THESIS

SURVIVAL AND PERSISTENCE OF *SALMONELLA ENTERICA* IN DRY BULB ONION PRODUCTION PRACTICES; A RISK ASSESSMENT APPROACH

Submitted by

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ABSTRACT

SURVIVAL AND PERSISTENCE OF *SALMONELLA ENTERICA* IN DRY BULB (DB) ONION PRODUCTION PRACTICES; A RISK ASSESSMENT APPROACH

The first chapter of this research thesis focuses on onion production practices and the potential risks associated with contamination of dry bulb (DB) onions along the cropping cycle with human enteric pathogens. Dry bulb onions are a widely consumed vegetable globally that has been previously thought to be safe from human pathogen contamination. This literature review summarizes the history of outbreak-related information, the biology of etiologies of interest, the body of literature associated with microbial risks and onions, and pre-and post-harvest production practices with respect to the risk of contamination with human pathogens. The information discussed in this review is useful for portraying the complex interactions of the microorganisms of interest and for industry professionals, producers, and consumers with respect to management and applicable risk mitigation efforts in the future for DB onions.

The second chapter of this research thesis focuses on determining the microbial risk factors associated with pre-and post-harvest commercial DB onion production practices. In the past 30 years DB onions have not been involved in foodborne illness outbreaks in the United States. However, two major multi-state foodborne outbreaks linked to *Salmonella* spp. (*Sal*) in 2020 and 2021 have altered the perception of producers and consumers about the microbial safety of this crop. Despite significant efforts to identify the source and route of contamination, little knowledge exists regarding the risk factors associated with enteric pathogen contamination along the DB onion cropping cycle. Thus, the goal of this research was to develop risk assessment profiles of

DB onion production practices capable of identifying and reducing *Sal* contamination in pre-and post-harvest activities. DB onion cultivars grown in the state of Colorado were used to determine the ability of these onions to potentially inhibit *Sal*. This was achieved by testing the minimum inhibitory concentration (MIC) with a cocktail of attenuated and pathogenic *Sal* at 3,600 mg/L of onion slurry. All evaluations indicated that there was no significant inhibition of *Sal* irrespective of the type of strain or DB onion cultivar. Pre- and post-harvest risk quantification was determined based on field inoculations of a 2-strain attenuated *Salmonella* (att*Sal)* cocktail. Survival and persistence of att*Sal* was assessed at multiple production stages including at the 3 leaf stage, lifting, topping, curing, harvest, transport, and packing house environments. From these evaluations, results indicate that att*Sal* is capable of surviving in both DB onions and soil for over a period of 64 days across the entire cropping cycle including harvest. Cultivar, agronomic practice, and UV index had no significant impact in our ability to recover att*Sal* and in the survival of these strains along the cropping cycle in soil or DB onion. At harvest, the population of att*Sal* on DB onion was 3.4 MPN/g at the three-leaf stage development (3LS), log 2.07 cfu/g at topping and log 1.87 cfu/g at lifting irrespective of DB onion cultivar. During interstate transport, the population of att*Sal* further decreased to undetectable levels (< 3.0 MPN/g of DB onion). This scenario was considered a low-risk event for packinghouse purposes.

Commercially grown DB onions were also included in all packinghouse evaluations. These onions were free from naturally occurring *Sal* and were inoculated with chalk containing att*Sal* to mimic soil dust contamination. This chalk had an initial att*Sal* population of log 5.5 cfu/g DB onion and for packinghouse purposes, it was considered a high-risk contamination event. A total of 14 locations within the packing line were selected to test the potential transfer of att*Sal* from inoculated DB onions to control treatments and food contact surfaces. Additionally, DB onions

from both high and low-risk contamination events were collected during sorting and packing. In both high and low -risk packing line contamination events, att*Sal* was not recovered from any food contact surface or DB onions (Total N= 897) over the course of 4 days of processing. A dry sanitation event was implemented in the packing line to assess whether such approaches could reduce contamination from att*Sal* or any other residues left by the crop or by previous activities at the packing line. Our dry sanitation cleaning protocol involved the cleaning of the crop contact surface with a dry brush-single use paper towel, followed by sanitation with a food grade alcohol wipe, followed by a spray of an ethanol alcohol solution (food grade) at 75%, followed by a final removal of alcohol residues with a dry single-use paper towel. This approach proved to be effective in reducing packing line residues Adenosine triphosphate (ATP) measurements and the population of two indicator organisms *Enterobacteriaceae* (EB) and *Escherichia coli* (EC). However, the effectiveness varied with the type of surface. Plastic and camel hair bristles were not cleanable. There was no correlation between the population of EB and EC and the presence of att*Sal* from high and low-risk contamination events. Dry sanitation events clearly indicated that it is a viable and useful practice that could be implemented on DB onion packing lines. The absence of cleaning and sanitation will be conducive to significant accumulation of DB onion debris and for the potential proliferation of indicator and pathogenic organisms. These findings are important to industry professionals, producers, and consumers regarding developing risk profiles and application of risk mitigation strategies to improve the microbial safety of DB onions.

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CHAPTER 1 Literature Review
Introduction

The global food trade system is an inherently complex supply chain, facing consistent demand increases for raw agricultural products. Human pathogen outbreaks due to foodborne illnesses have ascended with the rapid intensification of fresh produce production, processing, and distribution, expansion into new markets with different consumer habits and improved detection tools within our epidemiological system (Carstens et al., 2019; Gutierrez-Rodriguez & Adhikari, 2018; Smith et al., 2018). Food security and safety concerns must adapt in conjunction with consumer habits and the well documented increase in consumption of fresh produce (Aiyedun et al., 2021; Callejón et al., 2015). To prevent foodborne illnesses from happening, the point in which pathogens contaminate, persist and transfer to fresh produce must be understood during pre- and post-harvest practices. Numerous foodborne outbreaks have clearly indicated where microbial risk inflection points increase contamination risk (Harvey et al., 2017; Irvin et al., 2021; Weber, 2020). However, there are clear knowledge gaps in determining the source of contamination and the implementation of qualitative and quantitative risk assessment practices within federal and state regulations (Wahab et al., 2022).

Traditionally, management of microbial risks within food environments focus on characterizing the most important risk factors in the farm to fork continuum (Lammerding, 1997). The most important risk factors associated with fresh produce production and microbial contamination are of fecal origin within soils and manures, animal intrusion, contaminated irrigation water, health and hygiene of farm personnel, and sanitation practices before and after harvest activities (Alegbeleye et al., 2018; Luna-Guevara et al., 2019). Ideally qualitative and quantitative risk factors would mitigate potential foodborne outbreaks; however, risk assessment, management and

implementation are difficult to master or introduce into food safety systems due to cost barriers, lack of training and expertise in this field (Olimpi et al., 2019). Prevention strategies should be employed at different agronomic practices along the cropping cycle to mitigate the major known risk factors and to ultimately decrease the probability of foodborne illness occurring. The formal process of estimating risk was established by U.S federal agencies by means of standardizing regulatory decision-making specifically concerning human exposure to chemical substances; a predictive process that is developed to estimate the likelihood that a defined outcome could occur in a particular situation based on previous findings (Joint et al., 1995; Lammerding, 1997; World Health et al., 2006). However, for regulatory purposes associated with foodborne pathogens, this risk assessment process is significantly challenged because of the variability associated with the growing environment, how microorganisms grow and persist along different environments and the ability to define the source of contamination, which compounds the estimation of risk factors. Consequently, greater ambiguity around certain federal regulations impacts grower preventative practices, complying with regulations and significantly reducing risks within the farm that could positively impact consumers.

Human Pathogens on Plants (HPOPs) and the Environment

Considerable efforts to address reoccurring foodborne illness outbreaks have spurred research amongst food scientists, horticulturists, plant pathologists and some microbial ecologists. Finding the commonalities and differences between HPOPs and plant pathogens is in the interest to identify the best practices for mitigating produce contamination (Fletcher et al., 2013). Understanding the differences in epiphytic and endophytic colonization could provide insight into the possible routes of contamination that allowed human pathogens to colonize plants. Epiphytic or endophytic

colonization refers to undesired microorganisms colonizing plant surfaces or vascular tissues within the plant. Poor handling practices of fresh produce have been shown to increase the risk of fruit and vegetable contamination; and those challenging practices could introduce pathogens to the surface of crops that ultimately lead to internal colonization or harborage within plant microsites (Olaimat & Holley, 2012). Microsites within fresh fruits and vegetables can harbor multiple bacteria, protozoa and viral particles due to their heterogenous surfaces that serve as available niche space for these organisms allowing them to persist and find direct pathways into the plant. The hydrophobic nature of these sites, protects them against water soluble sanitizers that are not able to reach those crevices' and from desiccation and ultraviolet light (UV) exposure prompting research efforts looking to understand how enteric human pathogens' colonize and persist in non-host specific environments (Critzer & Doyle, 2010).

Enteric pathogens can cause intestinal illnesses that live within the epithelial linings of host specific human or animal intestines. Typically, host epithelial linings are rich in nutrients and favorable in temperature and osmotic conditions to promote biofilm formation and subsequent infection. The non-host environment of HPOPs found in crops is nutrient poor and fluctuates with diurnal temperature, osmotic conditions, and ultraviolet light (UV) (Smith et al., 2018). Microorganisms have evolved mechanisms to respond to environmental stress factors that would permit colonizing and persisting in niche space (Gálvez et al., 2010). *Salmonella* and other enteric microorganisms that associate with the highly competitive epithelial plane have gleaned a variety of cellular mechanisms through vertical and horizontal gene transfer that has developed microdiversity and subsequent ecotypes that display improved environmental fitness. These resistance mechanisms can be classified as adaptive, innate, or acquired (Guillén et al., 2021). Enteric microorganisms possess morphological, biochemical, and pathological mechanisms to

effectively succeed in colonization and bio-film formation at the epithelial plane of the gastrointestinal tract of humans and animals. These mechanisms have also been demonstrated to translate to improved epiphytic fitness in non-host environments and in some instances are the basis for recurring foodborne outbreaks associated with fresh fruits and vegetables.

Enteric pathogens like *Salmonella* spp (*Sal*), *Escherichia coli* O157:H7, *Campylobacter, Yersinia enterocolitica* and *Shigella* likely become associated with plants by direct exposure with contaminated manures or indirect contact with contaminated irrigation water, soil or other agricultural elements such as organic amendments, feces, manures and compost, insects and contaminated seeds, bioaerosols, and fungal, protozoan or nematode vectors (Critzer & Doyle, 2010; Fletcher et al., 2013; Liu et al., 2018; Smith et al., 2018). Contrary to enteric pathogens, many foodborne pathogens associated with environmental contamination including *Listeria monocytogenes* and *Clostridium* species, mainly cross contaminate produce through water, soil, and insects (Alegbeleye et al., 2018; Berger et al., 2010). In 2019 alone, globally *Sal* was responsible for 94 million documented instances of gastroenteritis and 155,000 deaths (Saw et al., 2019); and has become a significant concern for a variety of commodities and supply chains due to its gene-adapted properties to colonize, grow, survive, persist and transfer to non-host environment of HPOPs.

Sal is well equipped to adapt to the heterogeneous environment of the phyllosphere and rhizosphere of plants and is able to survive outside of the host gastrointestinal tract (Wiedemann et al., 2015). *Sal* has been previously described as natural endogenous components of the gastrointestinal microbiota of vertebrates and subsequent fecal matter evacuations can voyage into

aquatic systems runoff or via sewage discharge (Sha et al., 2013; Woodward et al., 1997). The persistence of *Sal* in conventional agricultural soils with diminished prokaryotic diversity has been shown to be longer in comparison to higher diversity systems; underlining the importance of maintaining soil health (Schierstaedt et al., 2020). Conventional agriculture practices that utilize mineral fertilization schemes have been shown to contain low microbiota richness, evenness and dispersion. Furthermore, these soil microbiota are characterized by their ability to adapt to nutrient limited environments (Hartmann et al., 2015). *Sal* may persist in this type of agricultural production systems due to numerous reintroduction events linked to production practices in conjunction with several different hosts and fitness characteristics including salt, acidity, desiccation, and chemotaxis. (Li et al., 2014).

Sal contamination is a complex public health concern in food (Besser, 2018; Popa & Papa, 2021). Disease patterns are determined by routes of *Sal* exposure, population dynamics, and climatic and ecological factors; driven by *Sal* biology and the impacted host population (Welch et al., 2018). Trends in *Sal* epidemiology has changed due to broad trends with regards to food production, consumption, types of products, and global industrialization and globalization of the food supply (Besser, 2018). *Sal*'s presence in crops is determined by an ecological cycle with key causal relationships that are difficult to predict salmonellosis outcomes in parallel to prevalence within the environment, climate change, microbial adaptation, and crop specific production practices (Akil et al., 2014). For example, in a five-year study of serovar diversity, distribution, and prevalence in a 500 square mile region of the central California Valley from 2011-2016, 91 *Sal* serovars were recovered and of the 24 most commonly identified serovars detected, sixteen were associated with those that commonly cause salmonellosis in the United States (Gorski et al., 2022). A deeper understanding of the causalities within local ecological cycles like those in the Central

Valley of California in which *Sal* establishes within production environments is critical in the underlying occurrence of large-scale foodborne illness outbreaks. Therefore, sources of human pathogen contamination in local agricultural production regions exists and understanding the ecological transfer of *Sal* from those environments into fresh produce is critical to reduce foodborne diseases associated with this pathogen. Our ability to identify the risks of contamination and minimize transfer based on risk factors of *Sal* during agronomic practices, packinghouse activities and transport has not been well characterized and significant knowledge gaps exist in this realm.

The biology of *Sal* plays an integral role in the acclimatation, persistence and distribution of this enteric pathogen along the cropping cycle and must be considered at every stage of production. First, *Salmonella enterica* is highly diverse and serovars have evolved adaptive responses to delicately regulate resource distribution between growth and survival within resource limited and dry environments to survive and persist in food contact surfaces, soil, and water (Andino & Hanning, 2015). Second, *Sal*'s ability to adapt and persist in non-host environments creates difficulty for risk management strategies, detection, and subsequent prediction of product contamination. Ecological persistence of *Sal* in farm environments and the risk posed in reintroduction events and persistence in soil and water compounds is underlined by *Sal* biology and critically may explain probable transfer from pre-harvest production to the latter post-harvest and distribution phases.

History of Foodborne Outbreaks Related to DB- and Green Onions

Acute diarrheal disease attributed to *Sal* continues to remain a major public health issue that presents a need for worldwide improvement in pathogen mitigation strategies (Popa and Papa, 2021). Nontyphoidal *Sal* bacterial foodborne illness is estimated to be one of the highest financial and health burdens globally (Gorski et al., 2022). The Centers for Disease Control and Prevention (CDC) has reported that between 2006 and 2017, 53.4% of all foodborne outbreaks in the United States (US) were attributed to *Sal* and of these, 32.7% were linked with the consumption of produce (Liu, 2018). In the past 30 years, the United States only experienced two multi-state *Sal* foodborne illness outbreaks associated with DB onions in 2020 and 2021, a rare occurrence in this industry.

Prevention of *Sal* outbreaks in whole DB onions is of critical importance to DB onion producers' success in the future. The root-causes of raw produce-associated outbreaks are difficult to identify as multiple routes and sources exist throughout the supply chain with numerous possible contamination points. In July of 2020 a bi-national foodborne outbreak linked to *Salmonella* Newport (*SalNewport)* was identified by the Canadian Food Inspection Agency, the Federal Drug Administration (FDA) and the CDC linked to California grown whole red onions and later considered to be one of the largest foodborne outbreaks in the last 10 years in the Nation (Food and Drug Administration, 2020). Such events have created significant effects across the industry, as no previously documented event had been recorded in over 30 years and the crop was considered of low risk by the industry and FDA understanding of crop production, handling, and storage practices. As the investigation of the outbreak concluded in late October of 2020 across states and North American countries, the industry began to question whether current practices and growing conditions could be a significant risk of contamination and initiated funding projects addressing

those needs and in updating the 2010 NOA commercial DB onion growing practice manual. One year after this initial outbreak, the unthinkable occurs and in October 2021, a new outbreak linked to DB onions was identified with whole fresh red and yellow onions imported from Chihuahua, Mexico (FDA, 2021b) and contaminated with *Salmonella Oranienburg* (*SalOranienburg)*. Since the reporting of foodborne outbreaks initiated in the United States, these outbreaks represented the first two events of their kind linked to the DB onion industry and represented a significant departure from the consumer and industry perception of this crop being safe and unlikely to be associated with a multistate and multiregional foodborne outbreak.

Increased foodborne outbreak occurrence in food and specifically in fresh produce has been at the forefront of federal legislation by the FDA and with significant focus and efforts since 2011 with the signing of the Food Safety Modernization Act (FSMA) detailing requirements and recommended measures to reduce and mitigate contamination along food supply chains. The FSMA, which includes within 8 new regulations the establishment of the Produce Safety Rule (PSR), has been the most significant reform in food legislation since the 1970's and has been implemented with a goal of preventing foodborne outbreaks across multiple food categories. The safety of fresh produce has and continues to be one of the most critical and difficult food categories to regulate because of four main issues: 1- fresh produce can be consumed raw and no additional kill steps exist within their supply chain, 2- short cropping cycles and mass quantities of land under production create significant challenges to protect the crops against contamination, 3- numerous potential routes of contamination in large quantities of product that prevent investigation efforts in pinpointing which source is to blame and most importantly how to prevent or eliminate this source from entering the system and 4- once contamination is within the farm environment, there are no concrete and tangible tools to eliminate contamination from farmland or irrigation water, making

prevention of crop contamination the key to reducing these outbreaks while also recognizing that there is a finite amount of resources to keep farming and feeding consumers.

The PSR provides requirements and recommendations for growers looking to mitigate risks associated with agricultural water quality, wildlife and domestic animal intrusion, biological soil amendments, worker health and hygiene and packinghouse sanitation practices. DB onions are subject to the PSR and a year prior to establishing the FSMA, in 2010, the National Onion Association (NOA) released commodity specific food safety guidelines regarding the DB Onion Supply Chain. These guidelines were updated in 2022 due to the outbreaks linked to *Sal;* however, both documents lack specific information regarding risk factors along the cropping cycle, mitigation strategies to reduce contamination during pre-and -post harvest activities including activities related to storage, handling, and packaging of DB onions. These limitations can be clearly associated with 3 factors: 1- an industry that believes in the safety of the crop, 2- lack of fundamental research associated with growing, harvesting and handling practices and 3- lack of urgency to expand these practices before the 2020 and 2021 multiregional or multistate outbreaks.

The two recent foodborne outbreaks of *Sal* linked to DB onions surpass in magnitude of human health effects on previous multiple outbreaks implicated with Sal and other human pathogens in green onions; however, both commodities have received significant national attention due to the impact of their respective outbreaks on consumer health. Concomitant with the 2010 DB onion commodity specific produce safety guidelines, in the same year, the green onion industry also released guidelines looking to reduce contamination of this crop in pre- and postharvest activities. However, the green onion producer guidelines originated from numerous outbreaks and applied research efforts looking to reduce contamination. Concern began in the fall of 2003, when the FDA

had announced 3 separate outbreaks of viral hepatitis A (HVA) associated with green onion consumption (CDC, 2003). Outbreaks later in the same decade in 2000 associated with Shigella and in 2006 and 2009 linked to *Escherichia coli* (*E. coli*) O157 H7 NM and *Sal* respectively and the 2010-2011 survey of crops of concern performed by the Canadian Food Inspection Agency (CFIA) triggered industry and federal responses to address sources and routes of contamination (CDC, 2000; CDC, 2003; CFIA, 2011; CIDRAP, 2006; CFIA, 2009). In the CFIA survey (n= 591) of both imported and domestic conventional and organic green onions from across Canada, one sample (0.2%) of domestic conventional produced green onions tested positive for *SalOranienburg*, and one other (0.2%) tested with an elevated level of generic *E. coli*, leading to a product recall in response to the positive *Sal* sample (CFIA, 2011). Green onions are labor intensive and highly perishable with a 7-10 day shelf life requiring storage conditions of 0°C and relative humidity above 95% (Smith et al., 2011). Sprinkler irrigation, hand harvesting and washing of the crop can create the necessary conditions for contamination as indicated by outbreak investigations and commodity specific green onion production guidelines (Calvin et al., 2004), (FDA, 2010). Further, they are frequently consumed raw, making personal hygiene in relation to Good Agricultural Practices (GAP) and Good Handling Practices (GHP) critical throughout harvesting and handling of green onions to minimize the risk of contamination. Overall, green and DB onion are mainly consumed raw, but their growing, harvesting and storage practices vary significantly. In green onions, a short growing cycle, mixed with sprinkler irrigation and hand harvesting practices provide clear potential routes for pathogen contamination that will allow enteric pathogens to gain access to nutrient reach microsites and moisture, with little to no environmental or human-based intervention strategies at harvest or beyond to reduce contamination. For DB onions a long growing season coupled with furrow and drip irrigation practices and mechanical harvest and curing procedures seems to provide conditions that could

expedite enteric pathogen die-off as it has been suggested by several researchers (Emch & WaiteCusic, 2016; Shock et al., 2013; Shock et al., 2016; Wright et al., 2018). However, hand topping and harvest practices, the use of burlap sacks and lack of sanitation practices in the packing line would provide that contamination event at the end of the growing season able to provide the necessary conditions for enteric pathogens to survive. Limited research around these practices and their persistence during storage, handling, sorting, and packing, compound our ability to understand the risk factors within DB onions.

Lingering questions around the effects of UV light exposure during the growing season and how it may or may not expedite enteric pathogen die-off is unknown and whether soil moisture could support persistence along the cropping cycle. There are, however, current efforts that provide insights on how soil moisture supports growth and persistence of *E. coli O157:H7 and Sal* (Gutiérrez-Rodríguez et al., 2012; Lee et al., 2019; Van Der Linden et al., 2014). Specifically, how these parameters (UV index and soil moisture) impact survival of these pathogens in DB onion practices is not known and could provide detailed risk factors along the cropping cycle.

From the perspective of storage, handling, and sorting practices, it's well described in the 2010 and 2022 DB onion guidelines, the importance of managing these activities under dry conditions, which implies dry sanitation practices to prevent moisture accumulation. The absence of dry sanitation, clean breaks and wet sanitation practices on human pathogen contamination at the packinghouse has been well documented in the caramel apple *Listeria monocytogenes* (*Lmono)* outbreak of 2014-2015 (Ward et al., 2022). In this instance, a gram-positive environmental pathogen was able to survive and persist inside a dry packinghouse for over 6 months. Outbreaks linked to low water activity foods, manufactured under conditions with little to no water, have highlighted the need to understand which genome-based mechanisms are used by *Sal* to survive in

those environments and which, if any, dry sanitation practices could reduce survival and transfer of *Sal* to different commodities. Significant efforts evaluating the survival and virulence expression of *Sal* on low-water activity foods, suggest 3 key mechanisms used by *Sal* for low moisture stress adaptation: 1- osmotic stress induces changes in cell membrane fatty acid and phospholipid composition (Chen et al., 2014), 2- desiccation increases the accumulation of trehalose; an important osmolyte capable of stabilizing proteins and supporting retention of enzyme activity (Li et al., 2012) and 3- the presence of small RNAs (sRNAs) that support and increase stress adaptation to desiccation (Barnhill et al., 2019). These 3 mechanisms could take place simultaneously in the transition from harvest to storage and packaging of DB onions since in these steps no sanitizers are used that could elicit other stress adaptation mechanisms. Further, most packinghouse sanitation practices don't involve water or typical sanitizer applications, which coupled with significant DB onion residue accumulation provide the ideal conditions for rich biofilm formation. It has been well characterized that dry sanitation conditions support biofilm establishment (Galié et al., 2018) and significantly increases the potential for pathogen growth and survival and subsequent transfer of enteric pathogens to multiple commodities. To that extent, the DB onion industry needs support in quantifying the potential transfer of *Sal* from contaminated onions to packinghouse equipment, an understanding of how dry vs wet sanitation practices impact survival and persistence of *Sal* and which cleaning, and sanitation practices could be implemented to remove this organism from low moisture environments.

Pathogens Characteristics Related to Outbreaks with DB and Green Onions

Salmonella

Sal, the causative agent of salmonellosis is an enteric pathogen of warm-blooded animals and humans that is a Gram-negative, rod-shaped, non-sporulating facultatively anaerobic microorganism (Fàbrega & Vila, 2013). Salmonellosis clinically presents as gastroenteritis, or symptoms akin to it, and incubation times typically are between 6 and 48 hours (Crum-Cianflone, 2008) The primary infection routes of *Sal* in humans is typically via fecal-oral transmission or through the consumption of contaminated products. Salmonellosis minimum infectious dose for certain members of the population could be less than 100-125 colony forming units (CFU) (Fatica & Schneider, 2011). *Salmonella enterica*, subspecies *enterica* (I) is one of six subspecies of *Salmonella* and contains the serotypes typically causing human disease (Fierer & Guiney, 2001). With greater than 2500 recognized serotypes, there is substantial variation in epidemiological and ecological characteristics of serotype diversity within *Sal*; determined by variable genetic characteristics in selective evolutionary processes such as gene acquisition/deletion, horizontal gene transfer, positive selection, homologous recombination, and changes in population size (Liao et al., 2019).

Sal possesses a variety of mechanisms to deal with exposure to variable environments over the course of dispersal in the host. Typically, levels of pathogenicity to the host are determined by virulence factors. *Sal* pathogenicity islands (SPIs) acquired via horizontal gene transfer, contain cassettes that encode important virulence factors that govern pathogenesis (Pradhan & Devi Negi, 2019; Wiedemann et al., 2015). Pathogenicity islands within the genome of *Sal* contain specific sets of genes responsible for clinical representations such as typhoid fever, enteritis, or non-typhoid infections (Fierer & Guiney, 2001). Type III secretion systems (T3SSs), a syringe like apparatus essential for pathogenicity in Gram-negative bacteria enhance invasion, inhibit phagocytosis, promote bacterial trafficking and activating host cell apoptotic pathways (Hallstrom &

McCormick, 2015). *Sal* encode genes for adhesion, invasion, and toxin genes in the SPI groups (Santos et al., 2003). Within larger gene cassettes, small endogenous mobile elements exist, where greater than 20 SPIs contain enteropathogenesis genes such as *inv*A, *spv, fim*A and *stn* (Sabbagh et al., 2010). The *inv*A and *spv*C genes importantly code for a protein necessary for invasion and survival within host animal epithelial cells, respectively (Chiu & Ou, 1996; Darwin & Miller, 1999). These genetic markers are effective in the rapid identification of *Sal* in sample matrices via multiplex PCR methods in clinical diagnostics and food safety applications.

Sal species are adept in colonization at their natural infection site, the lumen side of the epithelial lining of the human gastrointestinal tract and competing for suitable niche space. In animals and humans, *Sal* infection requires attachment and adhesion to host surfaces, the production of bacterial factors to aid with invasion, proliferation and evasion of host defense mechanisms (Wiedemann et al., 2015). *Sal* are capable of attachment to biotic and abiotic surfaces. In previously conducted experiments with tomato and lettuce, *Sal* were shown to be capable of attachment and colonization of plant tissue as an endophyte and epiphyte (Gu et al., 2011).Proteinaceous surface appendages called fimbriae, particularly curli fimbriae, ameliorate attachment to chicken intestinal linings and bio-film association of *Sal* on alfalfa sprouts, parsley, and tomato leaves (Barak et al., 2009; Barak et al., 2007; Cevallos-Cevallos et al., 2012; Jones & Dangl, 2006; Lapidot & Yaron, 2009; Ledeboer et al., 2006). *Sal enterica* Enteritidis has been previously characterized in forming biofilms on abiotic surfaces and has been shown to produce an O-antigen capsule expressed in coregulation with fimbria- and cellulose-associated extracellular matrix; a potentially conserved method of environmental persistence regulated by the *yih* group of genes (Barak et al., 2007; Gibson et al., 2006; Guard-Petter et al., 1996). Multicellular and aggregative morphotypes such as rdar, rugose, and lacy are often observed in conjunction with

collocated fimbriae, cellulose and extracellular polysaccharide secretion (EPS) to cooperatively form extracellular matrix and bolster environmental persistence and acid resistance in biofilms (Anriany et al., 2001; Cevallos-Cevallos et al., 2012; Guard-Petter et al., 1996; Römling et al., 1998). The *rpos* gene both *EC* and *Sal* possess is key to global regulation of larger groups of genes associated with environmental fitness (Nickerson & Curtiss, 1997). This highly conserved master regulator of the bacterial general stress response (GSR) *rpos* gene reflects the antagonistic pleiotropic tradeoff of long-term fitness in stressor adaption over the short-term cost to reproduction (Abram et al., 2021; Snyder et al., 2012; Somorin et al., 2016). RpoS is typically induced upon exposure to abiotic stressors such as acid, heat, oxidative stress or upon initiation of the stationary growth phase (Guillén et al., 2021).

Many environmental factors are stressors to bacterial cells, deleterious stressors of nutrient limitation, pH, osmolarity and temperature stratification, for example. Environmental stress can therefore be defined as any external factor to a bacterial cells that applies adverse consequence on the physiological state of cells, resulting in changes in reproductive growth rate, or inactivation or death of individual cells or entire populations (McMahon et al., 2007). The DB onion supply chain is complex and lacks uniform approaches that depend on local production practices. However, there exists similarity in environmental stratification across the production phases inherent in the open field production, interstate transport, storage, and packing house operations. However, *Sal*'s biology has led to its emergence as an etiology of concern for food safety managers in multiple commodities due to their evolving environmental fitness.

