1.571
LogIC50	15.29 to 17.22	15.44 to 16.58	18.28 to 20.41	16.83 to 17.94	20.60 to 23.58	16.90 to 18.06	24.11 to 27.44	22.03 to 25.58	21.92 to 25.00	21.99 to 25.1	34.65 to 41.02	32.86 to 39.86
HillSlope	-0.1630 to -0.0	-0.1981 to -0.1	-0.09970 to -0.	-0.2286 to -0.1	-0.08090 to -0.	-0.2210 to -0.1	-0.08033 to -0.	-0.08645 to -0.	-0.1065 to -0.0	-0.1011 to -0.	-0.06082 to -0.	-0.05371 to -0.02709
IC50	1.936e+015 to	2.756e+015 to	1.913e+018 to	6.705e+016 to	4.005e+020 to	7.915e+016 to	1.287e+024 to	1.082e+022 to	8.276e+021 to	9.743e+021 t	4.438e+034 to	7.245e+032 to +infinity
Span	4.484 to 5.401	4.968 to 5.593	4.718 to 5.489	4.815 to 5.262	4.924 to 5.800	5.028 to 5.538	4.734 to 5.527	4.200 to 5.002	4.859 to 5.763	5.058 to 6.03	4.576 to 5.922	4.971 to 6.650
Goodness of Fit												
Degrees of Fr	71	66	97	90	136	131	161	161	131	126	161	161
R square	0.9416	0.971	0.9607	0.9701	0.9407	0.9497	0.9226	0.9064	0.9061	0.9106	0.8374	0.8478
Adjusted R sc	0.9392	0.9697	0.9595	0.9691	0.9394	0.9485	0.9212	0.9047	0.904	0.9084	0.8344	0.8449
Absolute Sum	18.29	10.12	16.08	15.06	37.14	36.52	57.48	55.38	67.08	65.15	132.9	137
Replicates test for lack of fit												
SD replicates	0.4864	0.368	0.379	0.3551	0.3746	0.4988	0.5654	0.5261	0.7467	0.7337	0.9355	0.9555
SD lack of fit	0.6005	0.4932	0.5199	0.5986	0.945	0.6479	0.7259	0.8062	0.5462	0.6454	0.7733	0.7541
Discrepancy	1.524	1.796	1.882	2.842	6.365	1.688	1.648	2.348	0.535	0.7738	0.6834	0.6229
P value	0.1414	0.0771	0.0317	0.0012	< 0.0001	0.0389	0.0309	0.0006	0.9571	0.7502	0.8834	0.9308
Evidence of ir	No	No	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No
Number of points												
Analyzed	75	70	101	94	140	135	165	165	135	130	165	165

Survival in soils: Interpolation of Low Inoculum

	Q-E. coli	Q-Salmonella	L-E. coli	L-Salmonella	W-E. coli	W-Salmonella	S-E. coli	S-Salmonella	Sc-E. coli	Sc-Salmonella	C-E. coli	C-Salmonella
Sigmoidal, 4PL, X is log(concentration)			Interrupted									
Best-fit values												
Top	1.952	2.361	1.994	1.929	1.955	2.368	1.818	2.078	2.062	2.219	2.104	1.874
Bottom	-2.058	-1.963	-1.834	-1.797	-1.862	-1.727	-1.98	-1.744	-2.988	-2.09	-1.809	-1.807
LogIC50	14.47	12.56	16.74	12.66	19.69	14.95	28.96	23.27	26.21	23.76	34.64	33.95
HillSlope	-0.1373	-0.3326	-0.07047	-0.3695	-0.2143	-0.2559	-0.1131	-0.09428	-0.05813	-0.08631	-0.104	-0.156
IC50	2.97E+14	3.655E+12	5.539E+16	4.588E+12	4.914E+19	8.905E+14	9.112E+28	1.853E+23	1.634E+26	5.738E+23	4.33E+34	8.969E+33
Span	4.01	4.323	3.829	3.726	3.817	4.095	3.798	3.822	5.05	4.309	3.913	3.682
95% Confidence Intervals												
Top	1.743 to 2.162	2.218 to 2.503		1.760 to 2.099	1.823 to 2.086	2.210 to 2.526	1.684 to 1.953	1.778 to 2.377	1.684 to 2.439	1.905 to 2.533	1.937 to 2.271	1.741 to 2.007
Bottom	-2.288 to -1.82	-2.115 to -1.811		-1.978 to -1.61	-2.051 to -1.67	-1.898 to -1.55	-2.122 to -1.83	-1.969 to -1.51	-4.292 to -1.68	-3.128 to -1.05	-2.051 to -1.56	-2.002 to -1.613
LogIC50	13.71 to 15.23	12.22 to 12.90		12.14 to 13.18	19.11 to 20.28	14.40 to 15.50	27.97 to 29.94	21.47 to 25.06	22.11 to 30.32	20.44 to 27.07	33.20 to 36.08	32.94 to 34.97
HillSlope	-0.1693 to -0.1	-0.4054 to -0.2599		-0.4931 to -0.2	-0.2691 to -0.1	-0.3205 to -0.1	-0.1385 to -0.0	-0.1267 to -0.0	-0.08247 to -0.	-0.1275 to -0.0	-0.1358 to -0.0	-0.2055 to -0.1066
IC50	5.150e+013 to	1.664e+012 to	8.028e+012	1.376e+012 to	1.278e+019 to	2.523e+014 to	9.436e+027 to	2.975e+021 to	1.274e+022 to	2.783e+020 to	1.578e+033 to	8.712e+032 to 9.234e+034
Span	3.651 to 4.370	4.105 to 4.542		3.467 to 3.985	3.574 to 4.060	3.849 to 4.340	3.589 to 4.007	3.411 to 4.232	3.470 to 6.631	3.087 to 5.530	3.598 to 4.227	3.437 to 3.926
Goodness of Fit												
Degrees of Fr	63	60		58	76	67	121	116	75	70	121	116
R square	0.9591	0.9735		0.9509	0.9511	0.9599	0.9434	0.8609	0.9232	0.8631	0.8943	0.9122
Adjusted R sc	0.9572	0.9722		0.9484	0.9491	0.9581	0.942	0.8573	0.9201	0.8573	0.8917	0.9099
Absolute Sum	6.749	6.591		9.483	10.7	10.07	21.1	49.61	12.48	21.78	40.83	30.39
Replicates test for lack of fit												
SD replicates	0.2548	0.3183		0.4111	0.3112	0.2194	0.4144	0.6578	0.3814	0.5217	0.466	0.4641
SD lack of fit	0.5752	0.3976		0.3657	0.6122	0.8187	0.4325	0.6351	0.5159	0.7066	0.9538	0.6967
Discrepancy	5.097	1.56		0.7913	3.87	13.92	1.089	0.9322	1.829	1.834	4.189	2.253
P value	< 0.0001	0.153		0.6256	0.0002	< 0.0001	0.3722	0.549	0.058	0.0635	< 0.0001	0.0047
Evidence of ir	Yes	No		No	Yes	Yes	No	No	No	No	Yes	Yes
Number of points												
Analyzed	67	64	67	62	80	71	125	120	79	74	125	120