Some of the known 2,500 human pathogenic species of *Sal* have been previously shown to proliferate at temperatures between 8°C and 45°C, survive in 0°C temperatures, pH values as low

as 3.3 and as high as 9.5, and with water activity (a_w) as low as 0.35 (peanut butter) or in dry environments (Beuchat & Mann, 2014; Canakapalli et al., 2022; Foster, 1993; Liu et al., 2021; Medus et al., 2009; Yang et al., 2022). These adaptations are clearly viable means to withstand the variable environmental exposures in open field environments, storage, and packinghouse phases of the DB onion supply chain. *Sal* has been previously described in its ability to survive in aquatic environments by entering the viable but nonculturable (VNBC) state, existing within freeliving protozoa, facilitate extended survival within water biofilms, and exist as viable but not culturable cells (Guillén et al., 2021; Liu et al., 2018); although there is still debate on whether these cells are capable of reverting causing pathogenesis in more favorable environments. Currently, as the DB onion industry does not treat irrigation applications for potential microbial load, there lies inherent risk in irrigation supply, shared infrastructure, and late season applications as *Sal* is capable of existing in the environment in various physiological states. To effectively survive potentially fatal changes in microenvironments, S*al* encodes 21 regulator proteins/systems to respond to various environmental stress factors and these responses are correlated with environmental fitness and survival in commercial environments outside of the host (Spector & Kenyon, 2012). The antiquated method of keeping production methods dry is not sustainable for the DB industry as a catch all for minimizing bacterial load. The lack of dry sanitation events in packing houses may promote the formation of biofilms in food contact surfaces although there exists low moisture availability Interestingly, there are similarities in the mechanical phospholipid membrane fluidity in response to thermal and desiccated anhydrobiotic *Sal* cells (Pereira & Hünenberger, 2008).

Sal are capable of surviving in nutrient and moisture deficit environments and respond through alterations of their phospholipid bilayer. Of particular interest to DB onion curing and post-harvest

processing activities, *Sal's* desiccation response mechanism enables long term survival within low moisture food environments and surfaces. Cell viability and length of survival in is dependent on a variety of climactic factors (i.e., temperature, moisture, access to suitable nutrient substrates, UV, material, strain, etc.). *Sal's* physiological ability to survive in anhydrobiotic environments is evident in recent FBI outbreaks associated with low-moisture ready to eat products such as peanut butter, dried fruits, infant formula, raw almonds, dried coconut, dry seasonings, potato chips, and chocolate (Scott et al., 2009). Within two hours of drying on a surface, *Sal* globally upregulates fatty acid (FA) metabolism functional groups in nutrient scarce environments to degrade long chain FA and produce acetyl-coA to supply the tricarboxylic acid (TCA) cycle; an energy efficient method of producing cellular adenosine triphosphate (ATP); potentially altering phospholipid bilayer fluidity (Li et al., 2012). Furthermore, drying did not increase relative expression of genes associated with filamentous cellular morphologies of red and dry, or rough (*rdr)* aggregate cellular morphologies previously reported in the literature (Mattick et al., 2000; White et al., 2008). Trehalose, a glucose disaccharide, has been previously hypothesized to protect the phospholipid bilayer structure by maintaining fluidity from osmotic, shear, and hydrostatic stressors (Elbein et al., 2003; Pereira & Hünenberger, 2008). *Sal* expression of *fabA*, instrumental in unsaturated FA biosynthesis, showed an increased ability to survive, while an increased expression of *cfa* (instrumental in post- synthesis FA modification), showed decreased survival with exposure to low water availability food, respectively (Chen et al., 2014). The exact mechanisms and cascade of events in which *Sal* globally regulates stress responses associated with membrane fluidity and FA functional group expression remains uncertain. However, *Sal* has been described to coordinate gene expression in detection of low-moisture environments to maintain turgor pressure, primarily through altering fatty acid metabolism and potentially through physical alteration of the phospholipid bilayer through vitrification or via water entrapment; a function which may depend

on the polysaccharide availability within the environment or food. *Sal* expression of *fabA*, instrumental in unsaturated FA biosynthesis, showed an increased ability to survive, while an increased expression of *cfa* (instrumental in post- synthesis FA modification), showed a decreased of survival with exposure to low water availability food, respectively (Chen et al., 2014). Lowmoisture and low water available foods that are ready to consume such as peanut butter, dried fruit, and infant formula consist of a variety of simple and complex polysaccharides that could potentially enable vitrification or water-entrapment. The vitrification process hypothesizes that sugars exogenous to the cell may form a crystalline structure preventing structural or mechanical disruption of the phospholipid bilayer and subsequently prevent membrane fluidity of cells (Sun & Leopold, 1997). Water entrapment may result in maintaining phospholipid bilayer integrity by concentrating residual water molecules in proximity to the lipid-heads (Belton & Gil, 1994). Internally, cells may be coordinating global responses to desiccation with non-coding sRNA (ncRNA). *Sal* has become a robust model for RNA-mediated regulation of environmental stressors. Recently, 21 of 71 novel sRNA knockouts separately (sRNA1320429, sRNA3981754) separately showed significant reduction in the ability of *Sal* cells to survive desiccation while antagonistically showing an enhanced ability to excess heat (Barnhill et al., 2019). These descriptions have inherently changed the perceptions of food safety with regards to foods with low-moisture and therefore low water availability. These responses are instrumental to *Sal's* emerging environmental fitness and are of major concern to the pre-and post-harvest production and distribution phases of the DB onion supply chain.

Foods have been altered with moisture reduction agents to prevent microorganisms from surviving in production environments (Bourdichon et al., 2021). Sal is known to persist in low moisture conditions. The National Onion Association and conversational ideologies amongst DB onion

producers revolves around keeping DB onion packinghouse facilities dry; a viable food safety management guideline that minimizes the potential for microbial growth due to moisture. However, DB onions inner-scales are high in moisture content, between 80% to 90% of a raw onion's mass is water (Sidhu et al., 2019) and although the outer skins are dry, the whole bulb can become damaged throughout packinghouse activities depositing residues, nutritive organic material, moisture, and soil and plant material within surfaces of production equipment and potentially re-deposit residues to the outer skins. The DB onion industry must adopt and track trends in hygiene monitoring of surfaces and key production equipment where residues and potential biofilms may form from contaminants transferred from pre-harvest production into postharvesting processing. Without frequent sanitation events, the duration and volume of processing onions over time inevitably produces residues that may be conducive to microorganisms' growth and the potential for cross-contamination of mass quantities of DB onions is evident in the binational and multistate outbreaks.

In 2019, the Foodborne Diseases Active Surveillance Network (FoodNet), part of the CDC's Emerging Infections Program, found of 6,656 serotyped, *SalNewport* was one of the six most commonly found in clinical diagnosed presentations (Danielle M. Tack, 2020). In the past, *SalNewport* has been previously linked to foodborne outbreaks with beef, cheese, cucumbers and tomatoes (McCormic et al., 2022). Recovery of *SalNewport* in agricultural environments is evident, particularly in surface ponds intended for production agriculture and fresh produce. Li et al. (2014) previously recovered 50 of 170 (29.4%) water samples over a 27-month period from 10 selected surface irrigation ponds dedicated as sources of irrigation intended for vegetable production. Of these 50 samples, 16 *SalNewport* species were identified, and more than half of the isolates were multidrug resistant (MDR) (Li et al., 2014). Commonly associated with a wide

range of animals both wild and domesticated, antibiotic resistant *SalNewport* have distinct food-animalborne origins that are significant to the isolates recovered within the environment (Pan et al., 2018). This characterization is critical to understand in building risk mitigation strategies for vegetable crop producers in proximity to animal husbandry operations. Despite on-farm mitigation strategies, there are considerable ecological and spatial distribution aspects to *Sal* contamination that are not controllable even if they are known, such as interconnected irrigation infrastructure and proximity to concentrated animal feedlot operations (CAFO). The 2020 FIB implicating red DB onions and *SalNewport* serotype may be an example of both situations as the traceback efforts categorized environmental samples in production land proximal to the onion fields implicated in Bakersfield and Holtville, CA near shared irrigation infrastructure, and CAFO. Traceback efforts of 831 environmental samples, 26 testing positive for *Sal*, 11 for the serotype Newport, and zero positives for the outbreak strain (FDA, 2020) left largely the root cause and storyline to speculation. Because Sal is environmentally fit, they could be difficult to detect in low moisture conditions in pre- and post-harvest environments.

***Escherichia coli, Shigella* spp. and Hepatitis A virus (HVA)**

E. coli is also a Gram-Negative, rod-shaped, facultative anaerobe that is a naturally occurring microorganism of gastrointestinal systems of humans and animals (Croxen et al., 2013). Generic EC is considered an opportunistic pathogen only impacting immunocompromised individuals and within this species; 6 different pathotype groups are considered pathogenic. This pathotype groups include 1-Enteropathogenic (EPEC), 2-Shiga-toxin producing/enterohemorrhagic (STEC/EHEC), 3-Verocytotoxin (VTEC), 4-Enteroinvasive (EIEC), 5-Enteroaggregative (EAEC) and 6Enterotoxigenic (ETEC); and can cause diarrheal disease (Le Bouguénec & Servin, 2006).

The fresh produce industry has seen EHEC and STEC related outbreaks in both pre- and postharvest with contaminated animal feces and through preparation stages through cross-contamination via equipment, surfaces, and handlers. The serotypes contained within the EHEC group, attribute to the most severe disease cases such as bloody diarrhea, thrombic thrombocytopenic purpura (TTP), hemorrhagic colitis and hemolytic uremic syndrome (Forsythe, 2020). Particularly, the EC serotypes (O104:H4 and O157:H7) are of concern within fresh produce production worldwide. In 2011, 15 European countries witnessed a serious fresh produce outbreak associated with EC STEC owing to misinformation about the source, delay in traceback efforts, economic cost, and mortality (STEC Workshop Reporting Group, 2012), (Sprenger et al., 2011), (RKI, 2011). Between 2003-2012, 390 *EC* outbreaks were reported in the US resulting in 4,928 illnesses, of which 74% were attributed to foodborne disease outbreaks (Heiman et al., 2015). Low levels of contamination in raw vegetables are capable of causing infection and subsequent intestinal disease; *E. coli* O157:H7 has been suggested to result in incidences of disease in humans with consumption of less than 100 or even less than 10 cells (Ackers et al., 1998).

All 6 groups are commonly transmitted via the fecal oral and/or foodborne route and are typically associated with fecal matter contamination (WHO, 2018). Two key misconceptions from the consumer revolve around the belief that EC is a pathogen and that all fecal coliforms are from fecal origin. Most fecal coliforms are not associated with fecal matter and only EC has been found to have a direct link to feces besides the known environmental niches. Therefore, EC has been utilized as a fecal indicator organism (FIO) (Van Elsas et al., 2011) within multiple environments, despite knowing that only in food processing environments a clear link to the fecal oral route of contamination exists. In all other environments there is poor correlation of the presence of EC and that of enteric or Gram-positive human pathogens (Craven et al., 2021). Only does the

confirmation of EC in postharvest packing steps indicate the possible contamination that is of fecal origin and therefore the potential presence of human enteric pathogens (Luna-Guevara et al., 2019).

For green onions, soil amended with improperly composted manures are potential sources of preharvest contamination. In fact, *E. coli* O157:H7 has been shown to persist between 7 and 25 weeks depending on soil type and abiotic factors and can persist throughout storage and subsequent distribution (Duffy et al., 2005). Commonly found in the intestinal flora of cattle and other ruminants, EC can enter soils and irrigation sources from runoff. Wild animal encroachment and subsequent deposits of fecal matter within leafy greens are documented sources of pathogenic bacteria (Berger et al., 2010) and are an inherent risk in green onion production. Therefore, during hand harvest activities, personnel must not stack green onions in contact with the soil during harvest as the soil can potentially be contaminated with human pathogens (FDA, 2010). Late season irrigation applications to green onions with contaminated sprinkler irrigation carries inherent risk of persistence and transfer of pathogens during hand harvest activities of green onions as the time after irrigation is not sufficient in allowing significant die-off from abiotic field conditions. Therefore, careful considerations of personal hygiene in hands, harvesting equipment such as vehicles, implements and transport containers are of critical importance. Green onions and DB onions have very similar supply chains, and, in both cases, hand harvesting and trimming may be utilized in the field or in packinghouse settings. The general methods of removing green onions from production fields via harvest activities, packinghouse, storage, transportation, and distribution is similar to DB onion supply chains minus wash steps, value added processing and cool chain management measures to minimize spoilage and qualitative deterioration (FDA, 2010).

Good practices along the supply chain involves maintaining equipment and facilities in a manner that reduces the risk of cross contamination and there exists considerable parallel in methodologies and therefore concern for contamination events in both green and DB onion industries for future outbreaks.

Shigella

Shigella are facultative anaerobic, non-spore-forming, rod-shaped, non-motile Gram-Negative bacteria that are the etiological agent of bacillary dysentery or shigellosis in humans (Anderson et al., 2016). Natural hosts and reservoirs for *Shigella* spp. are humans and primates. A member of the *Enterobacteriaceae* family, *Shigella* is closely related to EC and can be delineated as dysenteriae and non-dysenteriae groups. Clinical manifestations characterized with four separate pathogenic species *Shigella dysenteriae, Shigella flexneri, Shigella boydii* and *Shigella sonnei* are the etiologies that result in human disease (Akhtar et al., 2014) and although *Shigella* has historically been characterized as a waterborne pathogen, foodborne illness outbreaks with raw and processed food products with hand preparation are familiar (Warren et al., 2006). *Shigella* spp. are well described contaminants of raw produce and have been studied in both water and food matrices. *Shigella* spp. are unable to survive pasteurization and cooking temperatures; but do display acid resistance and salt tolerance (Warren et al., 2006). Raw vegetable products are potential vectors to contamination with *Shigella* spp; inoculation studies with onion and bell pepper suggest a slightly negative growth potential at 4°C for a period of 12 days. Inanimate objects of various materials have been shown to act as vectors for transmission with *Shigella* spp. and are of concern in post-harvest phases of fresh produce production (Islam et al., 2001).

Hepatitis A Virus (HAV)

A small single stranded RNA virus lacking an envelope, hepatitis A virus (HAV) belonging to the *Picornaviridae* family, with six genotypes where genotypes I, II and III infect humans, and is the causative agent of acute self-limited hepatitis typically presenting as fatigue, jaundice, and in rare cases liver failure (Di Cola et al., 2021; Moon et al., 2022). Foodborne illness outbreaks have been characterized commonly with seafood and frozen foods since the early 1990's, but HAV infection has more frequently been an endemic issue in developing countries (Mohd Hanafiah et al., 2011). Endemics in developing countries have resulted in higher rates of immune individuals as they have been exposed to the virus in childhood. The most common routes of exposure to HAV involve the oral fecal route through wastewater or human feces, infected individuals mishandling food, and animal sources of infection (Hofmeister et al., 2019). Particularly, foods that require little preparation like bivalve mollusks and raw products are of concern for HAV contamination as the viral particle is more resistant to refrigeration, freezing, pH, desiccation, UV, heat and pressure, and disinfection than bacterial etiologies (Di Cola et al., 2021). There is a large at-risk population of people not vaccinated for HAV reflective of vaccine recommendations beginning in 1996 in the U.S. In light of this fact, there have been several large HAV outbreaks since 2016 which have resulted in 43,000 HAV infections, 26,290 hospitalizations, and 402 deaths (Moon et al., 2022). The typical incubation period of 2-6 weeks and subsequent viremia of 5-7 days is transmitted via the fecal-oral route through consumption of contaminated food such as shell fish, strawberries or water (Franjić, 2022).

DB Onion Morphology

DB onion (*Allium cepa* L.) is a common small seeded crop produced commercially as an annual for the edible bulb (Schwartz, 2012). Common scale colors widely distributed globally are red, white, yellow, and sweet. The industry provides whole DB onions for consumers as well as fresh

cut products that are processed and prepared in a ready to eat minimally processed food (RTEMP). There are a variety of mechanical harvest schemes in whole DB onion production, and most production is dedicated toward storage of DB onion as there is continual repackaging and distribution practices to ensure a steady supply. Small plots are typically sown to produce the biennial seed, where bulbs are formed the first year and flower and fruiting takes place the second year. Production revolves around Spring/Summer and Fall/Winter varieties, allowing the domestic supply of the major scale colors of yellow, red, and white onions to be available to the consumer on a yearly basis (Havey, 2017). Key milestone agronomic stages can be defined as seed germination and establishment, foliar production, and bulb initiation and development.

Bulbs consist of thickened modified leaves that mature partially underground. Root and shoot formation originate from a small stem, while the pseudo stem, tightly bunched leaves above the onion scales, also known as the "neck" lies below the leaf canopy. Vegetative growth of DB onions can be delineated into two phases: pre- and post-bulb formation. Pre-bulb initiation begins at establishment or the 2-3 leaf stage and continues until bulb initiation. Onions at bulb initiation (90-110 days post seeding) produce 8 to 12 leaves, and the size and yield of bulbs are correlated with planting density and the number of leaves present on plants; where bulb diameters of 2.5-4.0 cm (110-130 days post seeding), 4.0-7.5 cm (130-150 days post seeding) and greater than 7.5 cm (130-150 days post seeding) are considered mature and ready to harvest when 50% of crop is dried down (Schwartz, 2008, 2012). Onion leaves emerge in a circular fashion from this flattened small stem, where older leaves surround the emerging new growth. Bulb initiation is marked by newly developing leaves swelling into bladeless scales (Galsurker et al., 2017). Onion root systems are unbranched and 90% of root mass is located in the top 20cm of soil (Smith et al., 2011).

Irrigation methods vary depending on the production-type, soil type, evapotranspiration, and allowable depletion levels (Enciso et al., 2009), but may typically occur every 7-10 days until bulbmaturation and a dry-down period typically referred to as field curing. Sprinkler, furrow, and surface and sub-surface drip tape irrigation methods (highest to lowest microbial risk) typically depend on the end product (i.e. fresh cut or storage) (NOA, 2022). Irrigation scheduling and curing methods depend on local environments and seasonality. Field curing DB onion has traditionally occurred in drier climates while moister localities may utilize artificial curing due to nonconducive ambient environmental conditions. Exposure to ambient temperatures of 24-27°C for 2-3 weeks or heat between 29-35°C+ for 2 or more days has been shown to cure onions well (Schroeder & Du Toit, 2010). Dehiscent papery skins that form during the curing process of DB onions function to protect the fleshy, high-water content of inner-onion scales from soil and waterborne plant pathogens (bacteria and fungi). Producers utilize curing as a method to minimize water loss and to diminish the occurrence of fungal and bacterial rot issues during storage (VahlingArmstrong et al., 2016). Onion skin formation is through a combination of scale desiccation and senescence, and programmed cell death (Galsurker et al., 2017).

Plant pathogenic microorganisms of DB onions associated with these papery scales include *Enterobacteriaceae, Burholderia, Gluconobacter, Acinetobacter, Pseudomonas, Citrobacter, Nectriaceae, Botrytis, Wickerhamomyces, Penicillium* and *Candida* (Yurgel et al., 2018). Plant pathogen colonization may provide habitable niche space for simultaneously present human pathogens through plant tissue damage and subsequent release of available organic compounds suitable as substrate to survive and persist.

The specific morphological and physical nature of certain crops may play a role in the effectiveness in colonization, survival, and transfer of human pathogens along the cropping cycle. For example, the heterogeneous microsites contained within the outer-surface features of DB onions could provide suitable niche space for the potential contamination in pre-and post-harvest production phases. Maturing partially in the soil, the outer skin, basal plate, and neck have been postulated to be potential sites of endophytic contamination throughout production as these sites accumulate soil residues and damage. Although it is currently unknown whether *Sal* may act as an endophytic contamination in DB onion, studies with melon systemic transport of *Sal enterica* Typhimurium through root-uptake from furrow applied inoculum was deemed an unlikely source of internalization; however, direct contamination of the outer rind through the peduncle resulted in detectable inoculum within the sub-rind tissue below the application abscission (Lopez-Velasco et al., 2012). Although currently it is unknown whether internalization of *Sal* within DB onion is possible in open field environments, mechanized and hand-harvest activities could theoretically expose damaged tissues to soil, or aerosolized dusts and therefore provide a potential entry point. Physically, the layers of skin and inner scales upon external damage may potentially entrap soil and microorganisms or provide a suitable niche space for plant pathogens and human enteric pathogens to potentially exist. What is currently unknown is the growth response of pathogenic *Sal* in the presence of the metabolite profiles of widely produced DB onion cultivars. Anecdotal efforts have previously shown *Sal* proliferation was not observed on dried fruits and dried fruit homogenates (low a^{-}_{w}) at various concentrations and temperatures; although growth in date paste and raisin homogenates suggest marginal growth followed by rapid inactivation (Beuchat & Mann, 2014). Potentially, DB onions may exhibit similar growth response with enteric pathogens, but it is currently unknown with pathogenic *Sal*.

Onion inner and outer-scales contain a variety of phytochemicals, namely polyphenols, flavonoids, and anthocyanins (Albishi et al., 2013; Mnayer et al., 2014; Sagar & Pareek, 2020) Secondary plant metabolites contained within classes of organosulfides, phenolic compounds, and anthocyanins that are concentrated in onion skin have been previously studied in their nutritional aspects and bactericidal action. Quercetin mono-and diglucoside account for 80% of flavonoid content in onion and the action of alliinase and allicin derivatives is of particular interest in thiosulfate production and flavor profiles (Bahram-Parvar & Lim, 2018). Particularly, quercetin and allicin have been shown to possess an inhibitory function against Gram-negative and Gram-positive bacteria and some fungi, and viruses (Nguyen & Bhattacharya, 2022; Sharma et al., 2018). Investigations have previously shown that geographical location and meteorological conditions directly affect DB onion metabolite profile content and is also cultivar dependent (Böttcher et al., 2018; Rodrigues et al., 2011). Cultivar and annual production variability are important to consider with inoculation studies. Whole yellow onion skin inoculations with *Sal* produced on agar and broth at log7 cfu/mL showed a more rapid decline in die-off in the skins when prepared in broth; perhaps due to the lack of attachment gene expression and subsequent inability to adhere, colonize, and proliferate (Lieberman et al., 2015).

These same experiments with *Listeria monocytogenes* demonstrated a decline of bacterial population on yellow onion skins by greater than log 6 cfu per spot inoculation over 2-4 weeks at 23 °C, decline in population in whole onions spot inoculations of 2 to greater than 6 log cfu/ml over an 8 week period at 4°C, and growth was observed on diced yellow onion scales at 10 and 23°C but not at 4°C (Lieberman & Harris, 2019). The bactericidal activity of the skins is apparent, while the inner scales may lack similar concentrations of these ontologies and therefore may promote growth of both Gram-negative and Gram-positive human pathogens. Inner-scales alone

have been found to contain marked decreases in quercetin (40%) and anthocyanins (70%) content (Bahram-Parvar & Lim, 2018; Sidhu et al., 2019).

Artificial means of applying attenuated enteric pathogen inoculums has been effective in predicting natural contamination events and pathogen survival during open-field production practices of various crops when the attenuated strain growth rates and stress adaptation capacities have been previously compared to known pathogens (Lopez-Velasco, et al, 2015, Gutierrez-Rodriguez et al 2012, 2019). There currently lacks open field *Sal* inoculation efforts with attenuated *Sal* to potentially predict commercial pre- and post-harvest contamination and model die-off across production, harvest, transport, storage and packinghouse with regards to scale color and in response to the recent foodborne illness outbreaks. Previous efforts have looked to characterize the effect of scale color and metabolite profile and generic *E. coli* growth response. Particularly, *in-vitro* efforts with *Sal* and red and white DB onions suggested antibacterial and anticandidal activity through disc diffusion assays (Değirmencioğlu & Irkin, 2009). The issue here is that many producers may continue to rely on the inherent bactericidal metabolites contained within DB onions, although it is known that these profiles change on cultivar and annual basis. To supplement the lack of understanding, it is certainly pragmatic to screen widely produced DB onion cultivars' ability to inhibit or promote growth of human pathogens involved in multistate outbreaks and to gather metabolite profile data to elucidate potential correlations between ontologies and growth response.

Biofilm formation coupled with other plant pathogens on plant tissues has been suggested to play an important role in the scope of plant surface colonization by *Sal (*Castiblanco & Sundin, 2016*;*

Fletcher et al., 2013*)*. In particular, as *Sal* lacks the essential enzymes needed to degrade plant cell walls in order to acquire essential nutrients to proliferate, *Sal* may rely on essential nutrients and substrates of cell lysates and root exudates derived from plant pathogen activity (Barak & Schroeder, 2012; Teplitski et al., 2009). As DB onions are typically stored for various amounts of time prior to packing and distribution, storage conditions may provide an opportunity for low levels of *Sal* to survive and potentially proliferate in the onion. This phenomenon could occur in small batches of stored onions that subsequently contaminate sorting and packing food contact surfaces and machinery to create the potential situation where large quantities of DB onions necessary to produce multi-state outbreaks are cross-contaminated. However, a recent study found quercetin suppressed quorum sensing, virulence, and stress response gene expression in *Sal* and therefore could be considered as an antibiofilm agent (Wadhawan et al., 2021) as long as it's readily available to *Sal*. Cultivar and the various methods of curing and storage practices for DB onions may affect phenolic compound content in both skin and inner scales and subsequent periods of storage and therefore, may decrease quercetin content and subsequent suppressive skin properties besides the known access barrier linked to scale formation and direct access and contact with those pathogens. Theoretically, if bacterial contaminants were introduced to whole DB onions, there also exists consumer trend gaps in how individuals remove and prepare fresh cut whole DB onions. It is generally thought that consumption begins with removing the outer skins prior to cutting, but there lacks a clear consensus as to whether that is a general consumer trend (NOA, 2022). Studies with regards to these trends may help elucidate if in-home or commercial processing of whole DB onions plays a role in consumption of epiphytic bacterial contaminants, although the organoleptic properties suggest the likelihood of their presence to be low.

Dry Bulb Onion Pre-Harvest Production Practices

Good agricultural practices (GAPs) published by NOA in 2010 and 2022 for DB onion list key areas that producers and packers need to address to reduce the likelihood of DB onion contamination. These include field selection, agricultural water quality, unusual weather events (e.g., flooding), harvesting practices, onion curing, packing and storage conditions, cleaning and sanitation of food contact surfaces, transportation, and traceability. Most of these practices in DB onion have not been evaluated and studied to determine the quantitative risk factor associated with each practice. These recommendations originate from experiences drawn from the green onion industry and other commodities that have experienced significant contamination events and from a small body of research published over the last 20 years. To that end, the guidelines recommend that producers must be cognizant of common microbial risk control points within the cropping system; where concrete and pathogen reducing intervention events are put in place to reduce the risk, presence, or prevalence of human pathogens within farming operations. Some of these intervention events include, composting of soil amendments coming from manures or preconsumer vegetative wastes, treatment of irrigation water, training of workers on health and hygiene practices, using sanitizers at correct dosages as listed in the label and ensuring a registration number either for washing produce or for sanitation of food contact surfaces is obtained (James, 2006). Concomitant with these practices, handling produce in clean trucks and storing them in units that prevent dripping of condensation into the crop, preventing livestock from entering produce fields and monitoring and if possible dissuading wild animals from entering the farm (NOA, 2010, 2022; Olaimat & Holley, 2012; Suslow, 2003) are also critical to reduce the introduction of human pathogens to the farm. Further description of these pre- and post-harvest

control factors will be discussed in subsequent sections and for now our focus here will be on preharvest practices.

Primary production sites of DB onions should be carefully considered to prevent the known or presumptive presence of human pathogens that could in any likelihood transfer to crops intended for human consumption. In some cases, land use history is unknown and additional time is needed to reduce known pathogen levels; in either way, both approaches are unfeasible (Suslow, 2003). Animal rearing operations in close proximity to vegetable production are inherently problematic for crop contamination via direct or indirect forms of animal intrusion, bio-aerosols, or run-off (Brandl, 2006). The FDA investigation of the 2020 *Sal*Newport outbreak identified potential signs of animal intrusion, sheep grazing in adjacent fields, and a leading hypothesis suggests contaminated irrigation water used in pre-harvest growth phases may have led to contamination of onions (FDA, 2020). Suitable intervention strategies in preventing contamination in potentially adverse situations including the construction of diversion ditches, buffer areas, or vegetation strips to decrease the likelihood of microbial hazards has been recommended as potential mitigation strategies (Abu-Ashour & Lee, 2000). Characterization of the microbial quality of different irrigation water sources throughout the cropping cycle is critical and must be considered simultaneously with the selection of the field. Quantifying the microbial profile of irrigation water can provide information to a producer to strategically apply irrigation in a timely manner to reduce the risk of contamination as harvest approaches.

Producers of DB onions typically consider planting a cover crop, removing cover crops via herbicide or tillage, followed by disking and further tillage for bed preparation and shaping. Soil sampling is needed to quantify nutrient load available to DB onions and to inspect the soil for

nematodes, insects, and mites. Organic producers may elect to apply inputs of manures or other organic amendments. Untreated biological amendments of animal origin are of concern, and it has been previously shown that preharvest contamination of green onions with *E. coli* O157:H7 through contaminated manure, compost, and irrigation water can persist for several months in both green onions and soil (Islam, 2005). The PSR indicates that these types of amendments must not be applied directly to the harvestable portion of the crop unless treated (FDA, 2016). Thus, for organic producers, only treated amendments, mainly composting materials that follow a validated kill step as indicated by the PSR, could be the only significant source of nutrients applied directly to the harvestable portion of the crop if no other alternative practices are provided. As a best practice the PSR recommends growers to follow the 90-to-120-day rule established by the USDA national organic standards (NOP) to allow sufficient elapsed time between application and harvest for potential pathogen die-off (FDA, 2016; USDA, 2011) Particularly, a study conducted by Islam et al. has previously shown that heterogenous contamination of *EC* and *Sal* contained within poorly composted poultry and bovine manures can survive and persist in soils for several months (Bezanson et al., 2014; Islam, 2005). Interestingly, parsley grown in inoculated soils in these experiments persisted for 177 days, which is longer than the interval requirement within the PSR, which solidifies the importance of proper composting techniques and subsequent applications in fresh produce production.

Quality DB onions at harvest are determined through strategic pre-harvest planning. The critical timing of lifting/topping and subsequent curing processes is dependent on seeding date, establishment, vigor, and abiotic and biotic pressures throughout production. Producers must minimize additional applications of irrigation water at the initiation of the curing stage to dry down the crop once bulb maturation is complete. The curing process may create many layers of dried

scales or skin depending on the duration and abiotic conditions. The multiple layers of skin surrounding the inner edible scales protect the DB from the external environment. Late season applications of pathogen carrying irrigation waters can theoretically contaminate the edible portions of DB onions. On occasion, some irrigation applications may be used to soften or loosen the soil for lifting implement efficacy. A recent study was intrigued by this idea and found that the impact and timing of *EC* inoculation with sprinkler and drip irrigation at the initiation of topping and subsequent curing suggested finishing and curing after 3 weeks was effective in completely reducing the presence of the surrogate with respect to drip conditions, but a small portion of sprinkler inoculated samples were positive at the end of the 3-week curing period (Moyne et al., 2022). Irrigation applications and specifically sprinkler irrigation applications with the intent to softening or loosening the field in preparation for lifting therefore has considerable risk of crop contamination and persistence in latter post-harvest activities. Particularly, as late season lifting is marked in decreased abiotic stressors such as UV light and temperature, this could provide more suitable conditions of available moisture and organic compounds via masticated or wounded tissues for plant and human pathogens to utilize.

Typically, DB onions when directly seeded, need a well-structured soil profile and seed bed that is fine enough to promote imbibition, germination, and subsequent establishment of DB onion stands. DB onion yields are severely reduced by soil salinity, particularly at seedling emergence (Regessa et al., 2010) and by high plant densities. Prior to sowing, fertilizers, pre-emergent herbicides, and fungicides are applied to formed seed beds and typically focused on control of broad leaf weeds. Levels of phosphate (P) and potassium (K) added to the soil depends on preexisting levels but generally a high concentration of available P and K is key in satisfying DB onion requirements throughout establishment of DB onion stands; nitrogen (N) availability

throughout bulb initiation must remain above ~150kg/ha to attain maximum bulb yields and therefore is typically split into two separate applications equaling 180 kg/ha (J. L. Brewster, 2008). Precise P additions at early seedling development has also shown a considerable reduction in postharvest rotting and sprouting issues (Mishu et al., 2013) Primed or unprimed seeds can be utilized for direct seeding, or the process of transplantation with sets can be utilized to establish DB onion plants. Seeding rates depend on the local producer's soil nutrient profile, water availability, and yield projections. Stand establishment requires adequate nitrogen availability throughout the process of leaf canopy development and as observing early onset of bulb growth has been shown to result in fully mature, high-quality bulbs at the time of harvest that exbibit increased storability. Moreover, late season irrigation and N applications may negatively affect bulb quality by causing skin splitting, rotting, and susceptibility to spoilage organisms, especially in irrigation and/or fertilizer applications soon after periods of stagnant growth rates due to lack of water or nitrogen (Rabinowitch, 2018). Skin splitting, rotting, and other qualitative post-harvest related diseases may create favorable environments for which human pathogens may contaminate or if already present may find favorable environmental conditions to do so. Producers must critically address the pre-harvest related agronomic considerations to ensure quality plant pathogen free DB onions are produced to reduce the likelihood of human pathogen in the latter phases of post-harvest processing. Crops can become contaminated in pre-harvest production through contact with soil, feces from animal intrusion, irrigation applications from bacteria carrying water, applications of animal-based manures, fertilizer, dust, insects, or sanitation of personnel and container surfaces (Beuchat, 2002). Such practices could also be impacting the DB onion industry and could be the source of contamination as *Sal* has been shown to survived for lengthy periods of time.

Irrigation method, efficacy of application, and risk of microbial contamination during production of DB onions is of critical importance to the changing industry. In November of 2021, the FDA announced revisions to Section E of the PSR specific to the requirements for agricultural water used on the farm. This revision eliminated previous prescriptive metrics provided by the FDA for consumers to follow and moved to a risk-based assessments approach in which the grower is responsible for defining agricultural water microbiological standards, performing a risk assessment of the water source and methods of delivery and to monitor and control the microbial quality along the cropping cycle (FDA, 2021a). The water source, irrigation method and optimization of water application and timing are critical in decreasing the risk of microbial contamination of DB onions with plant and human pathogens during application events. Keeping DB onion stands adequately saturated without over application can be difficult due to evapotranspiration rates, seedling density, and to the unbranched nature of the root zone. Onion producers in Colorado utilize syphon, drip, and overhead sprinkler irrigation systems to deliver water to the crop at different stages of DB onions and this varies by district, but nationally overhead sprinkler irrigation is the most common method employed (NOA, 2022). Pathogen contamination by aerosolized water from sprinkler applications is of greatest concern right before harvest as the maximum opportunity for pathogen die-off is lost. Local on farm corrective actions to minimize the risk of potential contamination events can be achieved through system-based audits addressing application methods, timing, crop in production, and microbial risk of irrigation water (CDC, 2022).

Dry Bulb Onion Harvest Practices

Commercial DB onion production has locally diverse activities in preparation for harvest. Topping, lifting, or rolling mature bulbs and subsequent in-field windrowing or artificial curing processes improve bulb storability. As bulbs reach their final size, bulb ripening is marked by "fall-down' or 'soft-neck.' Foliage senescence as the plant desiccates is a general indicator of bulb maturity, and harvest timing at 50-80% fall down in wetter climates, and 100% desiccation in drier climates may improve skin quality and storage life respectively (Brewster, 2008). Delayed harvest of DB onions at 75% fall-down and subsequent field curing for one or two weeks prior to topping has been shown to improve qualitative appearance, storability, and retained moisture content (Kiura et al., 2021). The curing process effectively removes excess water from the outer neck and outer scales and creates a physical and chemical barrier that protects the semi-perishable bulb from desiccation and exogenous microbial plant pathogens (Petropoulos et al., 2017). Onion skin forms through a combination of scale desiccation, plant senescence, and programmed cell death (Galsurker et al., 2017). A general best practice is to keep harvest conditions dry, albeit this is not always possible for producers due to weather events but there have been studies that suggest that finishing and curing processes in DB onions significantly reduces microbial load (NOA, 2010). An instrumental study supporting conventional onion curing as a strategy to mitigate *Sal* and *EC* contamination from bacteria carrying water suggests curing as an effective means in bacterial control (Emch & Waite-Cusic, 2016; Wright et al., 2018). The inherent risk here, is that producers cannot always control for environmental conditions and reliance on curing as a method for bacterial load control is not sufficient due to seasonality, cultivar effects, and the heterogenous nature of contamination in pre-harvest phases of production. Typically, stochastic events of point source contamination in the field (i.e., manures that are not homogenously contaminated, rare

weather events, wildlife encroachment and deposits of fecal matter) may be more likely than events that result in homogenous contaminations (Raffo & Paoletti, 2022). Proper curing has been shown to significantly reduce bacterial load but there still exists inherent risk as curing methods and efficacy may vary on an annual and local producer level. Many will argue that pre- and postharvest field inspections required by the PSR can mitigate this risk, however the typical number of hectares and crop to survey is extremely large to effectively, through visual observation, assess the risk of contamination and mitigate the risk over this large-scale landscape. Without descriptive understanding of the individual risks associated with the practices of topping, lifting, and curing, field inspections are not a feasible means to prevent transfer of microbial contamination into the latter phases of production.

DB onions are harvested by hand or via mechanical implements or a mixture of both. Typically, short day onion types are highly prone to damage if mechanical harvest was to be used, leading to hand harvest activities to preserve quality and shelf-life. Rollers or spike rollers can be utilized to break the neck of the DB onion plant to speed up the dehiscent process of the leaf canopy. Removal of lodged onion leaf canopies can be accomplished by use of a rotary blade implement, or flail and is a pre-requisite to lifting and subsequent curing. Lifting or undercutting implements move beneath the DB onion root zone and lift the bulb from the soil. In some instances, onions can be lifted while the leaf canopy is still present and put into burlap bags (NOA, 2022). There exists a myriad of cultivar and locality dependent complementary methods and timings related to lifting/topping and subsequent curing practices. The inherent risk for the DB onion industry exists in the lack of standardized methods in these regards and substantial scientific data to support specific microbial load control measures for the different methods. Hand trimming or mechanical implements can be utilized to remove the roots and leaves of the bulbs. Then, bulbs are either

mechanically or hand loaded into high density polyethylene bins or into burlap sacks to be cured for a period ranging from a few hours to multiple weeks depending on the type of onion and local producers' cultural methods (NOA, 2022). The inherent risk in both mechanical and hand topping and harvest activities lies in proper sanitation practices for personnel participating in harvest activities and the potential for cross-contamination inherent in these activities. Heterogenous point sources of contamination may be spread by both mechanical topping implements which create aerosolized soil and debris, and hand methods which provide a more intimate risk to individual onions and food contact surfaces and provide a route for sick individuals to contaminate surfaces. Producers must have valid SOPs with regards to hand washing and general facility hygiene and sanitation respectively. However, many of the safety criteria and regulation of these risks are not strictly enforced and utilize verbiage within guideline materials that is indicative of recommendations rather than necessity.

Visual inspection of food contact surfaces within these processes is a recommendation, but any further microbial means of testing is not a requirement. Machine harvesting onions inherently causes undesirable damage and injury to DB onion. Mower implements typically are utilized first in machine harvest procedures to remove the leaf canopy of the plants. Windrowing machines can also simultaneously remove DB onions (previously mowed tops) from the ground, clean soil, and field debris via a conveyor belt and place two seed beds of bulbs into one 'windrow'. Windrows may undergo further field curing and are subsequently picked up with mechanized harvest equipment. Regardless of local production practices, variation on a year-to-year basis, there are key aspects necessary to mitigating microbial risk at the time of harvest. To successfully achieve control of microbial load and thereby ensure safety of fresh produce producers must consider the level of environmental contamination in the production fields, keen evaluation of growing areas

for animal and human intrusion and deposits of fecal matter or crop destruction, hygiene of personnel and food contact surfaces, and on farm implementation of these strategies in an effective and appropriate manner. There are a variety of production strategies depending on local producers' cultural methods and the type of onion in production, and the lack of consensus and standardized methods of maintaining the natural organoleptic characteristics of DB onions prior to harvest, renders the industries recommendation to rely on environmental log reduction prior to harvest activities in DB onions ineffective in preventing future outbreaks with *Sal*. The organoleptic properties of DB onions will inherently vary depending on cultivar effects, curing practices, the environment, and underlying microbial risks present on an annual and local basis. There exist many complexities here in terms of potential routes of contamination and cross-contamination risks, and it is pragmatic for the industry to identify the most critical aspects in risk mitigation strategies amongst pre- and post DB onion production practices to ensure the safety of distributed products to the consumer.

Dry Bulb Onion Post-Harvest Production Practices

The FDA investigation of the 2020 *Sal* Newport outbreak associated with DB onions identified plausible post-harvest contamination sources. The outbreak strain could have been physically transmitted to the Bakersfield packing line from farms in Holtsville, California (Commichaux et al., 2022). Pre-harvest investigations in Holtsville in primary production fields occurred after the outbreak, however; visual observation of the Bakersfield packing house confirmed plausible opportunity for *Sal* contamination via animal and pest activity along with food contact surfaces which had not been properly inspected, maintained or cleaned (FDA, 2020). Rather than leave this to speculation, commodity specific guidelines with quantified risk related to food contact

surface contamination are critical in providing post-harvest phase operators with sanitation guidelines that are effective in preventing accumulation of residues and biofilms.

Horticultural crops may encounter a variety of surfaces throughout pre- and post-harvest production and processing. Typically, these surfaces are made with stainless steel or plastic, but it is not uncommon they could be wood, ceramic, rubber or even glass (Skåra & Rosnes, 2016). Plastic and wood are considered standard food contact materials in commercial DB onion production (NOA, 2022). The FSMA PSR does not prohibit the use of wood, and research has investigated its' porosity, roughness, and microorganism activity and determined depending on research approach that wood may or may not possess unfavorable conditions for microorganisms and may or may not be suitable for use as a food contact surface under large scale production practices (Aviat et al., 2016; Cliver, 2006; Tebbutt, 1991). Antimicrobial properties may vary with different wood species and the hygroscopic properties therein may affect the hygienic properties of wood (Schönwälder et al., 2002). However, to the contrary it has been found that wood possess favorable conditions for microorganisms and biofilm formation, particularly in biofilms found in wooden cutting boards in wet markets and wooden shelves utilized in cheese production (Wadhawan et al., 2021; Ngan et al., 2020). Bins containers, and any materials that encounter DB onions must be maintained in good condition and stored in clean facilities to prevent contamination; by continually cleaning surfaces to remove feces, soil fomites, organic material, and moisture producers can alter these surfaces to decrease the potential occurrence of microorganisms (NOA, 2022). Visual observation as a preventative measure must be coupled with other means of determining whether surfaces need cleaning or sanitation as biofilms may be difficult to visually observe and remove. There is inherent risk in the lack of required SOPs for food contact surface materials utilized in field activities and post-harvest phases and the frequency

and type of sanitation events will truly influence risk reduction. Without clear scientific understanding of the risk of human pathogen occurrence in these phases, risk mitigation will not be effective.

Post-harvest treatments of horticultural commodities strive to slow down physiological processes of perishable and semi-perishable products to minimize both product loss and risk of microbial growth and contamination. The use of physical irradiation directly to DB onions, chemical peracetic acid (PAA) or ozone applied in storage facilities has been occasionally employed in onions to decrease storage losses due to sprouting and bulb-rots and to also decrease the presence of potential human pathogens existing in low levels. (Mahajan et al., 2014). Producers utilizing these types of methods are addressing the microbial load potentially transferring from pre-harvest activities into post-harvest phases. However, there exists a lack of consensus with regards to how many producers utilize these types of post-harvest treatments and whether their operations have access to the infrastructure and economic means to do so. There also lacks significant understanding into the effectiveness of these types of treatments regarding log reduction for the human pathogen etiologies of concern in DB onions such as *Sal, EC, Shigella*, and *HepA*. Therefore, there exists a need to understand the specific association with human pathogen risk and storage activities.

In storage facilities, plant pathogens like black mold (*Aspergillus niger*) fusarium basal rot (*Fusarium oxysporum* f. sp. *cepae*), white rot (*Sclerotium cepivorum*) can decay infected bulbs and contribute to storage loss (Schwartz, 2012). Storage conditions, particularly temperature and humidity management, are of primary concern with product loss. Poorly cured onions translating

from field or curing operations may promote plant pathogens proliferation and may result in an hospitable environment for human pathogen growth development (NOA, 2022). Of note, an association between plant pathogens and human pathogens has been observed in other types of plants; where *Sal* appears to successfully colonize other plant surfaces with the help of lesions, soft rots, or water soaking; particularly in combination with biotrophic plant pathogens such as *P. syringae* and *Xanthomonas campestris* which may promote proliferation and persistence of *Sal* and EHEC (Aruscavage et al., 2010; Barak & Liang, 2008; Potnis et al., 2014). DB onions in storage may develop qualitative issues with fungal pathogens and could increase the potential risk of human pathogen presence, but currently this phenomenon has not been studied in DB onions. Increased availability of organic substrate available on the surface of DB onions exhibiting soft rots may have the potential to create microsites able to enhance proliferation or persistence of enteric pathogens. The two separate foodborne outbreaks affected individuals within 38 states and 48 states respectively. Largely left to speculation traceback information attempted to answer how such a substantial amount of DB onions became contaminated and persisted throughout distribution in a manner consistent with outbreak information. Perhaps there is considerable risk in storage conditions, where low levels of *Sal* may find opportunity to grow within storage. There exists a considerable lack of study with regards to this phenomenon in stored DB onions specifically with *Sal*.

The supply chain of DB onions is complex and commonly stored bulbs are packed and repacked for various reasons. Storage and packinghouse facilities may be producer owned or independently operated. There is a lack of research addressing food contact surfaces of DB onions in both preharvest activities and packing and sorting equipment. Current verification methods for key aspects of food safety precautions with onion containers, bins, and food contact surfaces include

visual inspection and maintenance of activities in a clean and sanitary manner. Therefore, considerable revision is needed regarding microbiologically based standards with respect to equipment, storage, and packing lines is needed to mitigate microbial risk at the control points of packinghouse and storage processes. The inherent risk of cross-contamination with biofilms or contaminants throughout packinghouse activities could render clean onions contaminated. This phenomenon has not been studied and is thought to be a possible route of contamination that would result in large amounts of contaminated onions and subsequent foodborne illness outbreaks reflected in both the 2020 and 2021 events. Currently, wet sanitation processes in post-harvest related activities in DB onion are not recommended; but if they are conducted the water utilized must be absent of generic EC (NOA, 2022). Growers receive recommendations for and use commonplace practices for keeping onion packing and storage areas dry, which may coincidentally minimize the usage of wet sanitation practices. Utilizing vacuums and brooms may be conventional in the removal of debris such as soil fomites, onion culls/skin, and trash. Protection against aerosolization and cross-contamination of stored onions is a central consideration that is recommended while cleaning food contact surfaces Growers are recommended and conversationally idealize keeping onion packing and storage. Protection against aerosolization and cross-contamination of stored onions is a central consideration recommended for cleaning food contact surfaces. Alcohol based detergents and sanitizers are recommended to clean surfaces but microbial metric requirements for determining cleanliness are lacking. The Bakersfield packinghouse is a dry kept operation and the traceback efforts in 2020 suggested pest intrusion and food contact surfaces as potential sources of cross-contamination. With regards to the sanitation and hygiene practices implicated in their large-scale cross contamination, the frequency and type of sanitization employed during DB onion processing is presently unknown. The onion industry needs to quantify the risk of cross-contamination during post-harvest processing

activities, as accumulation of residues on food contact surfaces may produce suitable environments for *Sal* persistence or biofilm formation. Potentially, a positive correlation with duration of running processing equipment and residue levels might exist, indicating potential human pathogen presence. What is currently unknown here is the practical effectiveness of microbial indicator organisms *EB* and *EC,* and whether their use correlates with *Sal* presence in packinghouse environments along with the frequency and type of dry or wet sanitation events that are effective in preventing large-scale cross contamination from occurring. There may be considerable value for the industry to evaluate whether a cultivar effect exists in relation to cross-contamination due to morphological or metabolite differences and whether special precautions with scale color are necessary once materials reach the packinghouse.

Packinghouses utilize a variety of modalities to grade, sort, package, and ship DB onions. Grading tables, brush beds, blowers, and size and color distinguishing technologies aid in quickly sorting, grading, and packaging activities. Brush beds and consumers remove the outer-most dry skins which may inherently remove epiphytic contaminants, but it is uncertain if all modalities utilize brushes or means to remove the most outer-skins. Recommendations include keeping types of equipment dry and free of debris, and packing houses are required to determine a cleaning schedule based on the volume of product being processed (NOA, 2022). Packinghouse equipment certainly poses a microbial risk in cross-contamination but their lacks informative guidance regarding what frequency establishes meaningful and appropriate levels of cleanliness and sanitation to minimize risk beyond visual confirmation. In some instances, materials that reach packinghouses are stored for varied periods of time prior to sorting and packing (Petropoulos et al., 2017) Overall, the consensus of producer recommendations is lacking in standardized requirements that specifically address deficiencies that exist in DB onion pre-and post-harvest practices with regards to the

potential occurrence of multistate foodborne illness outbreaks in the near future.

Conclusion

Consumer trends have shown an increased consumption of fresh fruits and vegetables. During the pandemic, the WHO recommended a 400g mark for fruit and vegetable consumption needed for a healthy diet (WHO, 2020). Globally, the fresh produce industry responds to consumer consumption habits which fuels the sector's growth and development. However, recurrent foodborne illness outbreaks associated with raw and processed products decisively highlights the causal ecological, environmental, and legislative issues the industry must address in the future.

The fresh produce industry has developed a two-sided framework to straddle the control of bacterial contamination; the implementation of Good Agricultural Practices (GAPs) throughout pre-harvest production and Good Handling Practices (GHPs) throughout post-harvest phases and Good Manufacturing Practices (GMPs), Preventive Controls for Human Food and Hazard Analysis of Critical Control points during fresh-cut and food processing activities. Control measures are in some cases effective in ensuring the quality and safety of products reaching consumers. Yet legislation addressing these measures remains ambiguous which causes marked difficulty for producer compliance with the particulars of control frameworks that produces increased safety risks for consumers. Understanding the challenges, the legislative measures the industry must contend with is concomitantly met with private stakeholders need to formulate microbial testing measures that are complex, costly, and difficult to reliably report or indicate the presence of bacterial contamination. The DB onion industry is missing quantifiable risk profiles for the widely employed agronomic phases of topping, curing, lifting, curing, transport, sorting, packing, and storage. There is also a lack of risk-based tools available to producers that will enable identification of potentially consequential situations that will enable a higher level of safety to the

consumer. The challenge lies in coordinating a large-scale effort in updated legislation that absolves producer ambiguity with regards to implementation of pre-harvest and post-harvest measures and supplementing the ecological, environmental, and economic burden of producers ensuring microbial safety of their crops. Our goals root in updating our current scientific understanding and to provide tools producers can utilize along the production pipeline to navigate present existing issues.

The goal of this project is to contribute to our understanding of the best techniques to mitigate human associated microbial risks in pre- and post-harvest production practices of DB onions. Understanding the entirety of DB onion production from field to processing line can contribute to knowledge about the evolving risk of *Sal* contamination of this important crop. DB onions are a field produced row-crop with a variety of production schemes and these practices contain many processes that are known and unknown risks to pathogen contamination. These industry unknowns are reflected in grower interviews, the literature review conducted, and investigation into the recent foodborne illness outbreaks associated with DB onion. These efforts have led us to provide insight into the following unknown aspects within DB onions:

1. Although DB onions are known for their antimicrobial constituents, minimum inhibitory concentration (MIC) studies suggest that there is no correlation between the presence of these antimicrobials and inhibition of *Sal*.
2. *Salmonella* is capable of surviving and persisting along the entire DB onion cropping cycle and differences within topping, curing, soil moisture and UV index have little impact in expediting die-off.
3. Even contamination events do not lead to even distribution of *Sal* across the fields, highlighting the importance of preventing contamination from reaching the growing area. Once contamination occurs there are few intervention strategies that can be put forward to reduce the level of contamination, besides banking on the biology of *Sal* to impact its survival.
4. At harvest, contamination of DB onions with *Sal* persists through short term storage (3months) and short-term transport (3-days). However, significant die-off occurs at these

two stages within the DB onion food chain. This die-off could also vary based on environmental fitness of *Sal.*

5. High, medium or low contamination events at harvest do not lead to even distribution of *Sal* in the packing line and this can be attributed to a combination of die-off and dilution across the line.
6. Lack of dry or wet sanitation practices coupled with lack of clean breaks will lead to significant debris accumulation and bacterial growth as it was determined for EB, EC and ATP measurements.
7. A simple and cost-effective dry sanitation event was able to reduce debris and microorganisms from the packing line and should be implemented as a standard practice by the industry.
8. No correlation between the presence of EB, EC and *Sal* was determined at the packing line.
9. Plastic and camel hair bristles were not cleanable. Sanitation is unattainable and achieving an acceptable level of cleanliness (ATP< 30 RLU) was not possible.

Future research focus:

1. Quantifying *Sal* survival in various soil types and production locations with regards to UV light exposure.
2. Quantifying *Sal* survival in various irrigation sources and natural sediments contained within shared irrigation infrastructure and testing whether treating irrigation water prior to application is feasible and effective in mitigating contamination in pre-harvest production.
3. Understanding how the genetic make-up of *Sal* impacts its ability to survive under low moisture and low water activity conditions.
4. Describing how endo or epiphytic plant pathogens could increase the risk of contamination of *Sal* and other human pathogens along the DB onion cropping cycle.

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CHAPTER 2

Overview

Microbial risk assessment of dry bulb onion pre-harvest and post-harvest production practices based on *Salmonella* field contamination

Introduction: Dry bulb (DB) onion production practices have been involved in multi-state foodborne outbreaks linked to enteric human pathogens including *Salmonella* spp. (*Sal*).

Purpose: To develop risk profiles for DB onion production, we mimicked the cropping cycle through an open field inoculation trial followed by a packing line investigation.

Methods: Minimum inhibitory concentrations (MIC) Ten DB onion cultivars (10OCV) were used to determine whether onion tissue at 3,600 mg/L concentration could inhibit *Sal* (2, 3 and 5strain cocktail) inoculated at log 7 cfu/ml over a period of 24h of incubation at 37□C.

Field inoculation activities: MIC evaluations did not identify any significant reduction in *Sal*; consequently, 3 DB onion cultivars were selected based on scale color. At this location, risk factors were determined at 6 different stages during the cropping cycle 3-leaf stage (3LS), lifting, topping, harvest, storage, packing, and sorting activities. Risk quantification was based on initial inoculation of a two-strain cocktail of attenuated *Sal* (*attSal*) applied through furrow irrigation to a final nominal concentration of log 4.3 cfu/g soil and 2.5 cfu/g of DB onion. Bacterial recovery, enumeration (n=1063), and strain confirmation were achieved with selective and differential media, the *Sal*-specific MPN (MPN*Sal*), *Sal*-specific enrichment (NRCH*Sal),* and PCR methods to confirm inoculated strains.

Packing-house activities: Survival, persistence, and transfer of att*Sal* during sorting and packing activities of DB onions to food contact surfaces (FCS) was determined at a semi-commercial

packing line following the MPN*Sal* method. Additionally, the presence of indicator organisms *Enterobacteriaceae* (EB) and *Escherichia coli* (EC) and

Adenosine Triphosphate (ATP) residues were assessed using the Hygiena SystemSURE PLUS™ ATP system. A total of 897 swabs and 144 DB onion samples were collected, and the presence or absence of *attSal* was determined via NRCH*Sal.*

Results: Minimum inhibitory concentrations (MIC): The concentration of *Sal* remained the same after 24h of incubation and no statistical differences were determined between the initial and final inoculum concentration on all 10OCV (Tukey, $P<0.05$) with an average difference between time points of 0.164 log cfu/ml.

Field inoculation activities: The average population of *attSal* recovered at day 64 was 7.57 and 3.4 MPN/g of soil or onion respectively regardless of DB onion cultivar. Double Weibull (DWB) models for soil at early season (3LS), topping, and lifting indicates that 3LS meteorological conditions produce two distinct populations of *attSal* irrespective of cultivar. Later in-season treatments of topping and lifting did not produce two distinct populations of *attSal*, which suggests a marked increase in inactivation in comparison to 3LS. For DB onion samples, linear models suggested that cultivar, UV index, but not soil moisture content influence die-off. Differences in UV index between 7.8 and 3.8 generated significantly different die-off rates for *Sal*. **Packinghouse activities**: Dry sanitation practices were effective in removing DB onion residues from 12 out of 14 different FCS, and this effectiveness varied with material, timing, and risk level. Dry sanitation was marginally effective in reducing EB from FCS but ineffective in removing EC populations from those same areas. The average population of EB and EC taken from 14 FCS prior to dry sanitation was log 4.52 ± 0.18 cfu/location and log 3.28 ± 0.10 cfu/location ($n = 224$, $n = 224$) respectively. The average log reduction of EB and EC after dry sanitation was 1.54 and

-0.64 respectively. *Salmonella* was not recovered or detected (PCR *Sal*) from any of the swabs (Swab*attSal* < 3.0 MPN/area, n = 244) or composite DB onion (< 3.0 MPN/onion, n=144) samples collected over 4 days of sorting and packing DB onions. There was no correlation between the population of EB, EC, and *attSal* in DB onion or food contact surfaces.

Significance: Field contamination events closer in time to harvest activities increase the chance of onion contamination and subsequent transfer during storage, sorting, and packaging. Dry sanitation activities were effective in reducing microbial and DB onion residues from food contact surfaces. ATP was a good predictor of the accumulation of organic matter left in the packing line and can be used to determine the effectiveness of cleaning and dry sanitation events.

Highlights:

1. *Salmonella* was able to survive in soil and DB onions for over 64 days post-inoculation.
2. No difference in the survival of *Salmonella* was determined between onion cultivars.
3. No difference in the survival and persistence of *Salmonella* was determined between DB onion growing practices.
4. *Salmonella* recovered from DB onions at harvest persisted in the crop for at least 10 days after harvest.
5. Dry sanitation events are effective in reducing debris accumulation in the packing line.
6. Cross-contamination of food contact surfaces and DB onions was undetectable at contamination events with *Salmonella* populations in the range of log 2.31 cfu/g and 3.4 MPN/g DB onion.

Introduction

Enteric pathogens persisting within the proximity of confined animal feedlot operations (CAFO) have been known to be a contamination source of fresh produce (Gorski et al., 2022). Pathogenic enteric microorganisms evolve cellular mechanisms to facilitate their survival, adherence, colonization, and subsequent infection in niche spaces of epithelial linings of animal host gastrointestinal tracts (GIT) (Crum-Cianflone, 2008; Pradhan & Devi Negi, 2019). Ecotypes of these pathogens inherently possess morphological, biochemical, and pathological mechanisms assembled from interacting and evolving within the host's GIT, which has translated to improved epiphytic fitness (Barak et al., 2009; Barak et al., 2007; Guard-Petter et al., 1996; Nickerson & Curtiss, 1997; Römling et al., 1998). Microbial ecosystems of horticultural crops are complex, and their microbial consortia vary depending on the type of produce, agronomic practices, geographical area, and weather conditions (Beuchat, 2002; Brackett, 1999; Lund, 1992; Nguyenthe & Carlin, 1994). Human enteric pathogens becoming associated with endogenous phyllo-and rhizo-sphere consortia can occur anywhere throughout the duration of cropping cycles, in preharvest, harvest, and post-harvest environments. Enteric pathogens' adaptation to selective abiotic pressures within diverse niche spaces and subsequent prevalence within pre- and post-harvest production practices of fresh produce has increased the likelihood of foodborne illness (FBI) outbreaks.

Significant uncertainty encompasses the characterization and quantification of microbial risk factors regarding the prevention and mitigation of FBI outbreaks in many different fresh produce production systems including commercial dry bulb (DB) onion supply chains implicated in two major FBI outbreaks in 2020 and 2021 (FDA, 2020, 2021) and both linked to *Salmonella* spp. (*Sal)* contamination; which highlight how even low risk crops and their growing practices could become

a major problem to consumer health. The ability of *Sal* to survive, persist or proliferate within commercial DB onion pre-harvest and post-production environments is currently unknown, despite efforts to describe how generic *E. coli* and attenuated *E. coli* O157:H7 may contaminate the crop (Emch & Waite-Cusic, 2016; Islam, 2005; Shock et al., 2013; Shock et al., 2016; Wright et al., 2018). Improvement of DB onion safety regarding microbial human health hazards is of critical importance to its local industry in Colorado, the United States, and internationally. A binational FBI outbreak in the United States and Canada with *Salmonella* Newport linked to red onions in 2020 followed by a multistate outbreak with *Salmonella* Oranienburg linked to whole red and white DB onions in 2021, has prompted significant revisions of practices and risk factors associated with the crop. The presence of *Sal* in agricultural environments, such as soil, water, and on plants throughout pre-harvest production practices of DB onions could translate into cross-contamination of supply chains, starting at packing-house operations, interstate commerce, and subsequent consumer consumption.

The combination of the different varieties of DB onion cultivars, production regions, and the different production practices all add to our limited understanding of the complex parameters and factors that influence the life cycle of human pathogens persisting on the DB onions. Fundamentally, it is broadly accepted and supported *in vitro* that members of the *Alliaceae* plant family possess endogenous chemical constituents that result in antioxidant, antimicrobial, and antioxygenic properties (Anzabi, 2014; Mnayer et al., 2014; Reda et al., 2019; Sagar & Pareek, 2020), many of which are dependent on season, cultivar, and meteorological conditions throughout production cycles (Rodrigues et al., 2011). However, there is no clear understanding of the effects on how those metabolites impact survival and persistence of human pathogens like *Sal* in-vitro or in-vivo. Particularly, previous studies have suggested microbial inhibition of *Sal* in disk diffusion

studies with red and white onion extracts (Değirmencioğlu & Irkin, 2009), dehiscent papery skin and chopped inner-scale inoculations of yellow onions (Lieberman et al., 2015; Sagar & Pareek, 2020), and onion essential oil disk diffusion antibacterial activity measurements (Mnayer et al., 2014). Although DB onions possess intrinsic antimicrobial compounds, access to these compounds is limited to the inner layers away from the scale or neck of the crop, making this potential useful microbial hurdle limited in scope and applicability under commercial growing conditions, and highlighting the importance of identifying and addressing possible contamination routes along the cropping cycle.

Open field environment traceback efforts in the 2020 outbreak led investigators to suggest contamination may have originated from animal intrusion, proximity to CAFO's, irrigation sources, and particularly due to potentially persistent resident pathogens associated with the production area (FDA, 2020). Post-harvest traceback efforts identified the lack of sanitation practices, records, and training of personnel in two packing-house facilities, where visual observation of food contact surfaces suggested animal and pest intrusion, lacking inspection, maintenance, cleaning, or sanitization of food contact surfaces, and in some cases equipment installation issues may have collectively contributed to contamination (FDA, 2020). The DB onion industry widely does not utilize treated irrigation water in pre-harvest production and typically, packinghouses are dry operations that do not implement water in sanitization processes as it is critical to post-harvest quality and microbial safety of DB onions (NOA, 2022). The absence of frequent wet sanitation events may have prevented a "microbiological clean break" between sources of DB onions potentially providing the necessary conditions to cross contaminate multiple types of DB onions (FDA, 2020). Dry packing houses contain a variety of equipment dedicated to the cleaning, removal of skin, sorting, and grading of DB onions. A variety of materials are

used in this type of processing facilities. Currently it is unknown what frequency and type of sanitation of these materials will reduce the microbial contamination associated with DB onion packing facilities that could prevent future outbreaks.

Risk analysis determination relies on 3 separate parts, risk management, risk assessment and risk communication (Commission, 2003). Microbiological risk assessment (MRA) as the basis for implementing effective food safety risk management decisions in response to identified hazards relies on further scientific considerations including the likelihood of exposure and resultant risk profiles from these efforts as the basis for developing preventative strategies (LeJeune et al., 2021; World Health et al., 2006). Typically, in pre- and post-harvest agricultural production schemes, characterization efforts look to determine frequency of pathogen detection, variability in survival, and transfer rates with respect to major agronomic practices along the cropping cycle to better mitigate contamination through risk management efforts. A handful of previous efforts have looked to characterize prevalence and levels of *E. coli* throughout preharvest production of DB onions and curing processes (Emch & Waite-Cusic, 2016; Shock et al., 2013; Shock et al., 2016; Wright et al., 2018); but their currently lacks research findings to inform development of microbial risk assessments in DB onions with respect to prevalence and levels of *Sal* across pre-and postharvest production activities. It is currently unknown whether the inherent risk lies in vegetative growth, topping, lifting sorting and packing activities in dry packing house environments and whether dry sanitation events are effective in mitigating human pathogen prevalence and persistence. Therefore, assessment of risk within the DB onion food chain requires conceptualized pre-and post-harvest information coming from risk assessment efforts to determine the major contributing factors that increase the risk of contamination of DB onions.

This project looks to quantify microbials risk along the DB onion cropping cycle to identify actionable steps and thus, provide the industry with updated commodity specific production guidelines. Specifically, our goals were 1- to understand potential cultivar effects with regards to susceptibility to *Sal* contamination 2- to develop field experiments to evaluate the risk of *Sal* contamination in pre-harvest production practices 3- to evaluate the survivability of *Sal* in interstate transport and subsequent transfer of *Sal* into a semi-commercial packing-house setting 4- to quantify the use of dry sanitation practices in reducing transfer and cross-contamination of *Sal* to food contact surfaces within the packing-house and 5- to evaluate survival of *Sal* during storage.

Materials and Methods
Minimum Inhibitory Concentration (MIC)

Onion Growing Conditions

Ten DB onion cultivars (10OCV) were grown in 2021 and 2022 at the ARDEC South Station (ARDECS) located east of Fort Collins, CO USA (40°36'45" N, 104°59'46" W, 1521m). 10OCV included Blush F1, Vaquero, Sierra Blanca, Montessori, Red Delendro, Expression, Avalon, Super Star, Yellow Sweet, and Cabernet. Two 180-meter rows of each representative cultivar were sown using a 4-row Jang Automation Clean Seeder Precision planter. Soil physicochemical and fertility profiles were the following: pH of 8.1, 1.68 mmhos/cm soluble salts, 31.4 ppm nitrate (FIA) or 75lbs/acre nitrate, with 10 ppm bicarbonate content. Total cultivated areas were 1.503 acres receiving 78.33 lbs/ac 11-52-0 monoammonium phosphate and 200 lbs/ac 46-0-0 urea on 5/26/22. Pre-emergent applications of Induce, Roundup PowerMax, and Gunsmoke and post emergent Outlook herbicide were applied at label recommended rates when necessary. Rows were oriented east-west on a 1-2% slope allowing irrigation to leave the west side of the field. Seedbed preparation formed approximately 0.4m wide beds with 0.2m deep furrows. 20 rows of DB onions were planted, 2 rows per cultivar, 600 feet in length. Furrow irrigation was used to deliver 60% field saturation in manners specific to onion production. For minimum inhibitory concentration (MIC) and metabolite analysis, onions were hand harvested and topped after a two-week in-field curing process, labeled by waypoint number, repetition, and number of biological repetitions per sample per cultivar. A total of 3 collection bags per cultivar were used per analysis.

Weather Station Data Collection

A Davis Instruments EnviroMonitor Weather Station, which included a cabled Vantage Pro2 Plus

Instrument Sensor Suite (ISS) and three nodes, each configured with a Sentek 12" Drill and Drop Soil Temperature and Soil Moisture (SM) Probe and Davis Leaf Wetness Sensor were installed across the field to capture variation (Figure A3). The SM probe contains sensors for both parameters at the 4", 8" and 12" depth. A total of 3 SM sensors were placed in the fields. The ISS contained instrumentation which measures outside temperature, humidity, wind speed and direction, rainfall, UV, and solar radiation every five minutes, 24-hours a day. Barometer, temperature, humidity, dew point, wind speed and direction, wind chill, heat index, rain inches, rain rate, solar radiation, solar energy, evapotranspiration, UV index, UV Dose, heating degree days, cooling degree days, soil moisture and temperature at 4", 8", and 12", and leaf wetness parameters were measured on a five-minute interval over the course of all field experiments.

Salmonella Strain Selection

A cocktail of 5 strains was used on these evaluations. The cocktail consisted of 2 avirulent *Sal* genotypes *attSal* (PTVS 178 and PTVS 337) that lack the adenylate cyclase and cyclic AMP (cAMP) receptor protein due to deletion (delta) of the *cya* and *crp* genes respectively (Curtiss et al., 1988). Each *attSal* strain has deletions (delta) of the invA (-9 to +2057) gene contained within the T3SS-1 pathogenicity island and the deletion (delta) of *spiB* within theT3SS-2 pathogenicity island resulting in *Sal* genotypes unable to produced intestinal inflammation within host species (Crawford et al., 2012; Liou et al., 2022). Three pathogenic genotypes of *Sal*; specifically, *Sal enterica* serovar Poona (human isolate, CA Department of health (00A-3279), Montevideo (originally from Dr. Max Teplitski University of Florida), and Newport (originally from Dr. Swaminathan J1890, PA Dept of Health, Bureau of Labs, Leanville, PA, 2002 tomato). Mixed cocktail, which consisted of all five strains listed above mixed together. The goal was to elucidate

whether different slurries from 10OCV inhibit growth and survival of *Sal* and whether genotype differences impacted growth and survival of *Sal*. Comparison of attenuated and pathogenic behavior within minimum inhibitory concentration (MIC) assay will further elucidate the effectiveness of utilizing the attenuated *Sal* cocktail (*attSal)* as a model for pathogenic isolates associated with FBI outbreaks as listed by Lopez-Velasco, et al 2015. All strains possess rifampicin resistance (50 µg/ml) in both broth and agar settings to solidify our ability to select and recover *attSal* from the environment. The 2-train *attSal* inoculum was also used in field inoculation studies described below.

Laboratory Inoculum Preparation

Inoculum for MIC and field inoculations was prepared following the procedure described by Gutierrez-Rodriguez et al. 2012. In brief, stock cultures were streaked on selective and differential media TSA (TSA Rif^{50}), XLT4 (XLT4 Rif^{50}) and ChromAgar (ChromeAgar Rif^{50}) containing 50 mg/ml rifampicin (Rif_{50}) and incubated for 24h at 37 C. After colony morphology and phenotype were confirmed, 1-2 colonies from the TSA Rif^{50} plate were placed in duplicate 5ml m-Broth Rif_{50} 15ml tubes and incubated for 24h at 37°C and 200 RPM in a Excella E25 shaking incubator. Duplicate cultures were combined, pelleted at 7000xg for 5 minutes in a Thermo Fisher Sorvall Legend XIR centrifuge, washed two times and resuspended in appropriate volumes 0.01 M K_2PO_4 Potassium Phosphate buffer pH 7. The optical density (OD) of the resuspended pellet was measured (ThermoFisher Genesys 180 UV- Vis) and adjusted to an OD_{600} 0.750 ± 0.030 for each strain which represent a nominal concentration of Log 9 cfu/ml. Once the optical density was achieved, 100 □l of the bacterial solution was plated into triplicate TSA Rif^{50} plates and incubated overnight (ON) to generate bacterial lawn plates. Cells were removed from each plate by adding 2ml of Potassium Phosphate buffer pH 7 and using an L-shaped spreader to dislodge the cells from

the agar. Using a 2ml pipette, the entire liquid from the plate was removed and placed in 300ml of Potassium Phosphate buffer pH 7. A total of 3 plates (6ml of cell suspension) were placed in 300ml of Potassium Phosphate buffer pH 7. The OD of this solution was measured and adjusted to an OD_{600} 0.750 ± 0.030 representing log 9 cfu/ml for each strain and the 300ml solution from each strain was used to prepare a 2-strain (PTVS 178, PTSV 377), 3-strain (Poona, Montevideo, Newport) and 5-strain inoculum cocktail used on all MIC evaluations.

Minimum Inhibitory Concentration (MIC)

Representative DB onion cultivars (10OCV) were grown in the 2022 growing season at ARDEC South Research Station. Field samples of 10-15 onions were geotagged with a waypoint number to keep track of where in the field they originated. Three replicates per sample were retrieved from the field, with one sample within three separate 200 feet sections. Onions were subsequently processed in one day reflective of a short period of storage prior to repacking or distribution after field curing. DB onions were skinned using fresh nitrile gloves after the neck and basal stem were removed using a knife, leaving a halved edible portion of the bulb. Longitudinal cuts produced two separate halves of bulbs for each onion in the samples. Halves of each sample were then pooled separately to produce two sample sets per cultivar; one of these two sets was dedicated to the MIC assays while the other half was used for metabolomic analysis discussed below. Seventy percent ethanol was used to sanitize cutting boards and knives and items were dried using single use paper towels between cultivars throughout sample processing activities. Each sample set was stored at -80 ºC until further processing. For MIC evaluations, one sample ser was removed from the -80C and macerated into 0.1M potassium phosphate buffer to produce a 3,600 mg/L onion extract. The 3600 mg/L onion extract was subsequently filtered through a 0.45 µm cellulose acetate membrane inside a class II biosafety cabinet or near a Bunsen burner. DB onion extracts

were then inoculated in triplicate 1cm cuvettes samples with each of the 3 different bacterial cocktails described above to a final nominal concentration of log 7 cfu/ml. Three positive (inoculum cocktail) and negative (phosphate buffer and onion slurry at 3600 mg/L) controls were used on all evaluations. To determine the initial and final population of the cocktails, pre-and post-incubation cuvettes homogenized by pipette were serially diluted and direct plated on TSA Rif^{50} and XLT4 Rif^{50} to confirm strain morphology and bacterial population. DB Onion samples were at minimum representative of 3-6 biological repetitions per cultivar and were representative of only the edible portion of the crop, as skins were removed during sample processing.

Field Inoculations Studies

From MIC results, 3 DB onion cultivars were selected based on their color since none of the 10OCV slurries impacted die-off or growth of *Sal*. The two growing seasons lasted from April through October in 2021 and 2022 at ARDECS. Soil type at fields 3 and 4 is classified as clay loam soil (fine loamy, mixed, mesic Aridic Haplustalfs (Stewart et al., 2017). Rows were oriented in east west fashion at a 1-2 percent grade to allow irrigation to leave the west end of the field. The seeding rate was 140,000 seeds per acre. Field layout is described in Figure A1 and overall, it followed a split block design. Each block contained three repetitions per DB onion cultivar that were 100 feet in length and 12 rows wide. The 12 rows of each repetition were designated into four treatments: sowing-3 Leaf Stage(3LS), Topping, Lifting, and Control. Each treatment was separated by a buffer furrow and stand of DB onions in all field replicates. Each DB onion cultivar block contained three field replicates and was separated by a buffer zone of 10m. Field replicate seed beds and irrigation infrastructure were prepared to ensure similarity between split blocks. Within blocks, the treatments were fixed in order. Prevention of cross contamination between blocks and field replicates was achieved through the construction of berms and tail ditches to

ensure no flow of irrigation waters containing *attSal* between field plots. Placement of inoculation sachets and sampling locations for soil and onion were measured 3m apart along the furrow and flagged to ensure soil sampling per timepoint was uniform in location and will be described below. Field replicates were prepared in a manner sufficient to maintain high degrees of similarity with respect to seed bed preparation, herbicide and fungicide application, irrigation infrastructure and flow rate, sampling method, inoculation level, and plot size to isolate variables of interest.

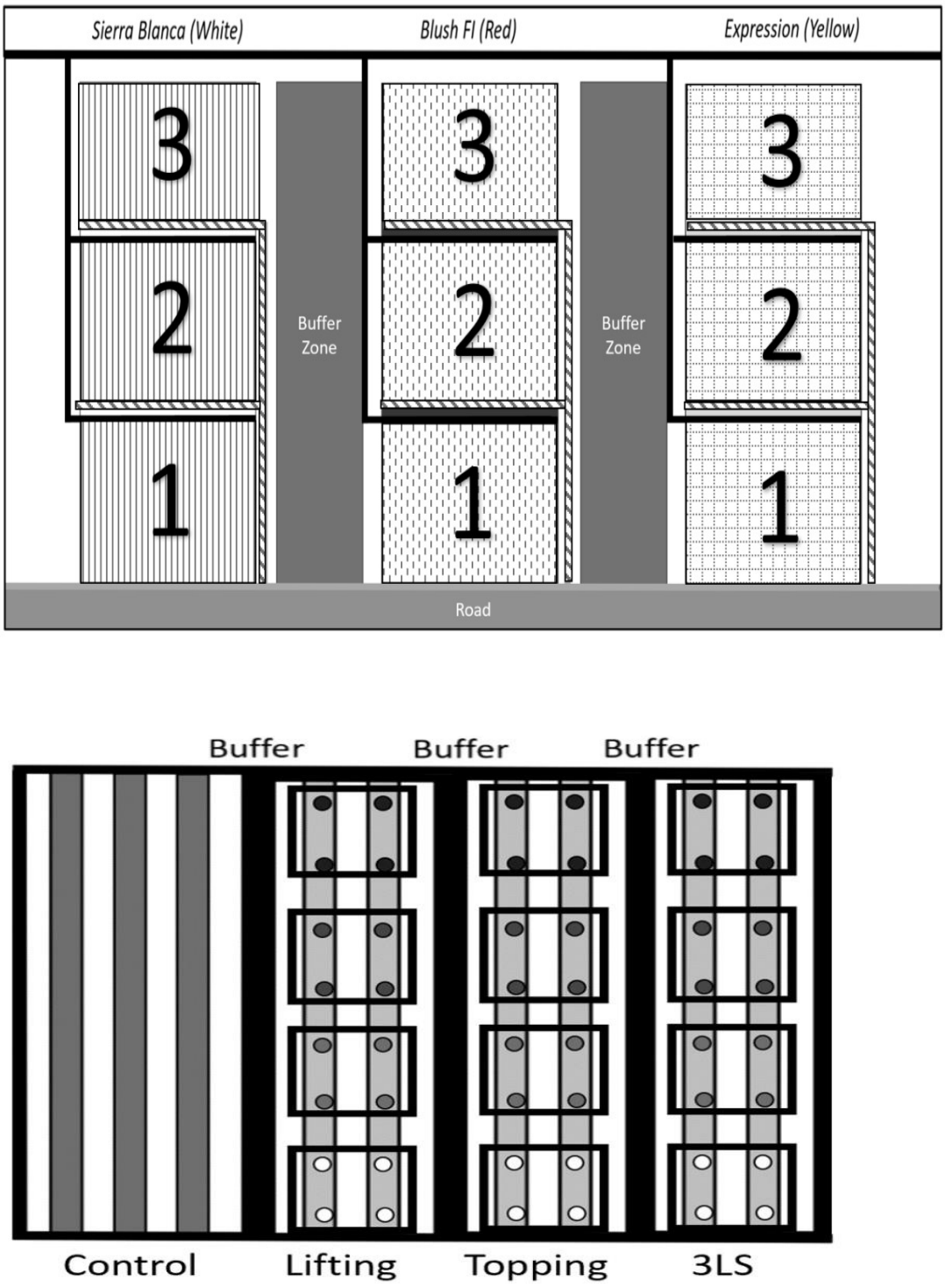

Figure 1. Top image: Field layout representation indicating location of the DB onion cultivars 2108 with 3 field replicates. The twelve rows were separated into treatments and are shown in the left image. Black lines are intended to show tail ditch and berms between treatments and striped

lines are intended to show furrow irrigation tubing. A 10m buffer zone separated each cultivar. Bottom image: The 12 rows were divided into four treatments to create a fixed DB onion development gradient within each field replicate to mitigate potential contamination across treatments and throughout sample collection efforts. Moving right to left, sowing-three- leaf development stage (3LS), Topping, Lifting, and Control are separated by a buffer row. Each group of four dots represents a single aggregate subsample for both soil and onion and is shown by a black rectangle surrounding the group of four dots. Each treatment contained four aggregate subsamples for both sample types, respectively. An aggregate soil sample of 150g represents four individual sterile scoops collecting soil at a depth of 20cm and an aggregate onion sample represents 4 individual whole onions, one from each dot respectively. Control onions were not inoculated with *attSal*.

Inoculum Preparation (Inoculation Sachet)

A cocktail of two *attSal* strains (PTVS 178, PTVS 337) was prepared as described before with two main modifications. TSA- Rif^{50} plates were amended with the fungicide pentachloronitrobenzene (PCNB) at a nominal concentration of 5mg ml^{-1} and after the first OD adjustment to OD_{600} of 0.750 ± 0.030, six to eighteen TSA Rif^{50} + PCNB plates were used to prepare the lawn plates. All other activities followed the procedure described before and by Gutierrez-Rodriguez et al. 2012. The combination of amendments of PCNB and Rif^{50} minimized interference with other bacteria that are not the model inoculant and help facilitate the detection and recovery of the strains contained within the cocktail (Beuchat et al., 2001; Gutiérrez-Rodríguez et al., 2012). After the second OD adjustments resulting in a nominal inoculum concentration of log 9 cfu/ml, this solution was diluted by one logarithm and the suspensions (total 3 L) was mixed with sterile sand (3kg) saturated with 2% non-fat powder milk (150g) and mixed vigorously before preparing the inoculation sachet. Sachets were prepared in the following manner. Three separate bins containing 3kg of sterile sand were autoclaved and allowed to cool to room temperature. Each 3Kg bin received 3L of *attSal* at log 8 cfu/ml. Next 150g of 2% non-fat powder milk were homogenized using sterile spoons to mix the sand inoculum powder milk mixture. Each inoculation process created 9kg of this suspension. After homogenization, 150-gram aliquots of the sand, inoculum, milk suspension were then placed into open sachets using a sterile spoon until the desired number of sachets were filled. After all the sachets had been filled, a heat sealer was used to seal the sachets to keep the sand mixture from escaping the sachet. At random, three representative sachets were selected to confirm the population of *attSal* inside the sachets. Confirmation was accomplished by adding 150ml of 0.1M sodium phosphate buffer + $Tween^{20}$ to the 150-gram sachet placed in a sterile Whirl Pak bag with filter. Hand homogenization for 1 minute aimed to dislodge attached *attSal* cells into suspension. Serial dilution and direct plating methods on TSA

Rif^{50} + PCNB and XLT4 Rif^{50} + PCNB were followed to determine the final concentration of *attSal* in each sachet.

Presence of Naturally Occurring *Sal*

Before inoculations took place samples were collected across all field replicates and DB onion scale cultivars to assess the potential presence of naturally occurring *Sal* within treatment plots. A total of 16 individual samples per field replicate per DB onion scale were collected for soil and onions at the 3LS. The same number of samples was collected when topping and lifting activities took place throughout the season. There was a 42- and 48-days sampling gap between the 3LS and topping and lifting, respectively. All samples were negative for the presence of naturally occurring *Sal (Data not shown).* After confirmation of negative results, field inoculations consisted in placing the sachets at 3m intervals along the length of each furrow. Inoculum distribution was achieved through furrow irrigation. Each irrigation event began at dusk, approximately 7pm and continued for 8h. The flow rate to each furrow was approximately measured at 7.5 gallons per minute (gpm), while the well head pressure was estimated to be 400 gpm. The initial inoculation provided in the sachets was targeted to apply approximately log 5.0 cfu/g to soil and log 3 cfu/g to DB onion stands per treatment plots by the conclusion of the 8-hour furrow irrigation application. Applications were controlled in terms of flow rate and duration to effectively suspend the cells from the sachets into the irrigation water to produce homogenized spread of inoculum. Confirmation of the presence of *attSal* in soil and onion samples was performed after 36h of the inoculation event at the 3LS, topping and lifting stages and described below. Additionally, 3 random sachets were collected from the field or from unused sachets to confirm the population of *attSal* delivered to the treatments. In all instances the population of *attSal* matched the targeted log 8 cfu/ml (Data not shown). Bacterial recovery from the sachets followed the modified procedure described by (Beuchat et al., 2001; Gutiérrez-Rodríguez et al., 2012) as described above.

Soil and DB Onion Sample Collection

Soil and onion samples were collected following aseptic techniques in a two-member team approach to prevent cross contamination. At all times each team member was wearing a Tyvek suit, boot covers and doble nitrile gloves. Samples were collected strategically to minimize the possibility of cross contamination of split plots and repetitions. Sampling took place at sunrise of each sampling event. An aggregate sample was representative of four individual samples. Four aggregate samples were collected per field replicate per DB onion cultivar. This process occurred for both soil and onion sample types. Aggregate soil and onion samples were in the range of 600g while aggregate onion samples varied in weight based on the size of the bulb. All collected bags were surface disinfected before placing them in coolers immediately after collection and subsequently transported to a 4°C walk in cooler located at the Nutrien Agriculture Science Building, Colorado State University. Samples were processed within 48 h of collection. Sampling events for the 3LS were as follows 1, 4, 8, 16, 32, and 64 days after inoculation, while for Topping and lifting were performed at 1, 4, 8, and 16 days after inoculation. Topping and lifting activities took place a week apart between them and started 42 and 48 days respectively after the inoculation of the 3LS.

Bacterial Recovery from Soil and Onion Samples

Aggregate soil samples: field samples were homogenized by hand for 1 min before collecting a 150 g subsample inside a new sterile WhirlPak bag with a filter. A 1:1 ratio of soil to 0.1M $NaPO_4$ (liquid) + $Tween^{20}$ was used to dilute the sample and to dislodge firmly attached bacteria from soil particles (Lopez-Velasco et al., 2015). The WhirlPak bag was massaged for 30s to effectively suspend the soil. The entire supernatant was extracted from the bag into a new bag for subsequent dilutions and detection of *attSal.*

Aggregate onion samples: field samples were weighed directly in the collection bags and a 1:1 ratio of DB onion samples to 0.01M K_2PO_4 (liquid) + $Tween^{20}$ was used to dilute the sample and to dislodge firmly attached bacteria to the onions (Gutierrez-Rodriguez et al., 2018). The WhirlPak bag was massaged vigorously for 30s and the supernatant was placed into a new bag for subsequent dilutions and detection of *attSal.*

Bacterial Enumeration for Soil and DB Onion Samples

Serial 10x dilutions from soil and DB onion supernatants were performed using 9ml tubes containing K_2PO_4 and plated on XLT4 + Rif^{50} + PCNB for enumeration of *attSal.* When samples were below the limit of detection direct enrichment of samples and the 3 tube MPN method for *Salmonella* (MPN*Sal)* described by (McLaughlin et al., 2010) were used to detect or enumerate viable populations of the *attSal* for each sample. Enrichment of samples followed the procedure described by Gutierrez-Rodriguez et al 2012 and 2018 and Lopez-Velasco et al 2015) with some modifications. In brief, an aliquot of the supernatant 1:1 (v/v) was mixed with 2X universal preenrichment broth (UPB) + Rif^{50} and incubated for 24h at 37°C inside a standup 500ml WhirlPak bag with no filter. After incubation, 10ml of the supernatant was transferred into 90ml of tetrathionate broth (TTB) + Rif^{50} and incubated for 6 hours. After the six-hour incubation, 20ml was transferred into 180ml of m-Broth + Rif^{50} and subsequently incubated for 24 hours at 37°C. Finally, m-Broth+ Rif^{50} enrichments were then streaked onto XLT4 + Rif^{50} + PCNB and incubated for 24 hours at 37°C for confirmation. Positive samples after enrichment or MPN method were saved in 26% glycerol tubes and stored at -20ºC for further testing and PCR confirmation of the inoculated strains. Probe based PCR confirmation followed the procedure described by LopezVelasco et al 2015.

Field Experiment Activities Statistical Analysis

Viable *attSal* recovered in aggregate whole onion plant samples and soil over the course of commercial DB onion pre-harvest 3LS, Topping, and Lifting phases were described using linear modeling in ggplot2 in R studio version 4.2.03 and constructed inactivation models produced within GInaFit linear log-tail (LLT), Double Weibull (DWB), and biphasic (BP) methods (Geeraerd et al., 2005). Models were constructed using an average of four sub-samples per field replicate per cultivar. Models were constructed using all subsamples contained within each field replicate to capture variability across sampling days. Analysis of variance between treatments within DB onion cultivars with regards to time series recovery of *attSal* showed no significant differences between cultivars using a one factor ANOVA, (data not shown). Consequently, the survival, persistence, and die-off of the inoculated *attSal* across production was evaluated based on soil moisture and ultraviolet (UV) index across the time series, where each DB onion cultivar block (total 3) was treated as single DB onion cultivar with 3 independent field replicates. These field replicates were separated based on differences in soil moisture content (Figure A3) and statistical comparisons were done based on soil moisture content and differences in the UV index along the cropping cycle.

Harvest and Interstate Transport of Onion Materials to California

DB onions were hand harvested the third week of October 2022 from each field plot based on DB onion cultivar and treatment (3LS, Topping, Lifting) and placed inside 38”x 40”x 31” polyethylene bins to approximately half of the 25 cubic foot capacity (Total of 3 bins per treatment per onion cultivar). These bins were placed in the buffer zones to avoid cross contamination. Harvest activities were completed based on inoculation events starting with the controls, followed by the 3LS, topping, and lifting to minimize potential cross-contamination between onion materials.

Once harvested, the plastic bins were moved inside a Cravo greenhouse, where they were transferred into carboard boxes that were used for transport to DB onions CA. This set of onions grown at ARDECS were considered low risk onions for all packinghouse activities listed below, based on the populations of att*Sal* recovered at harvest that range from 3 MPN/g +/- 0.15 to 9.6 using a 3tube 0.1, 0.01, and 0.001g inoculant and corresponding 95% CI.

Two additional treatments were incorporated in these studies and originated from commercially grown yellow and red onions cultivated by stakeholders in commercial fields in Colorado. These onions were mechanically harvested by our partners and brough to ARDECS inside 96x 102x 78.7 cm polyethylene bins. None of these onions were inoculated in the commercial fields. Once at ARDECS, the red and yellow DB onions were separated into two groups: 1- Commercial control and 2- Commercial inoculated. Each group was placed within separate carboard boxes with a plastic liner that was used for transport to CA. The commercial inoculated DB onions were inoculated inside the carboard boxes following the modified procedure described by (Glaize, 2021). In brief 0.75 kg of chalk inoculum was mixed with 0.36 cubic meters of DB red or yellow onions (Figure A4). Mixing was done by hand and by rolling the onions in the chalk containing *attSal.* The onions with chalk remained inside the plastic liner located inside the cardboard box. The initial population of *attSal* in this chalk was log 5.5 cfu/g after inoculum preparation and subsequently confirmed via serial dilution and direct plating on TSA + Rif^{50} and XLT4 + Rif^{50} seven days after inoculation showing identical *attSal* populations. These onions would be considered high risk onions for all packinghouse activities listed below, based on the artificial chalk inoculation of att*Sal*. This approach was undertaken based on how onions are mechanically field harvested from the ground in which the crop is exposed to significant dust and mixing of soil across the farm. All cardboard bins containing DB onions were stored inside a Cravo greenhouse

for 24h before subsequent shipment, to Fresno State California. The environment in the greenhouse was held to 18.3°C ± 10° and kept cool and dry. Travel from CO to CA took 60h and upon arrival in Fresno, all cardboard boxes were stored in a covered outdoor hallway. Carboard boxes remained in the covered hallway for 4-6 days before initiating packinghouse activities. A total of 11 days passed between harvest, chalk inoculation, transport, and the start of packinghouse activities. A small subset of DB onions from low and high-risk samples were collected before transport and placed in burlap sacks (30.5 x 50.8 cm). These burlap sacks were then stored for 3 months at 18.3°C ± 10° and 60% RH ± 10. After 3 months of storage, all burlap sacks (total 32) were removed from storage and tested for the presence of viable att*Sal.* All 32 samples from low and high-risk DB onions were negative for att*Sal* after enrichment and MPN enumeration (<3 MPN/g +/- 0 to 9.5) (Data not shown).

Packing House Handling and Sorting Activities

Before packinghouse activities were performed, the unit was calibrated to separate onions based on USDA grade. This was achieved using a small portion of commercial control DB onions. Once completed a total of 14 locations within the packing line were selected based on the frequency of contact of the DB onions with each location and visual observations where crop residues or color was being deposited from impacts, abrasion, or concentration of materials. The locations were marked to dedicate an approximate 0.25m x 0.25m area for swabbing for adenosine triphosphate (ATP) residues, indicator microorganisms *Enterobacteriaceae* (EB) and *Escherichia coli* (EC) and *attSal*. Before starting experimental procedures, each of the 14 locations was assessed for ATP, EB, EC, and the potential presence of naturally occurring *Salmonella* as the packing line had not been cleaned and sanitized in an estimated 3-year period. Once all swabbing activities were completed, each section was careful cleaned following a stepwise sanitation process which utilizes 1- scrubbing with single use paper towel to remove residues, followed by 2- application of food

grade 70% ethanol 3- followed by scrubbing with a single use paper towel, and finally followed by the use of a 4- food-grade alcohol-based wipe (WP+ Wipes Plus No-Rinse Food Contact MultiSurface Wipes) to remove any potential residues left on the surface. This procedure mimics a dry sanitation procedure that could be used by industry that will not leave any water residues on food contact surfaces. After alcohol evaporation (3-5min), the same areas were swabbed again for the same four parameters listed before and to evaluate the efficacy of this procedure in removing residues from DB onions and other components.

Table 1. Locations within the packing-line, description, material, ability to be cleaned and sanitized based on dry sanitation practices.

Location	Description	Material	Able to be sanitized
1	Leaf-eliminator start	Stainless Steel	Yes
2	Grading table rollers	Polyvinylchloride (PVC)	Yes
3	Brush bed start	Stainless Steel	Yes
4	Green brush	Brush	No
5	Brown brush	Brush	No
6	Divider	Plastic	Yes
7	Brush bed end	Stainless Steel	Yes
8	Belt	Rubber	Yes
9	Rollerstar beginning	Stainless Steel	Yes
10	Rollerstar chute	Plastic	Yes
11	Sorted Bin 1	Stainless Steel	Yes
12	Sorted Bin 2	Stainless Steel	Yes
13	Sorted Bin 3	Stainless Steel	Yes
14	Sorted Bin 4	Stainless Steel	Yes

ATP measurements were performed using the Hygiena SystemSURE PLUS™ ATP luminometer which provides real-time ATP measurements, while EB and EC enumeration was accomplished according to manufacturer standard operating procedures (Hygiena MicroSnap™ EB and EC). Any food contact surface with an ATP measurement below 30 RLU was considered cleaned based on ATP manufacture recommendations. The population of EB and EC was determined postincubation with the Ensure luminometer and enumeration was determined following procedure described by Ivancic et al 2008. The 3M™ neutralizing buffer swabs were utilized to recover *attSal* (Swab*attSal*) *from* all 14 surface locations and the supernatant was enriched following the NRCH*Sal* method. First, transferring 1ml of the supernatant to 9ml 2x Universal Pre-Enrichment Broth Rif^{50} (UPB) and incubated for 24 hours at 37 °C. Next, 1ml of the UPB

Rif^{50} enrichment was transferred to 9ml tetrathionate broth Rif^{50} (TTB) and incubated for 6 hours at 42°C. Finally, 1ml of the TTB Rif^{50} enrichment was transferred to 9ml of m-Broth Rif^{50} and incubated for 24 hours at 37°C. After enrichment, the supernatant was streaked on to XLT4 Rif^{50} to confirm the presence or absence of viable *attSal* inoculum. A total of 96 Swab*attSal* enrichment samples (43% of all collected samples; total 224) were also analyzed for the presence of *attSal* by probebased PCR as described above. All samples were negative for low and high-risk packing activities.

Besides ATP, EB, EC and Swab*attSal* samples, whole DB onion samples were also retrieved at random after each processing hour and consisted of 4-8 onions placed in a filtered WhirlPak bag. Samples were then weighed and diluted in an equal volume of 0.1M potassium phosphate Tween^{20} and homogenized by hand into suspension. Onion samples were enriched and enumerated following the NRCH*Sal* and MPN*Sal* method respectively. Morphologically consistent colonies from either NRCH*Sal* or MPN*Sal* XLT4 Rif^{50} plates were saved in 26% glycerol and stored at 20ºC for further testing and PCR confirmation of the *attSal* inoculated strain. A total of 60 enrichment samples (42% of all collected samples; total 144) were also analyzed for the presence of *attSal* by probe-based PCR as described above. All samples were negative for low and highrisk DB onions.

Sampling scheme High-risk Event Day 1 (Commercial Red DB Onions) and Day 2 (Commercial Yellow DB Onions)

Initial handling and sorting activities consisted of the following approach. The inoculum of these onions was log 5.0 cfu/ml. Day 1 activities included the following: 1- Dry Sanitation 2- swabbing of dry sanitation areas, 3- processing of commercial control and commercial inoculated samples after dry sanitation (DS1) for 4h. On day 1, commercial red onions were processed. In the first

hour commercial control red onions were sorted and packed (CR1). After 1h commercial inoculated red DB onions were sorted and packed for 1h, followed by sorting and packing of commercial control red onions at the 3h mark (CR3). Finally, the commercial inoculated red DB onions were sorted and packed for an additional hour to complete the 4h processing ran (CR4). Samples were collected from food contact surfaces and from DB onions at the end of the 1, 3 and 4h timepoints. No dry sanitation events were performed between day 1 and 2 activities and represent standard commercial practices for DB onions. Day 2 activities were identical to day 1 with the only modification that commercial yellow (CY) DB onions were sorted and packed during this second day. Sampling frequency and procedures were identical to day 1 activities and took place at the 1 (CY1), 3 (CY3) and 4h (CY4) timepoints. The number of swabs and DB onion samples retrieved during these hourly activities is described in Table 2.

Sampling Scheme Low-risk Event Day 3 ((ARDECS Yellow (AY)) and Day 4 and ((ARDECS White (AW))

These activities were considered a low-risk event knowing that the population of *attSal* at harvest was at or below 0.15 and 9.6 MPN/g (95% CI). In between the end of the high-risk activities and initiation of low risk sorting and packing DB onions a dry sanitation event (mimicking a clean break) was applied to the line at the 14 food contact surface locations marked in the line. The same dry sanitation approach described above was followed at these 14 locations (only) because of the size of the packing line and limited resources. The efficacy of dry sanitation practices was assessed by ATP, EB, and EC enumeration. Swabbed locations with ATP values below 30 RLU were considered cleaned per manufacturer specifications. After this dry sanitation event, the third and fourth day of sorting and packing DB onions started following this approach. On day 3 activities included: 1- Dry sanitation 2- swabbing of dry sanitation areas (DS2), 3- processing of yellow (AY) DB onions. Processing was also performed on a 4h long run, that included sorting and

packing of AY DB onions from lifting, topping, 3LS and the control. All AY DB onion samples were processed in this order and swan and onion samples were at the 1 (AY1), 2 (AY2), 3 (AY3) and 4-hour (AY4) mark. No dry sanitation events were performed between day 3 and 4 activities and represents standard commercial practices for DB onions. Day 4 activities were identical to day 3 with the only modification that white (AW) DB onions were sorted and packed during this fourth day. Sampling frequency and procedures were identical to day 3 activities and took place at the at the 1 (AW1), 2 (AW2), 3 (AW3) and 4-hour (AW4) mark.

The number of swab and onion samples retrieved during these activities is described in Table 2. After 4 days of sorting and packing DB onions from low and high-risk DB onions, a commercial sanitation company was hired to clean and sanitize the entire facility following standard wet and dry sanitation practices. No *attSal* was recovered from any of the 14 locations marked in the line (data not shown).

Table 2. Sampling scheme for sorting and packing DB onion.

Descriptor	**Risk Level**	**Identifier**	**Hour**	**ATP**	**EB**	**EC**	**Swab*attSal***	**DB onion samples**
Dry Sanitation 1	--	DS1	--	14	14	14	14	--
Commercial Red Inoculated	High	CR1	1	14	14	14	14	14
Commercial Red Control	--	CR2	2	--	--	--	--	--
Commercial Red Inoculated	High	CR3	3	14	14	14	14	13
Commercial Red Control	--	CR4	4	14	14	14	14	13
Commercial Yellow Inoculated	High	CY1	1	14	14	14	14	14
Commercial Yellow Control	--	CY2	2	--	--	--	--	--
Commercial Yellow Inoculated	High	CY3	3	14	14	14	14	13
Commercial Yellow Control	--	CY4	4	14	14	14	14	13
Dry Sanitation 2	--	DS2	--	14	14	14	14	--
ARDEC Yellow Lifting	Medium	AY1	1	14	14	14	14	8
ARDEC Yellow Topping	Medium	AY2	2	14	14	14	14	8
ARDEC Yellow3LS	Low	AY3	3	14	14	14	14	8
ARDEC Yellow Control	--	AY4	4	14	14	14	14	8
ARDEC White Lifting	Medium	AW1	1	14	14	14	14	8
ARDEC White Topping	Medium	AW2	2	14	14	14	14	8
ARDEC White3LS	Low	AW3	3	14	14	14	14	8
ARDEC White Control	--	AW4	4	14	14	14	14	8
Total	--	--	16	224	224	224	224	144

DS1 and DS2 were dry sanitation events. Each row is representative of hourly sampling sessions. The packing line was halted at each hour and swabbing of food contact surfaces and collection of DB onion samples occurred. Total number of swabs for ATP, EB, EC, and Swab*attSal* across the four days and 16 h of sorting experimentation are reported.

RESULTS

Minimum Inhibitory Concentration

Sal growth was not inhibited by any of the 10OCV despite identifying significant differences in their metabolite profiles (data not shown). The concentration of *Sal* (2, 3 and 5-strain cocktail) remained the same after 24h of incubation and no statistical differences was determined between the initial and final inoculum concentration on all 10OCV (Tukey, $P<0.05$) with an average difference in the final population of *Sal* between time points of 0.164 Log cfu/ml (Table A1).

Survival of *attSal* Across the DB Onion Cropping Cycle (64 Day Period)

For soil samples, there was a significant die-off of *attSal* within the first four days after inoculation (die off = 2.1 log cfu/g of soil, $p>0.05$), however, this log reduction was identical between onion cultivars (Figure 1, Panel A). After day 4 survival and persistence of *attSal* was identical between DB onion cultivars and remained consistent and in the range of log 2.5 cfu/g of soil for over 32 days. At harvest the population of *attSal* for all DB onion cultivars was below the limit of detection and was estimated to be 7.57 MPN/g of soil, (1.3-20 95% CI) with no differences between DB onion cultivars (Figure 1, Panel A). No significance difference ($p< 0.05$) in the survival and persistence of *attSal* was determined between DB onion cultivars (cultivar) (mean *attSal* recovery of 2.03 ± 0.09, 2.01 ± 0.09, and 1.94 ± 0.09 log cfu/g DB onion respectively, ($n = 144$ for red, white, and yellow) along the majority of the cropping cycle (Figure 1 Panel B). This period included vegetative growth, bulb maturation, topping, lifting, a two week in field curing, and hand harvesting. It represents the typical commercial DB onion cropping cycle and is intended to follow the survival and persistence of *attSal* from the 3LS to the packing line. The population of *attSal* for DB onion at the 3LS remained stable and in the range of log 2.3 cfu/g of DB onion during the

first 32 days post inoculation. This was not observed for soil samples during the same sampling period. At 64 days post inoculation the population of *attSal* was estimated to be 3.4 MPN/g of DB onion. (0.17-18 95% CI) (Figure 1, Panel B) while for soil it was 7.57 MPN/g of soil, (1.3-20 95% CI) (Figure 1, Panel A). Based on these results, and despite finding a marginal difference in the recovery of *attSal* based on DB onion cultivar in one single time point (16-day recovery, Figure 1, Panel A and B) the overall effect for cultivar was determined to be non-significant (Tukey HSD ($p<0.05$). All other evaluations were based on soil moisture content and UV index with cultivar not included in the description of the main effects.

When comparing the survival and persistence of *attSal* based on percent soil moisture content for node 3 average % soil moisture across treatments was 19.5% (Low), for node 2 it was 25% (Medium), and for node 1 it was 36% (High). No significant difference in the recovery of *attSal* was determined between low, medium, and high soil moisture content along the cropping cycle (population of *attSal* was 1.93 ± 0.09, 1.96 ± 0.09, 2.09 ± 0.09 log cfu/g of soil respectively). Cultivar and soil moisture had no impact on the survival and persistence of *attSal* along the cropping cycle. When comparing the survival and persistence of *attSal* based on the average UV index (Table A1) (mean UV index of the first 16 days (7.8), 32 day (6.0) and 64-day (3.8)) significant difference in the population of *attSal* were determine along the cropping cycle (log 2.46 ± 0.03, 1.44 ± 0.06, and 0.68 ± 0.05 cfu/g of soil, Tukey's HSD ($p<0.05$), respectively).

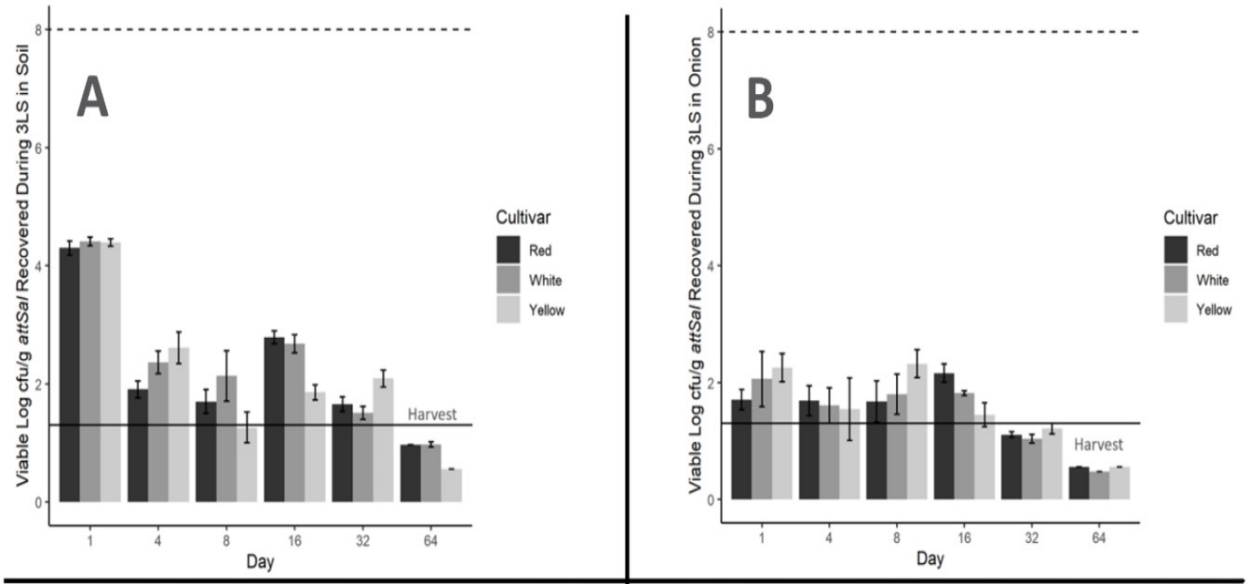

Figure 1. Distribution of *attSal* along the DB onion cropping cycle (64 days at ARDECS). Values represent the average population of *attSal* per timepoint and cultivar. N = 12 per timepoint and cultivar. Panels A and B describe the population of *attSal* from the 3LS until harvest for soil and onions respectively. No statistical difference using Tukey's HSD ($p<0.05$) was observed between cultivar along the 64 days of recovery. The dashed line represents the initial inoculation concentration in sachets of log 8 cfu/g of sand and the solid line represents the limit of detection of *attSal* by direct plating methods. Day 1 soil and onion recovery represents the population of *attSal* across the inoculated area. Values at Day 64 are indicative of MPN cfu/g of DB onion recovered at the time of harvest. Error bars are representative of standard error of the mean.

Survival of *attSal* During Topping and Lifting (Day 42 Through Day 64)

Both topping and lifting occurred the day prior to inoculation. The idea was to apply the inoculum as close to harvest and based on typical agronomic practices for both topping and lifting. At the topping and lifting stage, the same 2 log reduction observed at the 3LS after the first 4 days post inoculation was not observed (Figure 1 Panel A, Figure 2 Panel C and E). During both agronomic practices, survival, and persistence of *attSal* remained stable, with only 1 log reduction between inoculation and harvest activities (Figure 2 Panel C and E). During this time there were also significant differences in UV index, soil moisture content and overall temperature (Table A1). At harvest the population of *attSal* in soil for both topping and lifting was (3.31 ± 0.05 and 3.72 ± 0.04 respectively) with no significant difference between agronomic practices and DB onion cultivar (Figure 2 Panel C and E). When comparing the survival and persistence of *attSal* in soil

between the 3LS, topping and lifting after 16 days of inoculation, all 3 treatments had identical populations (3.06 ± 0.07, 3.31 ± 0.05, 3.72 ± 0.04 respectively).

For DB onions, the population of *attSal* after inoculation per cultivar (red, white, and yellow) for topping and lifting was log 2.10 ± 0.06, 2.26 ± 0.0, 2.20 ± 0.09 and 2.84 ± 0.08, 2.8 ± 0.09, 2.54 ± 0.18 cfu/g DB onion. The survival and persistence of *attSal* remained consistent and identical between DB onion cultivars and timepoints for topping (Figure 2 Panel D and F). At harvest the population of *attSal* in DB onions (red, white, and yellow) for both topping and lifting was log 1.72 ± 0.03, 2.31 ± 0.03, 1.83 ± 0.04, and 1.63 ± 0.14, 1.90 ± 0.07, 1.82 ± 0.04 cfu/g DB onion, respectively with no significant difference between agronomic practices and DB onion cultivar (Figure 2 Panel D and F). When comparing the population of *attSal* at harvest between the 3LS, topping and lifting, there was an average difference of log 1.875 cfu/g of DB onion between the 3LS and all other agronomic practices (Figure 1, Panel B; Figure 2 Panel D and F). As with the 3LS, the interaction of cultivar and soil moisture content ($p>0.05$) was not significant in the recovery of *attSal* and difference in the UV index (mean UV index (4.3, and 3.8 respectively) were used to determine if difference in the population of *attSal* at harvest were attributed to this factor. However, mean populations of *attSal* in soil (log 3.06 ± 0.07, 3.31 ± 0.05, 3.72 ± 0.04 cfu/g of soil respectively) comparison with agronomic treatment (3LS, topping, and lifting) was significant using Tukey's HSD ($p<0.05$). Mean populations of *attSal* (log 3.06 ± 0.07, 3.31 ± 0.05, 3.72 ± 0.04 cfu/ g of soil, respectively) and mean UV index was significant between the early vegetative inoculation and the two latter inoculation events using Tukey's HSD ($p<0.05$).

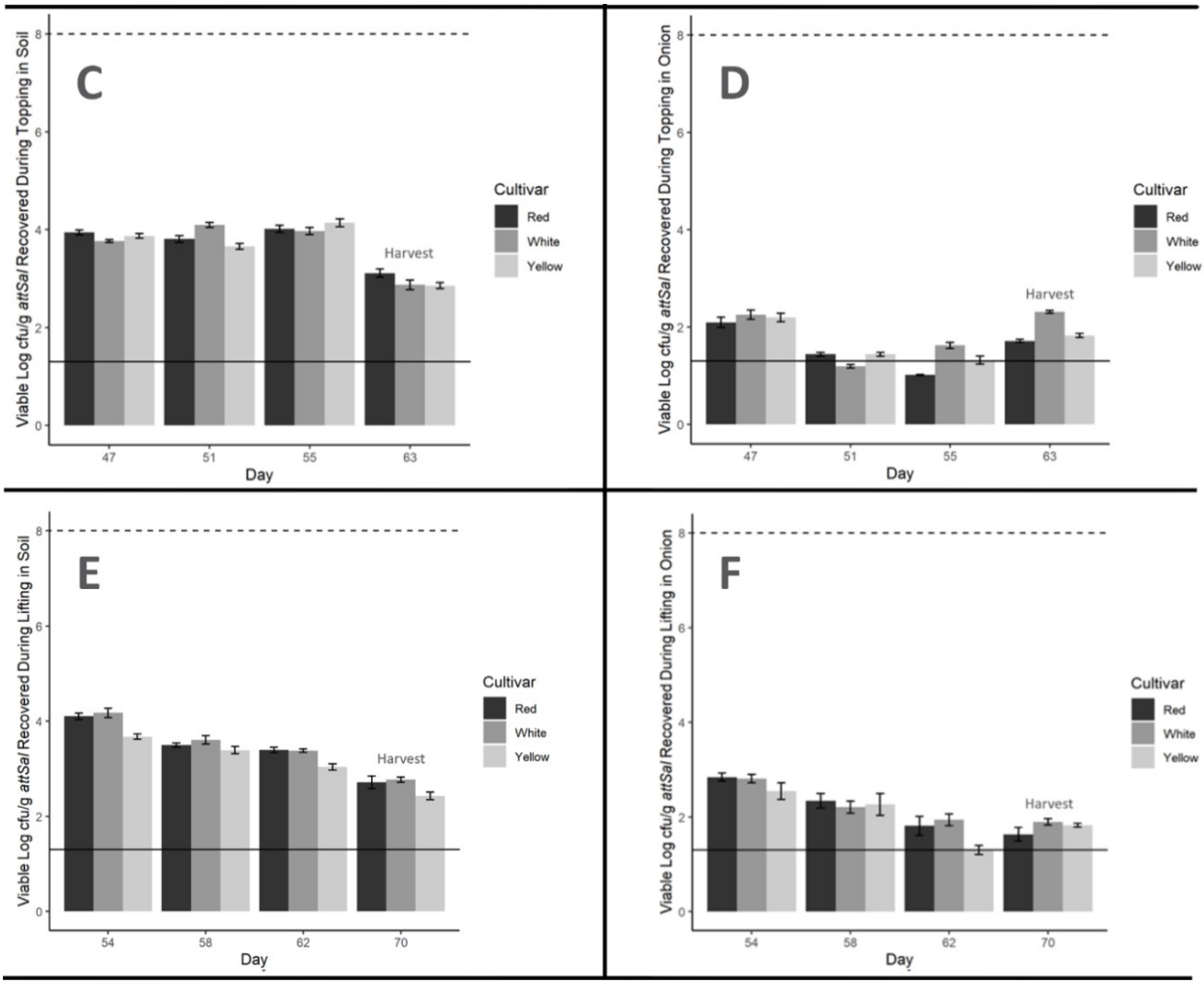

Figure 2. Distribution of *attSal* during topping and lifting activities (16-day period for both treatments). Values represent the average population of *attSal* per timepoint and cultivar. N = 12 per timepoint and cultivar. No statistical difference using Tukey's HSD ($p<0.05$) was observed between cultivar along the 16 days of recovery. Panels C and D describe the population of *attSal* from topping until harvest for soil and onions respectively. Panels E and F describe the population of *attSal* from lifting until harvest for soil and onions respectively. The dashed line represents the initial inoculation concentration in sachets of log 8 cfu/g of sand and the solid line represents the limit of detection of *attSal* by direct plating methods. Day 1 soil and onion recovery represents the population of *attSal* across the inoculated area. For topping and lifting there was a seven-day difference in the timing of the inoculation event.

Soil Die-off Kinetics of 3LS, Topping and Lifting

Die-off kinetics of *attSal* in soil samples across all treatments was best described by the Double Weibull model (DWB) previously described by Coroller et al., 2006. A comparison of the die-off kinetics was done between treatments (DB onion cultivar) during the first 16 days of inoculation.

There was a 42- and 48-day difference in the time of inoculation events between the 3LS and topping and lifting respectively. This coincided with a significant difference in the UV index and percent of soil moisture content during each inoculation event as described in Table 3. Despite these significant differences in atmospheric parameters, the shape parameter (p) for each treatment followed a convex approach consistent with biphasic bacterial survival (Table 3).

Table 3. Double Weibull survival model for *attSal* inoculated at Log 4.5 CFU ml^{-1} from 125 g aggregate soil samples collected from ARDECS over a period of 16 days along the DB onion cropping cycle.

Cultivar	Treatment	alpha	Delta 1	p	Delta 2	RMSE	UV index	Soil Moisture wfv (m^3m^{-3})
Red	3LS	1.6	3.0	6.0	212	0.64	7.8 ± 0.2 a	36.2 ± 1.7 a
White	3LS	1.3	3.1	6.0	121	0.61	7.8 ± 0.2 a	36.2 ± 1.7 a
Yellow	3LS	2.6	3.7	6.0	217	0.44	7.8 ± 0.2 a	36.2 ± 1.7 a
Red	Topping	0.8	0.8	6.0	199	0.15	4.4 ± 0.2 b	25.0 ± 1.6 b
White	Topping	1.2	15.8	6.0	166	0.22	4.4 ± 0.2 b	25.0 ± 1.6 b
Yellow	Topping	0.9	15.9	6.0	166	0.24	4.4 ± 0.2 b	25.0 ± 1.6 b
Red	Lifting	1.5	4.8	0.5	2.7	0.17	3.8 ± 0.3 c	19.5 ± 0.8 c
White	Lifting	1.3	2.7	0.4	2.7	0.14	3.8 ± 0.3 c	19.5 ± 0.8 c
Yellow	Lifting	0.7	9.2	1.0	21	0.07	3.8 ± 0.3 c	19.5 ± 0.8 c

Models were constructed with the first four sampling events Day 1, Day 4, Day 8, and Day 16 of each treatment (3LS, Topping and Lifting) N=12 per timepoint and treatment. DWB inactivation of *attSal* was identified to fit the equation: $N=N_0/(1+10\text{^}alfa) * (10\text{^}(-((t-1)/delta1)\ \text{^}p + alfa) + 10\text{^}(-((-1)/delta2)**p))$, and $\alpha = \log_{10}(f/(1-f))$. N represents the number of *attSal* survivors, N_0 and t represent the inoculum size and time, respectively. p is the shape parameter, when $p > 1$ a convex curve is observed; when $p < 1$ a concave curve is observed, when $p = 1$ a linear curve is observed. The scale parameter, delta, presents the time needed for the first decimal reduction. The f parameter fluctuates between 0 to 1 and indicates the fraction of subpopulation 1 in the initial population. α is obtained by log transformation and may exist from negative to positive infinity. The standard error of each parameter is shown in parenthesis and the root mean square error (RMSE) is reported. The average UV index, and soil moisture is representative of the 16-day period evaluated for each treatment. Different letters between treatments indicate significant differences in UV-index and moisture content Tukey Honest Significant Difference (HSD), $\alpha = 0.05$.

The RMSE parameter on all treatments was small and below 0.64 and these higher RMSE values correspond to those atmospheric events in which there is high UV-index, desiccation, and greater fluctuations in soil moisture content. Once UV-index and soil moisture content were below 5 and 25% respectively, there was little variation in the recovery of *attSal* from all soil samples. Delta values were similar between cultivar at each treatment, however, delta 1 and delta 2 were significantly different between treatments, indicating that the two sub-populations behave differently, but similar between DB onion cultivar and weather parameters (Table 3). Early season high UV-index and soil moisture content produce two very distinct populations of *attSal* irrespective of cultivar (3LS), where the second sub-population experienced a gradual and slow decline due to a significantly high delta 2 value. Late season low UV-index and soil moisture content didn't generate two distinct populations within treatments with delta 1 and 2 values almost identical within them. Delta 2 values for topping and lifting were significantly different and smaller than those from the 3LS, indicating different faster die-off rates between those stages. Delta 2 values were also different between topping and lifting, with higher delta 2 values for topping, indicating a faster die-off during this stage than at lifting. DB onion cultivar had no effect on delta values within treatments except for the yellow cultivar at lifting which presented significantly different delta 1 and 2 parameters during late season low UV-index and soil moisture content. All other treatments did not follow this trend at lifting and topping.

Onion Die-off Kinetics of 3LS, Topping and Lifting

Die-off or inactivation of *attSal* in DB onion samples across treatments was best described by linear regression modeling. This comparison was completed between DB onion cultivar during the first 16 days of inoculation for the 3LS, topping and lifting. Although there were significant differences in the UV index and percent of soil moisture content (Table 3) between cultivar seven out of nine die off-rates were negative, with only two showing a slightly positive slope (Table 4),

suggesting a continuous but uneven decline in the population of *attSal*. Linear mean DR for all treatments and onion cultivars were identical during the sampling period, with an average mean DR for all treatments of 0.182 ± 0.150 log cfu/day. No other kinetic model was able to consistently describe inactivation of *attSal* across all treatment cultivar combinations.

Table 4. Die-off of *attSal* inoculated at Log 4.5 CFU ml^{-1} from aggregate onion samples collected from ARDECS over a period of 16 days along the DB onion cropping cycle.

Treatment	Cultivar	Linear DR, R^2 (Log cfu/day)	Mean DR per Day (Log cfu/day)
3LS	Red	y = 0.0103x + 1.7903 R^2 = 0.0201	0.154 ± 0.137 a
	White	y = -0.0075x + 1.9652 R^2 = 0.0445	0.156 ± 0.129 a
	Yellow	y = -0.0339x + 2.0407 R^2 = 0.1942	0.155 ± 0.188 a
Topping	Red	y = -0.0182x + 2.5475 R^2 = 0.0597	0.154 ± 0.162 a
	White	y = 0.0219x + 0.6607 R^2 = 0.0657	0.166 ± 0.151 a
	Yellow	y = -0.0138x + 2.439 R^2 = 0.0396	0.151 ± 0.132 a
Lifting	Red	y = -0.0741x + 6.6787 R^2 = 0.4067	0.182 ± 0.149 a
	White	y = -0.052x + 5.3843 R^2 = 0.3782	0.185 ± 0.138 a
	Yellow	y = -0.0493x + 4.9926 R^2 = 0.1753	0.175 ± 0.159 a

Values represent average population of *attSal* ± standard error recovered from DB onions per treatment, cultivar and timepoint. DR= die-off rates expressed in a linear format, with N=12 per

timepoint and treatment. Limit of detection was < 3 MPN/g of DB onions. Mean comparison was made by day of recovery per cultivar and treatment using an analysis of variance (ANOVA) performed using Tukey HSD. Different letters represent significant difference ($p < 0.05$) between treatments. The average UV index, and soil moisture per treatment was 3LS: 7.8 ± 0.2 and 36.2 ± 1.7, Topping: 4.4 ± 0.2 and 25.0 ± 1.6, Lifting: 3.8 ± 0.3 and 19.5 ± 0.8, respectively and are representative of the 16-day period during which sampling events took place for each treatment.

Onion Die-off Kinetics Described by Moisture Content Grouping

Inactivation of *attSal* in DB onion samples was considered by grouping field replicates by soil moisture content since there was no effect associated with cultivar. This grouping was determined through soil moisture probes strategically placed across the field to capture variability in the percent of soil moisture content (Figure A2 and Table A1). Percent soil moisture content for node 3 on average across treatments was 19.5% (Low), for node 2 it was 25% (Medium), and for node 1 it was 36% (High) (Table A1). Die-off comparisons were done between soil moisture content as the driving factor for survival and persistence in DB onion samples and was completed using the first 16 days of inoculation for each node number (disregarding cultivar). Although there were significant differences in the percent of soil moisture content and UV index (Table 3 and 5), only significant differences in the inactivation of *attSal* were determined between lifting and the other two treatments (Table 5). Greater inactivation of *attSal* was determined for lifting that the 3LS and topping stages.

Table 5. Population of *attSal* based on soil moisture content grouping (Node 1, 2, and 3).

Treatment	% Moisture	Linear DR, R^2 (Log cfu/day)	Mean DR per Day (Log cfu/day)
3LS	19.5	$y = 0.0088x + 1.9308$ $R^2 = 0.0226$	0.111 ± 0.054 a
	25	$y = -0.0212x + 1.9519$ $R^2 = 0.0985$	0.112 ± 0.056 a
	36	$y = -0.0187x + 1.9135$ $R^2 = 0.0941$	0.125 ± 0.048 a

Topping	19.5	y = -0.0074x + 2.0461 R^2 = 0.0099	0.109 ± 0.06 a
	25	y = 0.0009x + 1.6756 R^2 = 0.0001	0.108 ± 0.071 a
	36	y = -0.0035x + 1.9255 R^2 = 0.0019	0.103 ± 0.064 a
Lifting	19.5	y = -0.0783x + 6.8375 R^2 = 0.4455	0.133 ± 0.087 b
	25	y = -0.0465x + 4.9969 R^2 = 0.1933	0.135 ± 0.091 b
	36	y = -0.0506x + 5.2211 R^2 = 0.2507	0.130 ± 0.010 b

Values represent average *attSal* population ± standard error recovered from DB onions by node number. DR= die-off rates expressed in a linear format, with N=12 per timepoint and treatment. Limit of detection was < 3 MPN/g of DB onions. Mean comparison was made by day of recovery by node. Different letters indicate significant differences between treatments, Tukey HSD test α=0.05. The average UV index, and soil moisture per treatment was 3LS: 7.8 ± 0.2 and 36.2% ± 1.7%, Topping: 4.4 ± 0.2 and 25.0% ± 1.6%, Lifting: 3.8 ± 0.3 and 19.5% ± 0.8%, respectively and are representative of the 16-day period during which sampling events took place for each treatment.

Recovery of *attSal* at Harvest, During Transport to CA and After Storage

The population of *attSal* at harvested for DB onions grown at ARDECS was different (P<0.05) between the 3LS and topping and lifting (Table 6) by one order of magnitude. Based on this difference, the DB onion harvest at the 3LS was considered a low-risk DB onion, while the DB onion harvested during topping and lifting were considered medium risk based on their population at harvest (Table 6). There was a 42- and 48-day difference in time of inoculation events between the 3LS and topping and lifting respectively. This coincided with significant differences in the UV index and percent of soil moisture content (Table 3) and the temporal aspects of open field production. At the end of transport and initiation of packing house activities, the population of

attSal from all ARDECS grown DB onions was < 3 MPN/g +/- 0 – 9.5 CI of DB onion and identical between all treatments and cultivars. The DR for these onions was different based on the initial population of *attSal* at harvest (Tukey HSD test α=0.05). Significant differences in DR were observed between low and medium risk DB onions with greater rates measured from medium risk DB onions (Table 6).

With respect to commercially grown DB onions, none of the tested samples was positive for the presence of naturally occurring *Sal* (data not shown). All commercially grown DB onions were inoculated with chalk containing a population of *attSal* of log 5.5 cfu/g chalk. The population of *attSal* remained stable for the first 7 days post inoculation and by the time packinghouse activities initiated it was < 3 MPN/g +/- 0 – 9.5 CI of DB onion and identical to all DB onions grown at ARDECS (Table 6). As expected, the mean DR for these onions was different from samples coming from ARDECS, however, because of the differences in inoculation approach differences in mean DR were not tested using the Tukey HSD test α=0.05. At the initiation of all packinghouse activities all DB onions had the same population of *attSal* < 3 MPN/g +/- 0 – 9.5 CI of DB onion irrespective of treatment and despite being ranked based on risk level at harvest/initiation of transport activities (Table 6).

A small portion of DB onions grown at ARDECS and from commercial partners previously inoculated with chalk impregnated with att*Sal* was stored for a period of 90 days at 18.3 °C ± 10° and 60% RH ± 10. After 3 months of storage, a total of 32 burlap sacks (50 x 90 cm) were removed from storage and tested for the presence of viable att*Sal.* All 32 samples from low, medium, and high-risk DB onions were negative for att*Sal* after enrichment and MPN enumeration (<3 MPN/g

+/- 0 – 9.5 CI) (data not shown).

Table 6. The population of *attSal* at harvest, at the end of transport (interstate transport between CO and CA) and at the initiation of packing activities in Fresno CA.

Treatment	Cultivar	Source	Risk	Population of *attSal* (log cfu/g DB onion)			Mean DR (log cfu/day)
				At Harvest/ Initiation of Transport	End of Transport (10 days)	At Packing (15 days)	
3LS	Yellow	ARDECS	L	0.5 ± 0.1 b	<3MPN/g	<3MPN/g	0.004 b
	White			0.5 ± 0.1 b	<3MPN/g	<3MPN/g	0.002 b
Topping	Yellow		M	1.8 ± 0.04 a	<3MPN/g	<3MPN/g	0.090 a
	White			2.3 ± 0.03 a	<3MPN/g	<3MPN/g	0.122 a
Lifting	Yellow		M	1.8 ± 0.04 a	<3MPN/g	<3MPN/g	0.090 a
	White			1.9 ± 0.1a	<3MPN/g	<3MPN/g	0.095 a
Grower	Red	Commercial	H	5.5 ± 0.2	5.4 ± 0.4	<3MPN/g	0.335
	Yellow			5.5 ± 0.2	5.4 ± 0.4	<3MPN/g	0.335

Values represent average *attSal* population ± standard error. N =12 per scale and treatment. The length between harvest and the end of transport was 10 days and packinghouse activities initiated after 15 days from harvest/initiation of transport. L= low risk, M= medium risk, H= high risk. Die off rates (DR) are expressed as the mean die-off that took place between harvest and the initiation of packing and sorting activities. Mean comparisons were only made between ARDECS DB onions because these group received the same inoculation method.

Packing House Evaluations

Recovery of *attSal* From Food Contact Surfaces and 14 locations Within the Packing line

Along the 6 days of cleaning, sanitizing, sorting and packing DB onions from low, medium and high risk contamination events, no food contact surface or DB onion sample tested positive by enrichment or MPN method for the presence of *attSal.* The population during those days was considered to be <3 MPN/g ± 0 – 9.5 CI of DB onion per location swabbed or DB onion sample (n = 224 and 144), respectively (data not shown). High, medium and low risk DB onions were significantly different in their size, and the amount of dry dehiscent onion skin and organic matter; however, this difference didn't translate in significant cross contamination with att*Sal* between the control and inoculated DB onions at 1, 3 and 4 h of processing. Despite significant accumulation of debris during packing and sampling of DB onions, measured as ATP residues in all treatments

(Figure 3 and 4), we were not able to recover the inoculated strain from food contact surfaces. No difference in the recovery of *attSal* was determined between the 3LS, topping and lifting activities or between ARDECS and commercial grown DB onions chalk inoculated with att*Sal*.

Accumulation of Debris and ATP Measurements

Across packinghouse experiments, the accumulation of onion chaff (skins, soil debris, dehiscent material) was visually observed in food contact surfaces on an hourly basis. Food contact surfaces varied within the packing line and the 14 separate locations were reflective of commonly used material. Irrespective of high and low/medium risk activities, a consistent accumulation of debris coincided with higher recovery of EB from 14 separate food contact surfaces despite swabbing the same location per timepoint and material type (Figure 5-9). There were no sanitation events between processing days, meaning the second dry sanitation event (DS2) occurred at the end of the high risk activities followed by an overnight period prior to the initiation of the low risk sorting activities. ATP residues measured in RLU's were recorded across all packinghouse activities. Our first initial goal was to establish whether a dry sanitation event would result in significant debris removal from different areas within the packing line. The dry sanitation procedure was effective in removing DB onion residues from all food contact surfaces except plastic and camel hair bristles. The latter two surfaces were impossible to clean despite significant efforts to remove debris (Figures 3 and 4).

For the high risk events, the accumulation of DB onion residues increased over time when no dry sanitation activities were performed irrespective of the characteristics of the food contact surface (Figure 3). Between day 1 and 2 of processing, debris accumulation at least doubled in most swabbed locations (Figure 3). All food contact surfaces after one day of processing with no dry sanitation activities accumulated significant debris (Figure 3). At the culmination of day 2, a dry sanitation event was able to remove the majority of residues accumulated from days 1 and 2 of

processing bringing all swabbed food contact surfaces to RLU values at or below 446. Although some areas didn't achieve values at or below 30 RLUs, all swabbed locations had significant reductions in the accumulation of DB onion debris (Figure 3) coming from high risk events. These DB onions also had significantly higher amounts of dry dehiscent onion skin and organic matter when compared to ARDECS samples (low/medium risk).

For low/medium risk events, an identical pattern was observed between surfaces, however, the amount of residues was an order of magnitude lower than high risk events as indicated by ATP measurements (Figure 4). Concomitant with this observation, accumulation of debris did not increase between day 3 and 4 of processing and in most instances remained at the same or slightly lower (Figure 4) ATP values. Significant differences in mean ATP measurements with respect to day and location were observed between dry sanitation events and processing days irrespective of risk level (Figure 3 and 4). ATP residue accumulation comparison between processing day ($p < 0.05$), location ($p < 0.05$) and dry sanitation events ($p < 0.05$) were significant for day 1 and 2 of processing. Both day and location affected the final debris accumulation in processing of high risk materials (Figure 3). Low/medium risk materials were analyzed using the same modeling and statistical comparison. Both day ($p < 0.05$) and location ($p < 0.05$) affected the final debris accumulation.

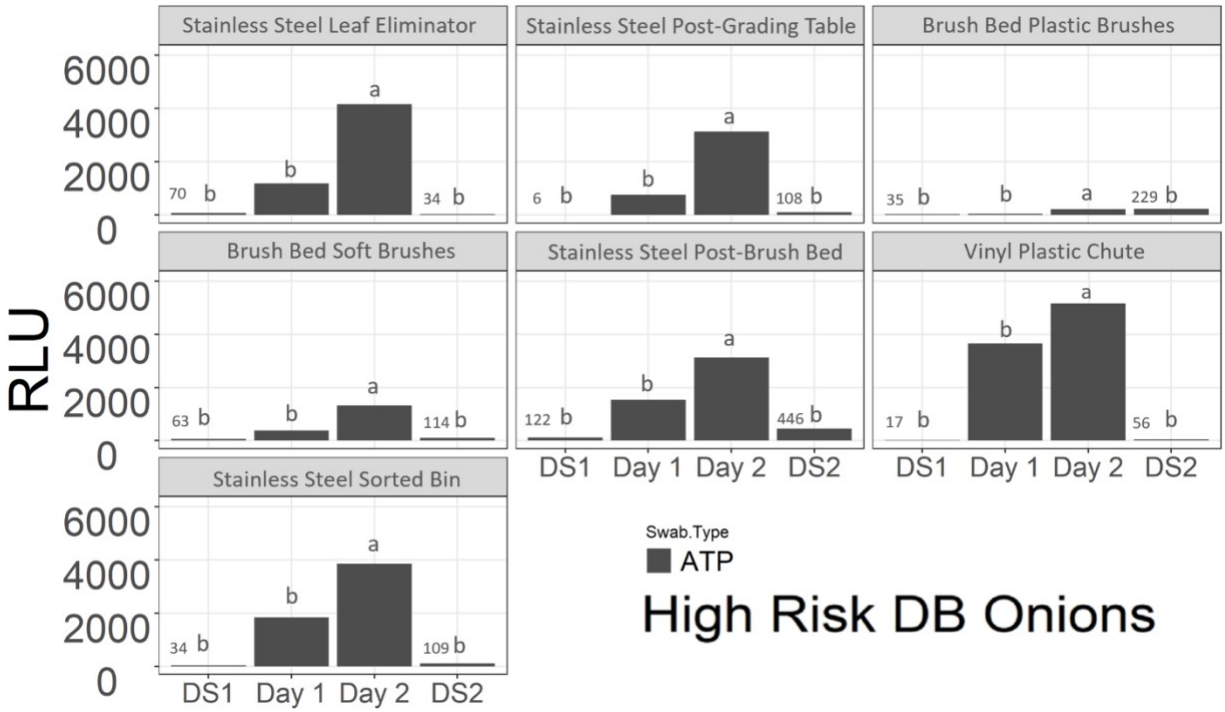

Figure 3. Average ATP measurements (RLU values) for dry sanitation events (DS1 and DS2) and after processing DB onions from a high-risk contamination event (Day 1 = Chalk Inoculated commercial red DB onions, Day 2 = Chalk inoculated commercial yellow DB onions). N = 42 per Day. N = 24 for dry sanitation measurements. Each day consisted of 4h of sorting and packing DB onions per day of processing. A total of 14 swabs per hour for 3h were collected from all food contact surfaces. A total of 8 hours of storing and packing DB onions coming from the high-risk contamination event were processed in the packing line. Reported values greater than 30 RLU for each location indicate significant accumulation of residues on food contact surface and are not considered cleaned. An analysis of variance (ANOVA) was performed using Tukey HSD test for means comparison. Different letters represent significant difference ($p < 0.05$) by day and location and are representative of the main effects of the model.

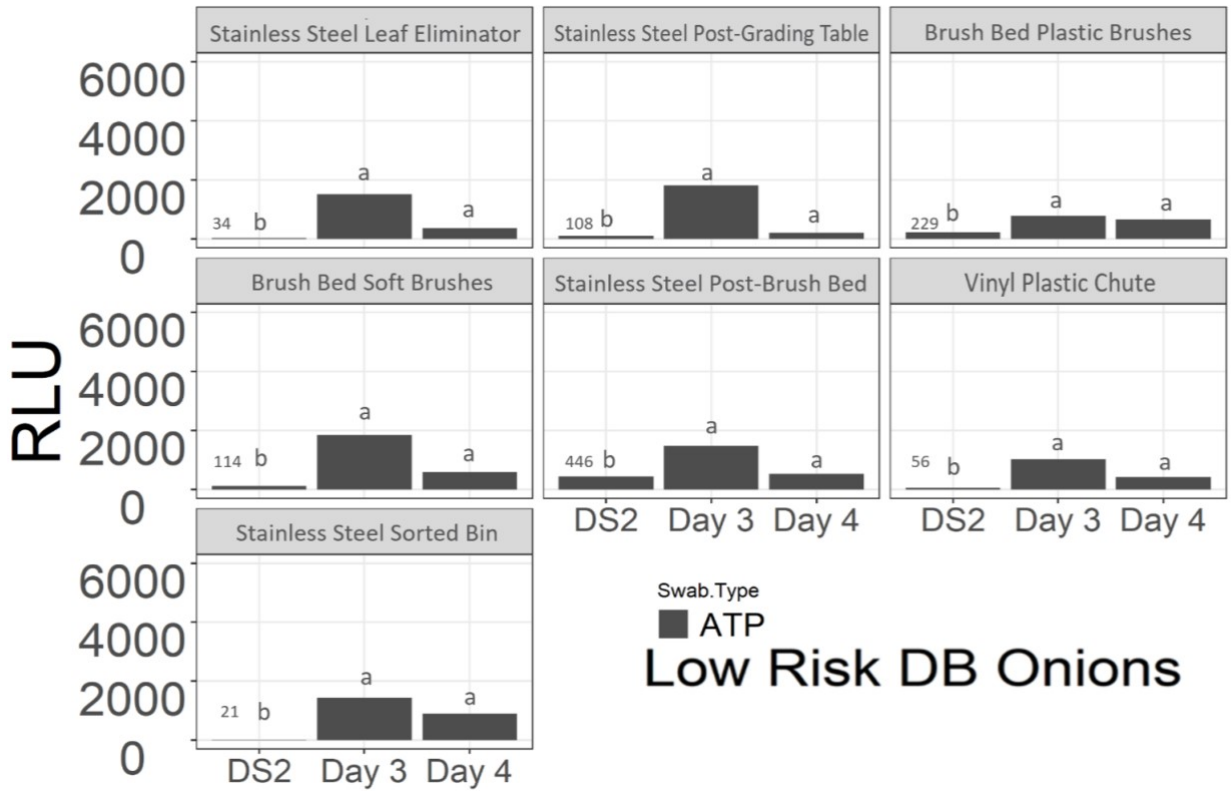

Figure 4. Average ATP measurements (RLU values) for the second dry sanitation event (DS2) and after processing DB onions from a low-risk contamination event (Day 3 = ARDECS yellow DB onions, Day 4 = ARDECS white DB onions). N = 56 per Day. N = 14 for dry sanitation measurements. Each day consisted of 4h of sorting and packing DB onions. A total of 14 swabs per hour for 4h were collected from all food contact surfaces. A total of 8 hours of storing and packing DB onions coming from the low-risk contamination events were processed in the packing line. Reported values greater than 30 RLU for each location indicate significant accumulation of residues on food contact surface and are not considered cleaned. After day 4 a commercial sanitation company cleaned and sanitized the packing line leaving all food contact surfaces free from residues except for locations 4 and 5 (brush beds) (data not shown). An analysis of variance (ANOVA) was performed using Tukey HSD test for means comparison. Different letters represent significant difference ($p < 0.05$) by day and location and are representative of the main effects of the model.

Determination of EB and EC From High-and Low Risk Processing Events

The population of EB and EC was determined from all 14 food contact surfaces marked across the packing line and collected from high and low/medium risk activities. The goal was to determine whether processing day and dry sanitation event impacted the recovery of both of EB and EC. All mean comparisons were performed within populations of the same organism between dry sanitation and processing days. Day ($p < 0.05$), location ($p < 0.05$), and the interaction between day

location ($p < 0.05$) significantly affected the final recovery of EB and EC accumulating during processing of high and low/medium risk materials respectively. Accumulation of EB in high risk activities was significantly different between Day 1 (log 3.58 ± 0.13 cfu/mL) and Day 2 (log cfu/mL 4.43 ± 0.08) and were significantly different from both dry sanitation events. DS1 (log 1.3 ± 0.5 cfu/mL) and DS2 (2.03 ± 0.06) were not significantly different in high risk activities, however the second dry sanitation event was lower than the first day by an average of log 0.6 cfu of EB (Figure 5). Accumulation of EB in low/medium risk activities was significantly different between Day 3 (log 3.84 ± 0.11 cfu/mL) and Day 4 (log cfu/mL 4.16 ± 0.10) and also were signifiantly different from DS2 (Figure 6). No major and significant differences were determined in the population of EB between equipment materials (Figure 6)

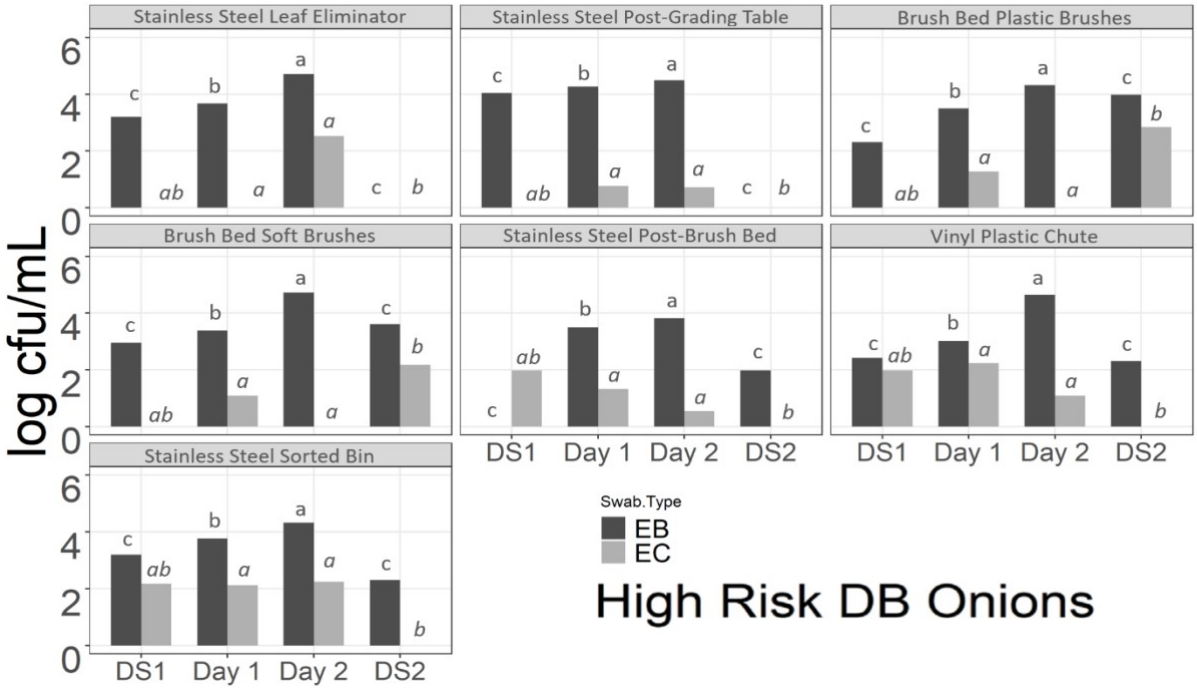

Figure 5. Population of *Enterobacteriaceae* (EB) and *Escherichia coli* (EC) recovered from 14 different food contact surfaces after dry sanitation events (DS1 and DS2) and processing DB onions from a high-risk contamination event (Day 1 = Chalk inoculated commercial red DB Onions, Day 2 = Chalk inoculated commercial yellow DB Onions). N = 42 per Day. N = 24 for dry sanitation measurements. Each day consisted of 4h of sorting and packing per day. A total of 14 swabs per hour for 3h were collected from all food contact surfaces. A total of 8 hours of storing and packing DB onions coming from the high-risk contamination event were processed in the

packing line. An analysis of variance (ANOVA) was performed using Tukey HSD test for means comparison. Different letters represent significant differences ($p < 0.05$) by day and individual location and show the main effects of each separate model for EB and EC respectively.

Accumulation of EC in high risk activities was not significantly different between Day 1 (log 1.47 ± 0.15 cfu/mL) and Day 2 (log cfu/mL 1.44 ± 0.23); however they were significantly different from both dry sanitation events. DS1 (log 1.3 ± 0.23 cfu/mL) and DS2 (0.76 ± 0.30) were significantly different in high risk activities (Figure 5). For low/medium risk activities the accumulation of EC was significantly different between Day 3 (log 1.22 ± 0.19 cfu/mL) and Day 4 (log cfu/mL 3.33 ± 0.13); however, Day 3 and DS2 were not significantly different (Figure 6). No major and significant differences were determined in the population of EC between equipment materials (Figure 6).

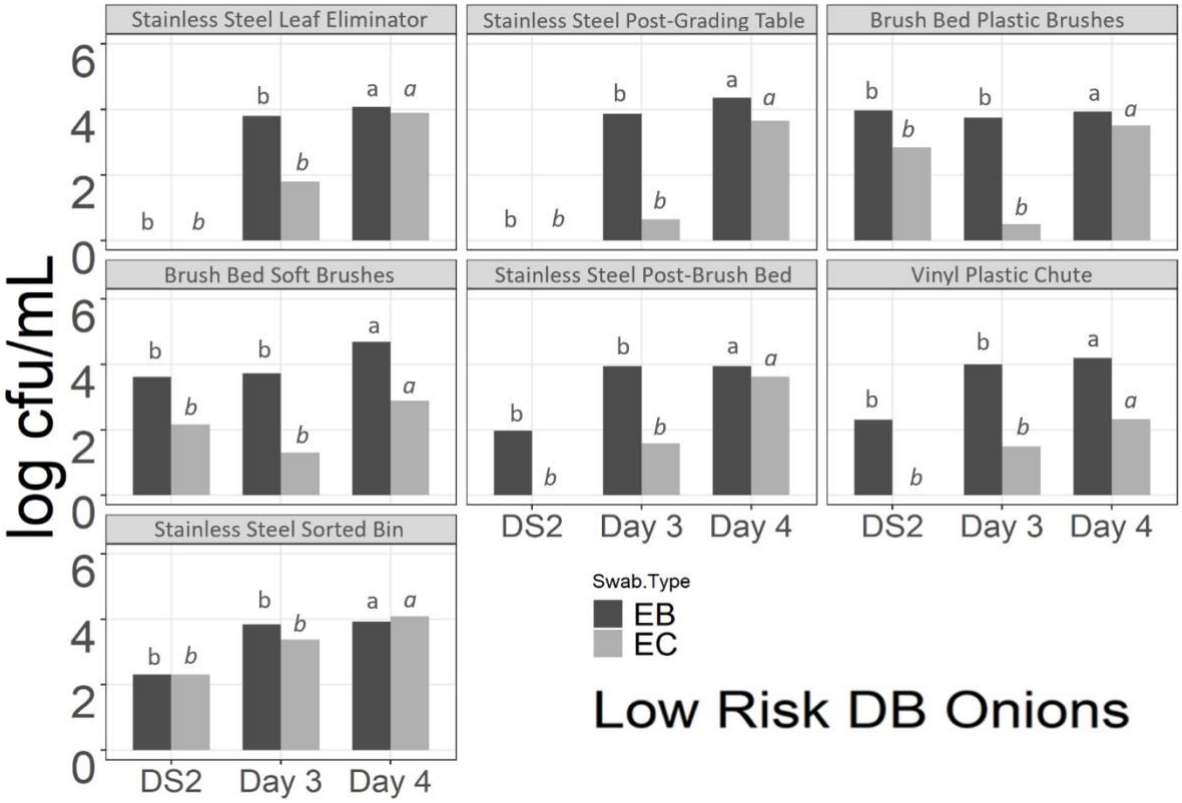

Figure 6. Population of *Enterobacteriaceae* (EB) and *Escherichia coli* (EC) recovered from 14 different food contact surfaces after a dry sanitation events (DS2) and after processing DB onions from a low-risk contamination event (Day 3 = ARDECS yellow DB onions, Day 4 = ARDECS white DB onions). N = 56 per Day. N = 14 for dry sanitation measurements. Each day consisted of 4h of sorting and packing per day. A total of 14 swabs per hour for 4h were collected from all

food contact surfaces. A total of 8 hours of storing and packing DB onions coming from the low risk contamination event were processed in the packing line. After day 4 a commercial sanitation company cleaned and sanitized the packing line leaving all food contact surfaces free from residues except for locations 4 and 5 (brush beds). An analysis of variance (ANOVA) was performed using Tukey HSD test for means comparison. Different letters represent significant difference ($p < 0.05$) by day and individual location and show the main effects of each separate model for EB and EC respectively.

Determination of EB by Location and Material from Food Contact Surfaces.

Recovery of EB from 14 different locations within the packing line was also evaluated based on the material of each food contact surface. The packing line was constructed from an array of metals and plastics and our recovery efforts for *attSal* and EB focus on 318 stainless steel, vinyl plastic, rubber-belting and brush bed bristles. The bristles were made of plastic and camel hair. For stainless steel, accumulation of EB across all packing line activities was identical between high and low/medium risk activities (Figure 7). Each sanitation event was able to reduce the population of EB from stainless steel to populations in the range of 0 to 3 log cfu/location. Accumulation of EB was not significantly different between hours of operation, but they were different between days of sorting and packing (Figures 5 and 7). The level of risk had no significant effect ($p > 0.05$) on the recovery of EB; however, processing day did affect the final population ($p < 0.05$). The sanitization events (DS1/DS2) had the lowest average EB log 2.8 ± 0.49 and 1.6 ± 0.42 cfu/location when compared to Day 2 and 4 which presented the highest mean EB counts log 4.26 ± 0.13 and 4.15 ± 0.11 cfu/location. DS1 and DS2 were significantly different ($p <0.05$) and the mean EB difference was approximately log 0.8 cfu/location.

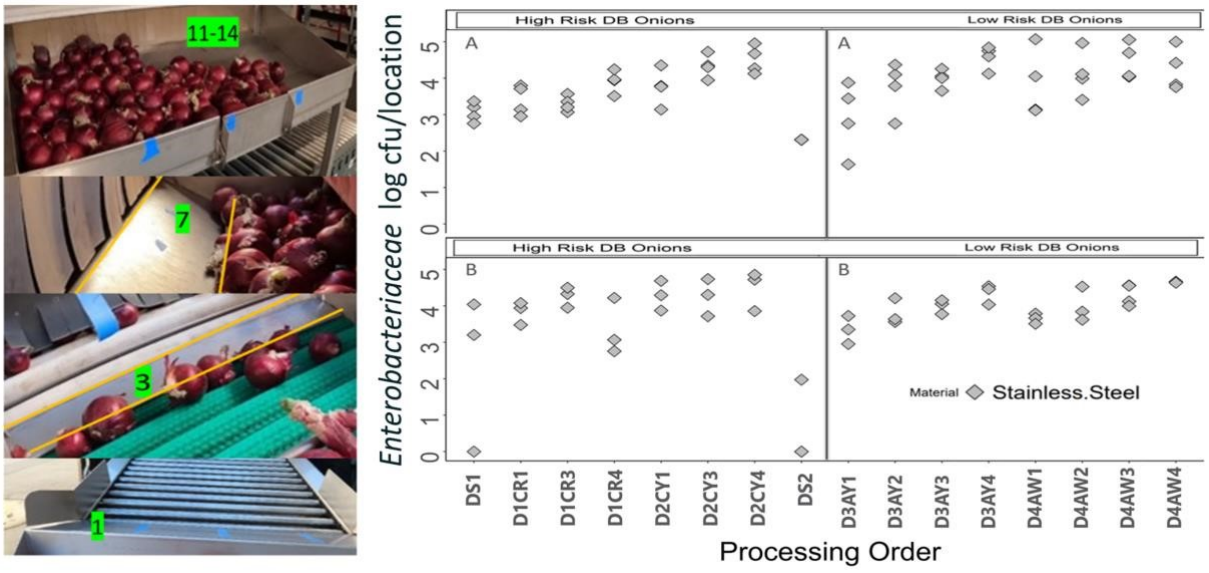

Figure 7. The hourly population of *Enterobacteriaceae* (EB) log cfu/location recovered from stainless steel food contact surfaces inside the packing line. 'A' portions of this figure are representative of FCS 11-14, and 'B' portions of this figure are representative of FCS 1, 3, and 7. Processing order of DB onion materials began with Dry Sanitation 1 (DS1), Day 1 commercial red hour 1 (D1CR1), Day 1 commercial red hour 3 (D1CR3), Day 1 commercial red 4 (D1CR4), Day 2 commercial yellow hour 1 (D2CY1), Day 2 commercial yellow hour 3 (D2CY3), Day 2 commercial yellow hour 4 (D2CY4), Dry Sanitation 2 (DS2), Day 3 ARDECS yellow hour 1 (D3AY1), Day 3 ARDECS yellow hour 2 (D3AY2), Day 3 ARDECS yellow hour 3 (D3AY3), Day 3 ARDECS yellow hour 4 (D3AY4), Day 3 ARDECS white hour 1 (D4AW1), Day 3 ARDECS white hour 2 (D4AW2), Day 3 ARDECS white hour 3 (D4AW3), Day 3 ARDECS white hour 4 (D4AW4). After day 4 a commercial sanitation company cleaned and sanitized the packing line leaving all food contact surfaces free from residues. There was an overnight period between DS2 and low risk processing.

For vinyl plastic, the population of EB recovered from the packing line was lower from the high than the low/medium risk events (Figure 8). Accumulation of EB in vinyl occurred across and between processing days. Dry sanitation events were able to significantly reduce the population of EB in vinyl plastic. The level of risk had no significant effect ($p > 0.05$) on the final population of EB; however, processing day did affect the final population ($p < 0.05$) of EB. Both sanitization events (DS1/DS2) had the lowest mean EB populations (log 2.41 ± 1.21 and 1.91 ± 0.72 cfu/location) whereas Day 2 and 4 had the highest mean EB populations (log 3.84 ± 0.20 and 4.33

± 0.18 cfu/location). DS1 and DS2 were significantly different ($p < 0.05$) and the mean EB difference was approximately log 0.5 cfu/location.

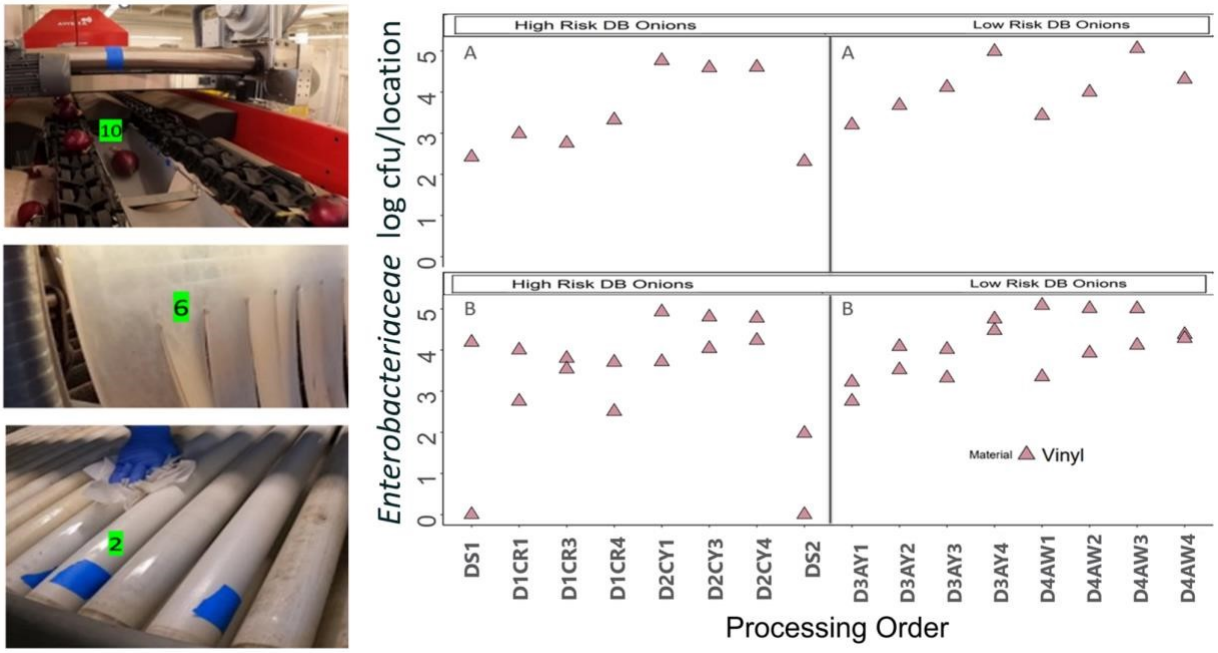

Figure 8. The hourly population of *Enterobacteriaceae* (EB) log cfu/location recovered from vinyl plastic food contact surfaces inside the packing line. 'A' portions of this figure are representative of FCS 10, and 'B' portions of this figure are representative of FCS 2 and 6. Processing order of DB onion materials began with Dry Sanitation 1 (DS1), Day 1 commercial red hour 1 (D1CR1), Day 1 commercial red hour 3 (D1CR3), Day 1 commercial red 4 (D1CR4), Day 2 commercial yellow hour 1 (D2CY1), Day 2 commercial yellow hour 3 (D2CY3), Day 2 commercial yellow hour 4 (D2CY4), Dry Sanitation 2 (DS2), Day 3 ARDECS yellow hour 1 (D3AY1), Day 3 ARDECS yellow hour 2 (D3AY2), Day 3 ARDECS yellow hour 3 (D3AY3), Day 3 ARDECS yellow hour 4 (D3AY4), Day 3 ARDECS white hour 1 (D4AW1), Day 3 ARDECS white hour 2 (D4AW2), Day 3 ARDECS white hour 3 (D4AW3), Day 3 ARDECS white hour 4 (D4AW4).

After day 4 a commercial sanitation company cleaned and sanitized the packing line leaving all food contact surfaces free from residues. There was an overnight period between DS2 and low/medium risk processing. For rubber belts, the population of EB recovered from the packing line was similar between high and the low/medium risk events (Figure 9). Accumulation of EB in rubber belts occurred across and between processing days. Dry sanitation events were able to

significantly reduce the population of EB in rubber belts. The level of risk had no significant effect ($p > 0.05$) on the final population of EB; however, processing day did affect the final population of EB measured at each location ($p < 0.05$). Both sanitization events (DS1/DS2) had the lowest populations of EB (log 2.41 ± 0.27 and 1.90 ± 0.24 cfu/location) whereas Day 2 and 4 had the highest mean EB populations (log 4.54 ± 0.09 and 4.54 ± 0.16 cfu/location). DS1 and DS2 were not significantly different ($p > 0.05$). Both days 2 and 4 corresponded to the second day of packing and sorting of DB onions. In both instances an earlier packing and sanitation event occurred before day 2 and 4. No dry sanitation procedures were performed between processing days from high and low risk activities. Both day 2 and 4 correspond to the accumulation of EB after two days of packing and sorting DB onions. Similar trends were determined for vinyl and stainless steel (Figures 7, 8 and 9).

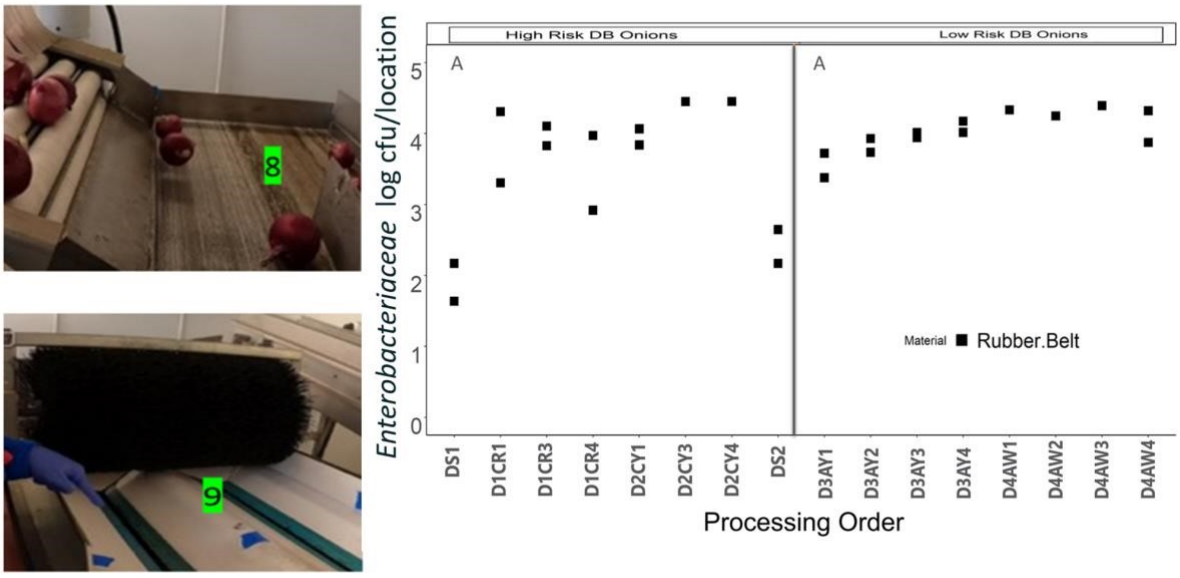

Figure 9. The hourly population of *Enterobacteriaceae* (EB) log cfu/location recovered from food contact surfaces within the rubber belt equipment inside the packing line. 'A' portions of this figure are representative of FCS 8 and 9. Processing order of DB onion materials began with Dry Sanitation 1 (DS1), Day 1 commercial red hour 1 (D1CR1), Day 1 commercial red hour 3 (D1CR3), Day 1 commercial red 4 (D1CR4), Day 2 commercial yellow hour 1 (D2CY1), Day 2

commercial yellow hour 3 (D2CY3), Day 2 commercial yellow hour 4 (D2CY4), Dry Sanitation 2 (DS2), Day 3 ARDECS yellow hour 1 (D3AY1), Day 3 ARDECS yellow hour 2 (D3AY2), Day 3 ARDECS yellow hour 3 (D3AY3), Day 3 ARDECS yellow hour 4 (D3AY4), Day 3 ARDECS white hour 1 (D4AW1), Day 3 ARDECS white hour 2 (D4AW2), Day 3 ARDECS white hour 3 (D4AW3), Day 3 ARDECS white hour 4 (D4AW4).

After day 4 a commercial sanitation company cleaned and sanitized the packing line leaving all food contact surfaces free from residues. There was an overnight period between DS2 and low risk processing. Finally for brush bed bristles, the accumulation of EB across processing hours and days was mainly impacted by dry sanitation events and processing days ($p > 0.05$). As with stainless steel, vinyl, and rubber belts, the level of risk had no significant effect ($p > 0.05$) on the final population of EB (Figure 10). Brushes were not able to be cleaned with any of the proposed dry sanitation activities. As expected, the population of EB increased between activities. In the case of dry sanitation events, the population of EB was significantly different between DS2 and DS1 (log 3.80 ± 0.18 and 2.63 ± 0.32 cfu/location, $P<0.05$), Figure 10). A similar trend in the accumulation of EB in Day 2 and 4 was also observed for brushes. Day 2 and 4 had the highest mean EB population when compared to all other packing and sorting days (log 4.52 ± 0.16 and 4.31 ± 0.20 cfu/location), however, both EB populations were identical (Figure 10).

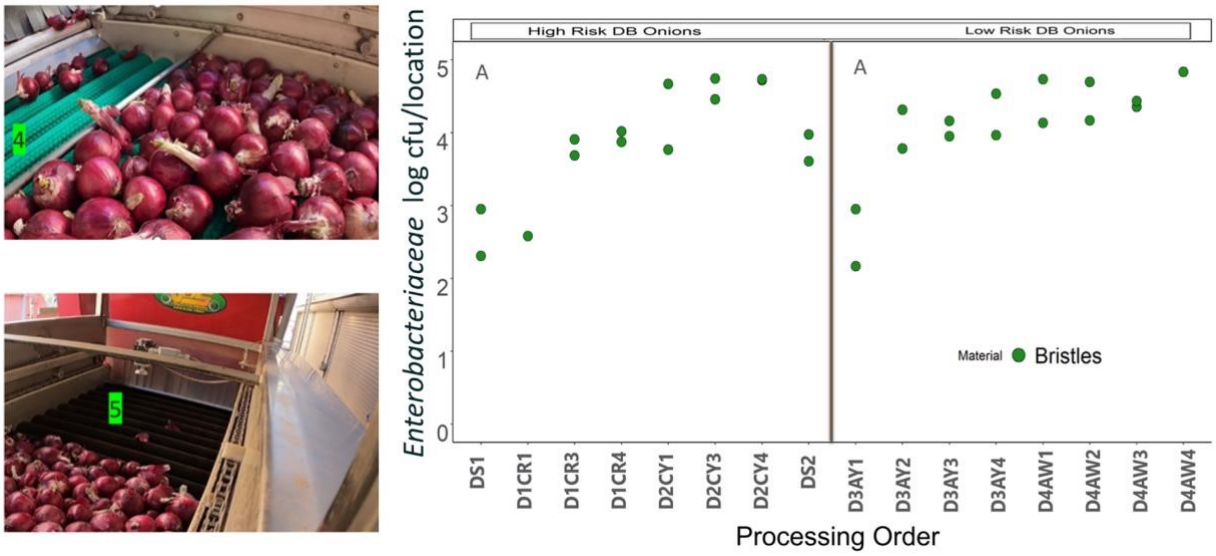

Figure 10. The hourly population of *Enterobacteriaceae* (EB) log cfu/location recovered from food contact surfaces within the bristle equipment inside the packing line. 'A' portions of this figure are representative of FCS 8 and 9. Processing order of DB onion materials began with Dry Sanitation 1 (DS1), Day 1 commercial red hour 1 (D1CR1), Day 1 commercial red hour 3 (D1CR3), Day 1 commercial Red 4 (D1CR4), Day 2 commercial yellow hour 1 (D2CY1), Day 2 commercial yellow hour 3 (D2CY3), Day 2 commercial yellow hour 4 (D2CY4), Dry Sanitation 2 (DS2), Day 3 ARDECS yellow hour 1 (D3AY1), Day 3 ARDECS yellow hour 2 (D3AY2), Day 3 ARDECS yellow hour 3 (D3AY3), Day 3 ARDECS yellow hour 4 (D3AY4), Day 3 ARDECS white hour 1 (D4AW1), Day 3 ARDECS white hour 2 (D4AW2), Day 3 ARDECS white hour 3 (D4AW3), Day 3 ARDECS white hour 4 (D4AW4). After day 4 a commercial sanitation company cleaned and sanitized the packing line leaving all food contact surfaces free from residues. There was an overnight period between DS2 and low risk processing.

Discussion

Human pathogen contamination can occur anywhere along cropping cycles. Traceback efforts in response to the 2020 and 2021 *Sal* outbreaks with DB onion has allowed researchers to focus on pre- and post-harvest contributing risk factors of animal and pest intrusion, sanitation, and irrigation sources to update current commodity specific guidelines (NOA, 2022). DB onion cultivar was identified as a potential source of contamination; however, it was not possible to identify whether contamination of the crop occurred at the field or the packinghouse level for each outbreak (FDA, 2020, 2021).

Minimum Inhibitory Concentration

In this study we evaluated pre-and post-harvest DB onion production practices, looking to determine those risk factors that increase the risk of contamination of DB onion. Per outbreak investigation outcomes, red, yellow, and white onions (FDA, 2020, 2021) were identified as potential sources of *Sal* contamination and in current research associated with DB onions has not provided insight into how DB onion metabolites (Değirmencioğlu & Irkin, 2009) impact growth, survival, and persistence of *Sal* along the cropping cycle. Our MIC results indicate that irrespective of metabolite profile (data not shown) and onion cultivar, highly concentrated onion slurries (3600 mg/L), were not able to reduce the population of *Sal* under ideal conditions for bacterial growth and survival (24h at 37ºC) and maximum exposure to DB onion metabolites. All MIC evaluations were performed with cocktails of 2, 3 and 5 strains per cocktail. Each included *Sal* strains previously used in fresh produce research (Fatica & Schneider, 2011; Greene et al., 2008; Wadamori et al., 2017) or from past outbreaks associated with human illness. Comparison of the pathogenic (T3, T5), and attenuated (T2) cocktails growth response at 3600 mg/L onion slurry for 10OCV, showed no significant difference between T3, T5 and T2, ($p<0.05$); indicating that the attenuated surrogates behave in a similar manner to the pathogenic strains and suggested the

attenuated strains could be used as model organisms in subsequent field inoculation and sorting experiments. These observations coincide with results provided by Lopez-Velasco et al. 2013 in their studies with cantaloupe fruit. Irrespective of the cocktail used, all MIC evaluations provided identical outcomes as illustrated on Figure 1S. Although previous anecdotal references and experiments (Benkeblia, 2004; Değirmencioğlu & Irkin, 2009; Lieberman & Harris, 2019; Lieberman et al., 2015; Sagar & Pareek, 2020) with members of *Alliaceae* has suggested potentially bactericidal ontologies present in their matrices; results from this study indicate that differences in metabolite composition from 10OCV had no effect in reducing the population of *Sal* from onion slurries. Further, the inner scales constituents irrespective of cultivar can sustain viable populations of *Sal* even at atypically high concentrations (log 7 cfu/ml) where organisms may be transitioning to the lag and death growth phases due to sublethal damage (Bertrand, 2019; Bréand et al., 1997, 1999; Swinnen et al., 2004). Inoculation preparation methods in our studies produce cells with differential expression in both attachment (agar), antibiotic resistance (Rif^{50}) and free-cell suspension (broth) that equate to a robust and environmentally fit inoculum. This was evident in the MIC negative controls, as the initial and final population without onion slurry (food) showed no significant difference in 10OCV ($n = 240$), ($p<0.05$). These results suggest that traceback efforts that implicated cultivar as a contributing factor or source to the outbreaks may not reflect what is happening in open field and packinghouse environments and DB onions could mainly be a physical vector of the pathogen coming from field or packinghouse crosscontamination events.

Field evaluations: Survival of *attSal* Across the DB Onion Cropping Cycle (64 Day Period)

No naturally occurring *Sal* was recovered in any aggregate control soil or onion samples. However, post-inoculum recovery was attained across a 64-day period in both soil and onion samples. Recovered populations from aggregate soil samples were significantly different in

magnitude than recovered populations from aggregate onion samples, ($p < 0.05$). Even contamination events did not lead to even recovery of *attSal* from soil or onion samples; however, greater evenness was determined for all soil samples when compared to DB onion. Similar observations were reported by Lopez Velasco et al 2013 and Gutierrez-Rodriguez et al 2012. Comparison of sub samples were not significantly different for both onion and soil, suggesting sachet application and sampling homogeneity was achieved across all field replicates (Figure 1,2). Similar observations were determined by Gutierrez-Rodriguez et al 2012 and 2018 and by Shock et al 2016 as the physical positioning of DB onions adjacent to the furrow and soil particulate size may influence the deposition of the inoculum closer to the furrow and the source of the flowing irrigation water rather than within the seed bed. Although *Sal* are typically flagellated and may be able to move to some extent, their very small Reynolds number in water due to cell size and lack of significant inertia, suggests that the inoculum and water suspension carries *attSal* into the seed bed in a passive colloidal manner through attachment to particulates, or as freely suspended planktonic cells whose movement is determined by the viscous forces of water itself (Rapp, 2017; Vaccari et al., 2018). Therefore, the capillary effects determined in soil variation and subsequent infiltration over the 8-hour furrow irrigation application may explain evenness in contamination. This phenomenon was evident at the 3LS when onion plants were very small in contrast to the fully mature, bulbed plants at topping and lifting which may provide less variability (Figure 1). Moreover, previous studies with water carried enteric pathogen surrogates in open field production experiments have found soil particulate size may determine bacterial movement as the larger particulates may filter freely suspended cells (Bech et al., 2014; Belias Alexandra et al., 2020; Lee et al., 2019; Lopez-Velasco et al., 2012; Lopez-Velasco et al., 2015; Shock et al., 2016). Physical interactions of the inoculum and the soil in the clay loam of ARDECS and the mean difference in inoculum recovered in soil and onion suggests partial filtration and attachment of the inoculum in

the soil located in the furrow most proximal to the sachet. Recovery of *attSal* 64-days post inoculation in soil was on average 7.57 MPN/g (1.3-20 95% CI) in comparison to 3.4 MPN/g in DB onion ((0.17-18 95% CI) irrespective of cultivar. Although this difference may be an artifact of sampling the soil in the middle of the furrow rather than in the stand of the onions. However, considering survival and persistence of *Sal* in soil is pertinent to the DB onion industry. Particularly, prolonged survival of *Sal* in soil has been attributed to several factors such as soil type and physicochemical characteristics (Peng et al., 2022), reciprocal interactions with soil microbiota (Schierstaedt et al., 2020), and *Sal's* entry into the viable but not culturable (VBNC) state with exposure to deleterious stress conditions (Jacobsen & Bech, 2012). Particularly, finer textured soils may possess greater pore space for both native and foreign bacterial communities, which could allow for improved water interactions (Liao et al., 2021).

When comparing the survival and persistence of the inoculum based on percent soil moisture content, no significance was determined ($p<0.05$) despite a range of 16.5 percent across node measurements (Table 3). Although previous efforts have suggested available soil moisture as a significant determining factor in prolonged survival (Alegbeleye et al., 2018; Leifert et al., 2008; Peng et al., 2022); UV index and time elapsed post-inoculation determined the significant differences in inoculum recovery in soil along the cropping cycle. Particularly, the exposure to UV in topping and lifting is altered as the leaf canopy of the fully mature bulbs (12-18 leaves) (Schwartz, 2012) is effectively removed.

Survival of *attSal* During Topping and Lifting (Day 42 Through Day 64)

Prolonged time periods of survival across pre-harvest production practices may extend into the latter portions of topping and lifting DB onions where soil is exposed or physically disrupted by

farm implements. These alterations in the environment where *Sal* may exist could result in changes to die off/inactivation rates or distribution of non-homogenous sources of contamination. Although the soil profile is disturbed in the undercutting of the basal root zone of the DB onion with lifting and is not altered in topping, similar post inoculation recoveries were observed (Figure 2, Panel C and E). Both treatments no longer possess the shading the leaf canopy provides, however the mean die off rate was found to be significantly larger ($p<0.5$) in lifting. This is also confirmed in the DWB inactivation kinetics (Table 3). Ultimately, the time of inoculation, mean UV index (Table A1, 3) and physical alterations to the DB onion leaf canopy were the driving factors in these findings.

Soil Die-off Kinetics for the 3LS, Topping and Lifting

To elucidate the change in *attSal* population in the open field environment across DB onion production, Double Weibull (DWB) modeling was found to best describe the inactivation of the artificial inoculation *attSal* in soil. The field environment contains abiotic environmental conditions and stresses deleterious to the survival and persistence of human bacterial pathogens and the risk of these contaminants at the time of harvest is determined by our ability to estimate their inactivation. Important in the scope of the FSMA PSR (FDA, 2016) which has in the past presented a catch all 0.5 log cfu decline based on indicator EC and recommends a 4-day interval of last irrigation and harvest to effectively decrease the potential microbial load by 2 logs to ensure safety of harvested crop; calculated soil and onion DR were well below this threshold in all instances (Table 4 and 5). DWB models assume two sub-populations that exhibit different abilities to resist the abiotic stressors, or physiological state of the cells and to the biphasic deactivation nature of pathogens on bacterial populations over time (Corroller et at., 2006), and altered survival rates. Our results were best described with this assumption, and therefore, *attSal* behaved in a biphasic manner irrespective of cultivar within ARDECS soil. Furthermore, these models indicate

the latter population selected by environmental stressors may survive for an increased amount of time in comparison to the initial inoculum dose (Delta 2 > Delta 1, Table 3) for the 3LS. Delta 1 values closer to zero indicate an increased rate of inactivation, while Delta 2 values with larger magnitudes indicate a decreased rate of inactivation. This latter effect is reflective of conditions in field environments, where contamination is low and localized. This was determined for the 3Ls but not for topping and lifting treatments suggesting the initial population (delta 1) for the latter two stages in the cropping cycle will decline significantly slower than those same populations from the 3LS (Coroller et al., 2006). This was expected because of the high initial inoculum dose, reduce UV index and lower field temperatures reflective of end season conditions and highlights the importance of preventing cross contamination of the soil or crop close to harvest. Further, when harvest occurs, any localized contamination that could survive at slightly higher populations than early in the season due to weather conditions, will now be distributed across the field as lifting, topping and harvest operations scatter soil and plant residues across the field. It also represents a significant event in which farm equipment could also be cross contaminated, and is equipment is moved and used across fields, this contamination could be easily transferred across multiple locations.

The larger Delta2 values described in the 3LS stage are reflective of *attSal* recovery over a 64 period through MPN*Sal* method, although Day 32 and 64 were removed from the modeling (Figure 1). Significantly smaller values for Delta 2 in constructed models for Topping and Lifting signify decreased rates of die-off. Previous studies (Lopez-Velasco et al, 2015) with BSL1 enteric strains used in these experiments besides those performed with MIC have been shown to behave in identical manner as BSL2 strains and therefore these results may be more reflective of survival and persistence of *Sal* in pre-harvest production of DB onions.

The split block design at ARDECS with fixed DB onion growth gradient treatment layout first looked to elucidate whether cultivar is influential in the survival, persistence, and transfer of *attSal* in the open field environment. Early inoculation at the 3LS stage allowed comparison of inoculum recovery to be completed across the entirety of the pre- and post-harvest production cycle. A 5way ANOVA model: mean *attSal* recovery ~ Cultivar (scale color) + Soil Moisture + Replicate + UV index + Sample was constructed in R studio version 4.3.0 for both sample types (onion and soil). Recovery of *attSal* over 64-days post inoculation suggests that no significant difference ($p<0.05$) between cultivars at different vegetative stages of bulb maturation including curing and harvest in either sample type. Particularly, the replicate sub samples across field replicates ($p > 0.05$) were not significantly different by sample type. However, significant difference in *attSal* recovery across field replicates between sample types ($p<0.05$) was observed. This comparison suggests that inoculum was spread across field replicates and sampled in a homogeneous fashion, however capillary effects, duration of irrigation application, and filtration in soil particulates may have determined the variability of inoculum recovery in aggregate onion samples. No significant difference in *attSal* recovery was observed with respect to soil moisture content regardless of substantial mean soil water content differences over the 64-day period in both sample types. This open field inoculation experiment would benefit from multiple locations, and differences in soil types. These data suggest that *Sal* may survive and persist in moisture limited situations regardless of available moisture content. These results are supported by well-known environmental fitness characteristics associated to *Sal* in low moisture foods and environments and are not in agreement with results associated with att*E. coli* O157:H7and EC, described by Gutierrez-Rodriguez et al., 2012. These differences could be attributed to the known differences in the genomic make up of *Sal* and *E. coli* O157:H7, where the former has over 1000 genes associated to environmental fitness compared to *E. coli* O157:H7. These environments may expose *Sal* to deleterious environmental

conditions and elicit sensory and cellular responses to promote survival. Particularly, osmotic stress may potentially induce changes in cell membrane fatty acid and phospholipid composition (Chen et al., 2014). Furthermore, increased accumulation of trehalose; an important osmolyte capable of stabilizing proteins and supporting retention of cellular enzyme activity (Li et al., 2012) and the coordinated upregulation of small RNAs (sRNAs) may support and increase stress adaptation to desiccation (Barnhill et al., 2019). The average UV index over the course of the production cycle differed significantly in the early-stage inoculation at the vegetative stage of development (3LS) between the 16-, 32-, and 64-day sampling points (Table 3, Figure A2). Significant difference in mean *attSal* recovery and UV ($p<0.05$) suggest that time from the initial inoculation and subsequent changes in UV index potentially affect the *attSal* population in both soil and onion samples respectively. Persistence in soil for lengthy amounts of time carries inherent risk for reincorporating inputs, tillage, and for seed bed preparation for rotational cropping systems.

Onion Die-off Kinetics for the 3LS, Topping and Lifting

No naturally occurring *Sal* was recovered in any aggregate DB onion samples. Mean comparison of recovered *attSal* in onion samples of the first 16 days of each agronomic treatment was completed to elucidate the driving factors. A five-way ANOVA analysis indicated marginal differences in one timepoint in the recovery of *attSal* between cultivars. However, this difference was very small and biologically speaking in terms of risk of contamination, for white, red, and yellow cultivar (scale color) must be considered similar and small. Statistically but not practically significant, these small differences in mean *attSal* recovery between cultivars are reflective of a non-biologically significant result and suggest that recovery of the inoculum was not substantially affected by cultivar. Similarly, comparison of *attSal* recovery across all treatments with respect to soil moisture grouping had no significant difference ($p < 0.05$) although there were considerable

differences in soil moisture content across field replicates (Figure A2). However, comparison of *attSal* recovery in onion samples between 3LS, topping, and lifting showed significant difference ($p<0.05$). Differences in mean *attSal* onion recoveries ($p<0.05$) were significantly affected by average UV index across treatments, and not by cultivar or moisture content. These outcomes juxtapose the widely held beliefs in the industry that 1- cultivar may contribute to the likelihood of contamination, 2- moisture content is deleterious to the survival of *Sal*, 3- field contamination events later in pre-harvest production (topping, lifting and curing) can significantly decreased rates of pathogen die-off in comparison to the early events during vegetative growth stage, 4- log 0.5 cfu/g die-off per day intervals with irrigation contamination may not account for multiple populations and extended survival of human pathogens in open field environments as *Sal* has evolved genetic mechanisms to survive the abiotic stressors of the open field environment. 5- UVindex does not impact die-off of *attSal* in DB onions only in soil. However, regarding the persistence and survival of *Sal* in natural locations it is necessary to consider seasonal variation between production locations and these findings must be supplemented with repeated open field production experiments.

Onion Die-off Kinetics Described by Moisture Content Grouping

Cultivar or cultivar metabolites have previously been hypothesized and colloquially thought to potentially inhibit human pathogen survival. In all instances across field studies, cultivar was found to be marginally statistically different on one single sampling event (Figure 2). However, biologically speaking these differences observed across sampling days were most likely influenced by sampling variability; despite having a significant set of replicates and subsamples per replicate collected along all sampling points. Therefore, cultivar effects in survival of *attSal* were not significant. These findings suggest that other abiotic factors may be the driving forces responsible

for *Sal* contamination of DB onions irrespective of cultivar and scale color. Previous open field inoculation studies have suggested that abiotic features of growing environments may play a significant role in the survival of human pathogens (Fatica & Schneider, 2011; Ongeng et al., 2015; Shock et al., 2013; Shock et al., 2016). Of particular interest, soil moisture content and UV index have been hypothesized as potentially lethal constraints to bacterial survival outside of the host GIT in open field production environments. Growing conditions at ARDECS were monitored across production with instrumentation contained with a Davis Instruments EnviroMonitor system. Soil moisture at 10cm, reflective of the location of the root zone for most edible crops including DB onion, and UV index across treatments showed significant differences over the production cycle (Figure A2). ($p< 0.05$). Comparing the effects and interactions of soil moisture, replicate, UV index and cultivar in our ability to recover and detect Sal indicated that although there was significant difference in soil moisture content across field locations, there was no significant difference ($p > 0.05$) in the recovery of *attSal* from soil samples. However, significant difference ($p < 0.05$) in the recovery of *attSal* across agronomic treatments in soil samples with respect to UV index were observed. Particularly, the two latter stages of topping and lifting where UV index is lower had identical populations of Sal on DB onion and significantly different to DB onions with identical vegetative growth coming from the 3LS. Physically, the removal or disruption of the shade providing leaf canopy for light to percolate to the soil surface in topping and lifting respectively may contribute to these findings. These data suggest that there was no significant contributing effect to *attSal* survival with regards to moisture content in soil samples recovered across all treatments, but there was a significant effect regarding UV index and time elapsed across production.

Cultivar and moisture content did not significantly contribute to the survival of the inoculum in DB onion samples. Modeling also suggests that there is no significant ($p>0.05$) interaction between soil moisture and treatment and the recovery of the inoculum from DB onions. These data suggest that contamination during vegetative growth early in production where UV remains high and topping and lifting/curing processes where UV index remains low are the main contributing factors affecting the recovery of the inoculum. This does, however, suggest late in season contamination events increase the risk of transferring pre-harvest contamination through contaminated irrigation waters into harvested DB onions destined for storage or immediate sorting and packing.

Recovery of *attSal* at Harvest, During Transport to CA and After Storage

At harvest the mean population of *attSal* for medium and low risk DB onion samples was log 2.0 and 0.5 cfu/g DB onion (0.17-18 95% CI), respectively. High risk onions were inoculated in a manner to mimic contamination at harvest with populations in the range of log 5.5 cfu/g DB onion, much higher than typical environmental populations determined in some agricultural environments (Barak Jeri et al., 2008) but on par with other inoculation studies with *Sal* (Alegbeleye et al., 2018). Risk ranking was made based on the population of *attSal* and the infectious dose needed for onset of illness. Our expectation was that these populations would remain stable across rapid transport and short storage while DB onions waited to be sorted and packed, however, this was not the case and all DB onions from different risk levels ended with populations of *attSa*l at < 3 MPN / DB onion (0 to 9.5, 95% CI). Such low level of *Sal* survival typically would reflect in marginal transfer to other environments or equipment.

Packinghouse activities looked to determine if cross contamination took place between control and inoculated treatments and transfer to food contact surfaces. However, the low level of contamination present in all DB onion materials destined for packing and sorting was not high enough to account for physical dilution effects in packing equipment despite utilization of MPN detection methods.

At the time of harvest, a total of 32 burlap sacks containing approximately 15kg of DB onions were stored for 3months mimicking typical storage conditions followed by the industry before onions are sorted and packed. These other group of DB onions also presented populations of attSal at < 3 MPN / DB onion (0 to 9.5, 95% CI) and in both instances no *attSa*l was not detected by enrichment, suggesting that either no more viable cells were available in the DB onions and/or some could have entered the VBNC state and not recovered by the selected approaches. Die off rates calculated between harvest, transport and packing line activities (Table 6 and 7) illustrate the temporal and inactivation aspects associated with *attSal* die off and in this case match closer those suggested by the PSR in the chalk inoculated red and yellow DB onions.

Bacteria exposed to low moisture environments must sense and respond to the difference of internal cell composition to the external environment to avoid water loss. A variety of mechanisms have been characterized in *Sal* such as osmoprotectants, alternative sigma factors, rRNA degradation, filamentation, biofilm formation, and entry in to the VBNC state (Finn et al., 2013). During transport, the environment typically fluctuates with however the crop or commodity is being transported and may play an important role in bacterial inactivation. DB onion are typically transported without temperature controls or chemical interventions (NOA, 2022). Source microbial load and transfer and persistence in transportation of commodities is complex and variable, which makes the control environmentally fit etiologies difficult. In other commodities

such as broiler chickens, the industry has been searching for mitigation strategies in *Sal* prevalence and transfer from on-farm production and the transition to transport and processing locations. Commonly, higher persistence and transfer in the broiler production cycle is observed with farm samples rather than transport and processing plant samples (Bailey et al., 2001). In our postharvest production transition from harvest to processing facility, survival and persistence was not observed in interstate transport or 3-month storage. No recovery of inoculum in the packing line processing or storage suggests die off during the transition from harvest to processing facility and in long term storage (Table 6). Transport environment and dilution aspects related to the volume of onions and sampling area per location also could have compounded this large decline in inoculum and resulted in the absence of recovery of *attSal* during interstate transport. Consequently, any small but continuous contamination events later in the DB onion supply chain coupled with environmental factors during sorting, packing and consumer handling could continue to provide the necessary conditions for some of those small but resilient populations to grow and cause illness since the infectious dose for Sal could be as low as 125 cells (Fatica & Schneider,
2011).

Packing House Evaluations

The packing house located in Fresno CA had not been cleaned or sanitized in over three years prior to these experiments and may reflect some of the conditions observed in the packing houses typically in use in the industry or those implicated in the 2020 outbreak (FDA, 2020). Sorting experiment efforts looked to determine the level of *attSal* contamination on food contact surfaces, and whether non-inoculated controls became cross-contaminated. Further the use of indicator organisms as a tool to predict contamination was also explored when evaluating the populations of EB and EC and whether there was any correlation with the presence of the inoculum, dry sanitation efforts and DB onion residues (ATP measurements). Fourteen different locations were

sampled in the same location for each respective swab to decrease variability and ensure sampling location did not interfere with variability and sampling scheme. Particularly, as red onions were implicated in the 2020 outbreak as the source of contamination, the high risk red and yellow chalk inoculated materials were alternated between inoculated and control DB onions to elucidate whether the control DB onions or the machinery and FCS became cross-contaminated with *attSal.*

Irrespective of risk level, *attSal* was not recovered from 144 DB onion and 224 swabs samples (<3.0 MPN/area) and their respective enrichments, over the course of 6 days of sorting and packing DB onions. Over a 15-day period of harvesting, loading, interstate transport and delivery it was determined that the low, medium, and high-risk DB onion materials had decreased to < 3 MPN/g of DB onion (Table 6 and 7) from log 0.5, 2.0, and 5.5, respectively. We were not aware of this decline during the first 3 days of processing. Potentially, there also existed a physical dilution aspect to the detection of the inoculum due to the volume of DB onions processed and the magnitude of the FCS area.

As high-risk DB onions were processed, ATP values increased over time in day 1 and day 2 regardless of material type (Figure 3). However, low risk DB onion processing did not produce the same magnitude of accumulation (Figure 2). This distinction was visually observable in the chafe and size differences in the onions. ATP values significantly higher than 30 RLU were not observed in all surfaces since several locations were above this threshold (Figures 3 and 4). However, dry sanitation efforts were effective in reducing the magnitude of ATP accumulation irrespective of risk and material (Figure 2 and 3). High risk processing did exhibit observable accumulation of skins, chafe, and debris and this correlated with the accumulation of ATP residue across Day 1 and Day 2 (Figure 3). Dry kept-processing facilities that observe organic matter accumulation potentially may increase the risk of pathogen presence. Particularly, desiccation

studies on abiotic surfaces with STEC and *Sal* were detectable for 35 and 60 days at 35 and 25ºC respectively and where organic substrate had been added resulted in a 79-fold increase in survival (Hiramatsu et al., 2005). This increase in survival suggests that an effective mitigation is to limit the accumulation of organic substrate. The packing line data suggests that the accumulation of organic material and ATP residue accumulates over processing hours until dry sanitation events which, could become actual clean breaks if applied consistently and after daily operations.

EB and EC proliferation increased in conjunction with ATP residue accumulation despite sampling continually revisiting the same location. However, it is clear in this study that EB and EC were not effective in predicting the presence of *attSal* as there was no recovery of the inoculum across six days of processing, although elevated growth of these microorganisms was observed irrespective of FCS material type (Figure 7-10). Mitigating viable substrate accumulation for bacterial growth is a necessary step in reduction of risk in DB onion packing lines and ATP is a great indicator for usage in determining when sanitation of FCS must be completed and whether sanitation has been effective in reduction of residue accumulation. Such a beneficial approach must be accompanied by further pathogen testing of zones 1 and 2 to accurately reflect the potential level of safety of these activities. Under our experimental conditions both, ATP and indicator organism populations complemented pathogen detection of *attSal* and clearly demonstrated that focusing on indicator organism testing will not support safety. Sanitation of low moisture processing facilities such as peanut butter products with isopropanol and quaternary ammonia has been found to effectively reduce *Sal (*Grasso *et al., 2015)*; and 70% ethanol and food grade ethanol wipes and paper towels may be effective in the low-moisture kept packing lines of DB onions.

To further elucidate whether dry sanitation events were effective in reducing debris and indicator microorganisms, ANOVA models were constructed that included the major risk factors including,

Day, and Location. EB/EC ~ Risk + Day within individual material types; as well as models constructed as ATP ~ Risk + Day and Tukey's HSD mean comparison indicated significant difference ($p<0.05$) between hourly and daily ATP residue and EB and EC indicator accumulation. The main effects of all models suggest the highest accumulation in days furthest removed from dry sanitation events. Dry sanitation events were effective in reduction of ATP residue and EB and EC presence (Figures 7-10) irrespective of material type. These findings suggest that dry sanitation is a viable sanitation practice that when performed daily, reduces the risk of potential food safety hazards present on DB onions. However, there needs to be continual reconsideration in the effectiveness of assessing the presence of indicator organisms in dry packing houses which process DB onion as no correlation with enteric pathogens was determined despite elevated populations of EB and EC (Figures 7-10). Dry sanitation efforts without the use of water therefore may decrease or reduce the inherent risk of human pathogen presence in food contact surfaces and equipment used in the sorting and packing of DB onions.

In some cases, indicator organisms are limited in evaluating safety of raw or minimally processed food products and depends on a variety of microbiological criteria related to the product in question (Busta et al., 2003). EB is often utilized as an indicator of hygienic processes and EC is typically utilized as an environmental fecal contamination indicator (Santos et al., 2020). In the recent past, numerous studies have shown that EC, coliforms, fecal coliforms and EB are unreliable when utilized as indicators of pathogen contamination in food products (Kornacki & Johnson, 2001). Although the criteria are numerous and characterized to be specific to certain food products and processing situations in fresh produce, instances where generic EC utilized as an indicator of environmental fecal contamination has been found with greater probability of enteric pathogen

presence (Kornacki & Johnson, 2001; Park et al., 2013). There are numerous instances where positively correlated indicator microorganisms are effective in environmentally recovered samples in soil and water, however the usage within dry kept environments needs further experimentation to solidify whether their use is predictive in pathogen presence in commercial FCS in packing lines. The accumulation of EB and EC was significant and suggests that dry sanitation of packing line FCS is necessary daily and is effective without the use of water. However, the frequency of dry sanitation events needed to effectively control levels of indicators microorganisms is currently unknown and further exploration of this parameter may be needed on different packing house operations.

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Appendix 1

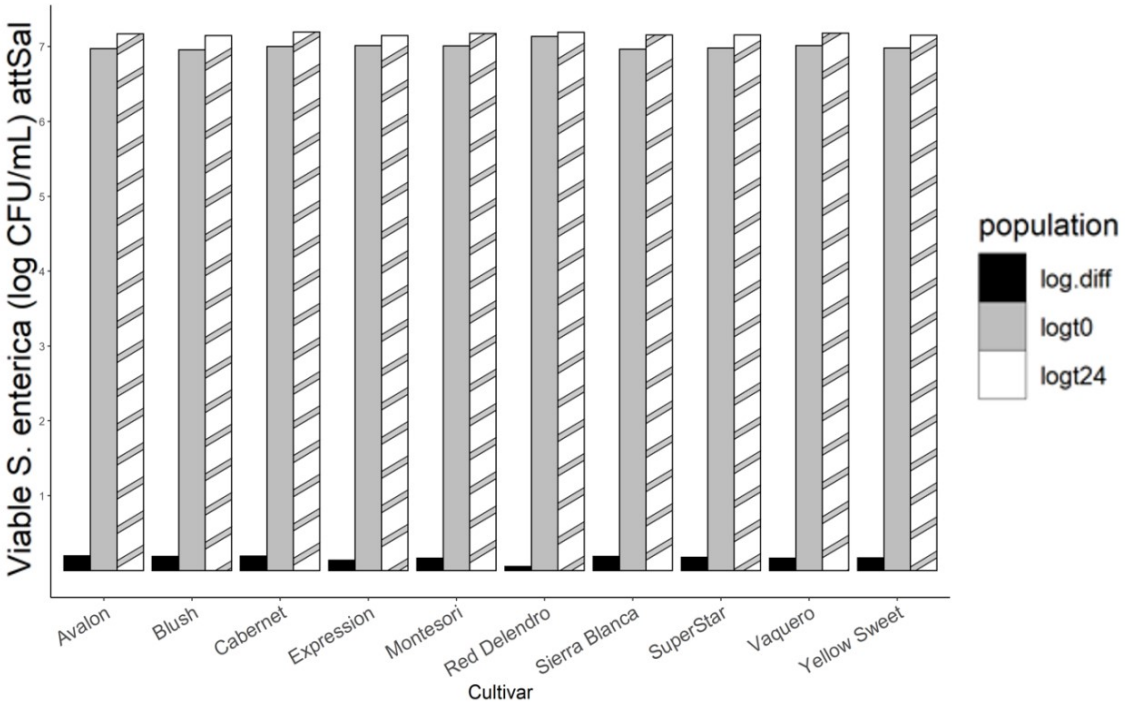

Figure A1. Minimum Inhibitory Concentration (MIC) of 10 cultivars of DB onions widely grown in Colorado. Viable *attSal* log CFU/ml prior (logT0) and after a 24-hour incubation at 37°C (logT24) with 3600 mg/L suspension in 0.1M potassium phosphate buffer and onion slurry from each cultivar. The log difference cfu/ml is representative of the average of 3 repetitions prior to 24-hour incubation subtracted from the post incubation (log t24) cfu/ml, respectively. Positive controls (n = 270) showed no significant change and negative controls no presence of *Sal*.

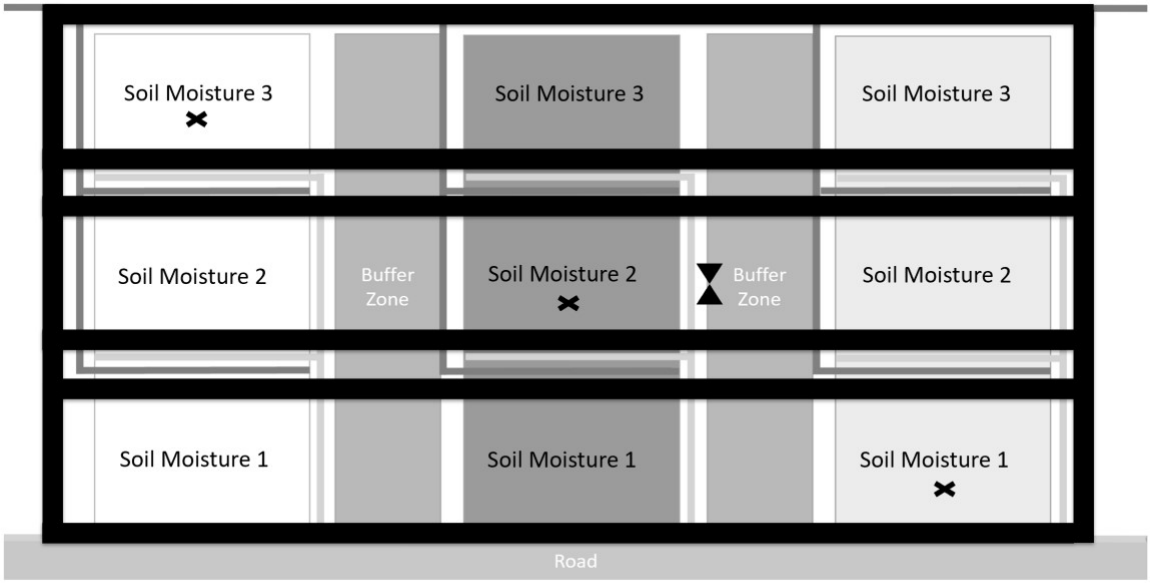

Figure A2. The cultivar replicates were separated based on differences in soil moisture content determined by moisture probes indicated by black 'X'. Statistical comparisons were done based on high, medium, and low soil moisture content (Soil Moisture 1 = high (36%), Soil Moisture 2 = medium (25%), and Soil Moisture 3 = low (19.5%) and difference in the UV index along the cropping cycle as cultivar was found to be insignificant in the recovery of *attSal*. The black hourglass signifies the location of the Davis Instruments Integrated Sensor Suite (ISS). Blocking of the field configuration by moisture was made from individual node measurements per area and direction of water flow per field replicate.

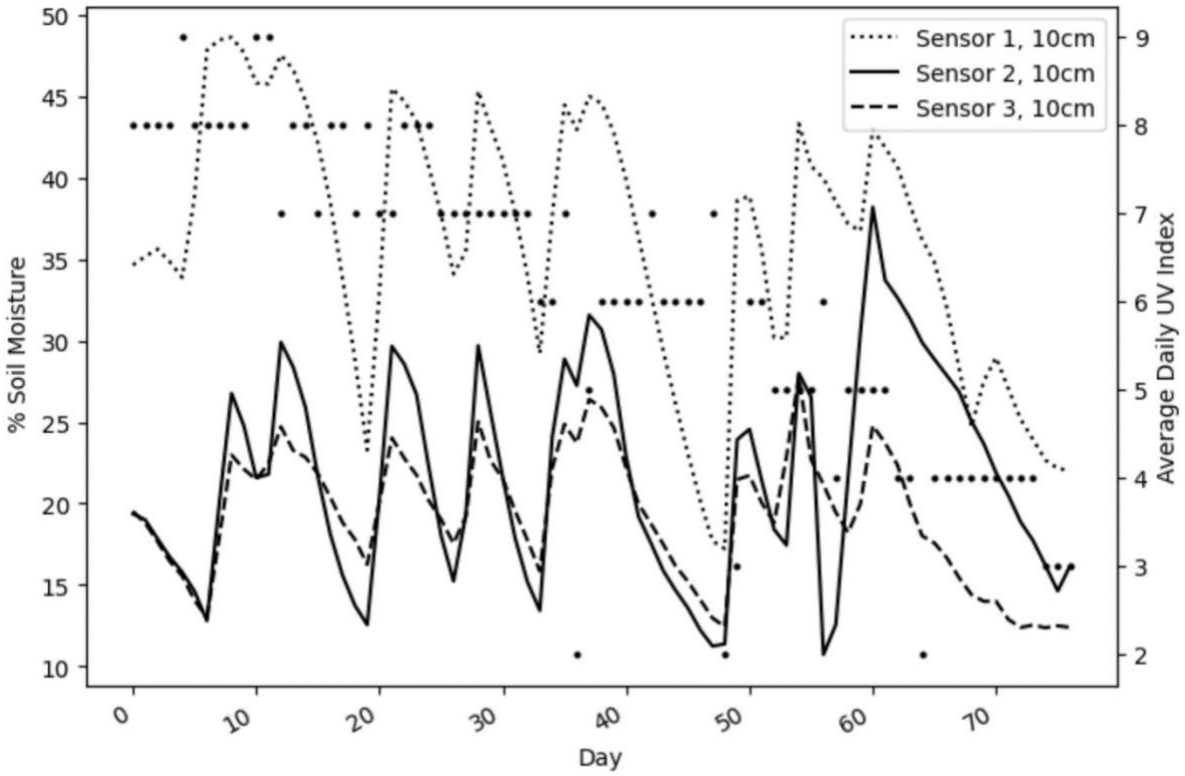

Figure A3. Average % soil moisture content and daytime UV index over the course of 74 days at ARDEC South Research Station, Fort Collins, CO. Davis Instruments EnviroMonitor ISS and 3 nodes logged data on a 5-minute interval, 24-hours a day across DB onion production. The 30.5 cmm Drill and Drop Sentek soil moisture and temperature probes were placed across the field in a manner to capture variability across the split block design. Sensor 1 average soil moisture, Sensor 2 average soil moisture, and Sensor 3 average soil moisture was representative of daytime measurements only. Average UV index was representative of daytime high measurements only. Dotted line represents the soil moisture gathered from moisture node 1, solid line represents the soil moisture gathered from moisture node 2, and dashed line represent the soil moisture gathered from moisture node 3.

Table A1. Mean soil moisture content and daytime UV index over the course of each agronomic treatment and cultivar at ARDECS.

Cultivar	Treatment	Mean High UV Index	Mean High (%) Soil Moisture 10cm depth	Mean High Temperature ºC
Red	3LS	7.81 ± 0.16	36.66 ± 1.36	28.8 ± 1.1
White	3LS	7.81 ± 0.16	31.09 ± 1.78	28.8 ± 1.1
Yellow	3LS	7.81 ± 0.16	40.76 ± 1.83	28.8 ± 1.1
Red	Topping	4.44 ± 0.22	26.37 ± 1.87	21.8 ± 1.9

White	Topping	4.44 ± 0.22	25.96 ± 1.57	21.8 ± 1.9
Yellow	Topping	4.44 ± 0.22	22.60 ± 1.38	21.8 ± 1.9
Red	Lifting	3.82 ± 0.30	20.33 ± 0.90	20.0 ± 1.8
White	Lifting	3.82 ± 0.30	16.85 ± 0.99	20.0 ± 1.8
Yellow	Lifting	3.82 ± 0.30	21.31 ± 0.57	20.0 ± 1.8

Davis Instruments EnviroMonitor Instrument Sensor Suite (ISS) and 3 nodes logged data on a 5-minute interval, 24-hours a day across the DB onion cropping cycle. Mean UV index was representative of daytime high measurements only. Data was filtered to exclude values at night and are representative of the high number reached per day over the course of 16 days per treatment and cultivar for soil moisture values and ambient temperature ºC, respectively.

Abbreviations

Abbreviation	Meaning
CAFO	**Confined animal feedlot operation**
GIT	**Gastrointestinal tract**
FBI	**Foodborne illness**
DB	**Dry bulb**
Sal	**Non-typhoidal *Salmonella enterica* spp.**
LM	***Listeria monocytogenes***
PEC	**Pathogenic *Escherichia coli***
10OCV	**10 DB onion cultivars widely grown in Colorado**
3OCV	**Three representative DB onion cultivars of white, red, and yellow cultivar (Sierra Blanca, Blush F1, Expression)**
3LS	**Three-leaf vegetative growth stage**
GCMS	**Gas-chromatography mass spectrometry**
XLT4	**Xylose Lysine Tergitol-4**
TTB	**Tetrathionate Broth**
UPB	**Universal Pre-Enrichment Broth**
MPN	**Most Probable Number**
Tween20	**Polysorbate 20 Wetting Agent**
Rif50	**Rifampin 50 µg/ml**
LOD	**Limit of Detection**
GPM	**Gallons per Minute**
OD600	**Optical Density Measurement at 600nm**
DWB	**Double Weibull Model**
FCS	**Food Contact Surface**
DS	**Dry Sanitation**
3SC	**Three Strain Cocktail**
ATP	**Adenosine Triphosphate**
EB	***Enterobacteriaceae***
EC	***Escherichia coli***
PCR	**Polymerase Chain Reaction**
ARDECS	**Agricultural Research Development and Education Center South**
MPN*Sal*	**Most Probable Number Enumeration Method Specific to *Salmonella* spp.**

Swab*attSal*	**3M^{tm} Neutralizing buffer swab utilized to sample for the presence of the inoculated strain *attSal***
OD	**Optical Density**
VBNC	**Viable but Non-Culturable State**
att*Sal*	**Cocktail of Two Attenuated Strains of *Salmonella enterica***