CENTER for PRODUCE SAFETY

CPS 2021 RFP
FINAL PROJECT REPORT

Project Title
Assessing the potential for production practices to impact dry bulb onion safety

Project Period
January 1, 2022 – December 31, 2023 (extended to January 31, 2024)

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Objectives

1. *Quantify Escherichia coli contamination rates, die-off, and potential growth in onions due to application of crop protection sprays, including clay application to prevent sunburn.*
2. *Quantify E. coli contamination rates, die-off, and potential growth in onions due to overhead irrigation with contaminated water.*

Funding for this project was provided partly through the CPS Campaign for Research

FINAL REPORT

Abstract

The 2020 *Salmonella* Newport outbreak linked to onions demonstrated the potential for a significant contamination event of foodborne pathogens during dry bulb onion production activities. The intensive investigation did not reveal the cause or mitigating factors that led to the contamination event; however, agricultural water quality and use was suggested as a likely contributor to crop contamination. Previous research by our group has demonstrated that drip irrigation with poor quality water does not constitute a significant contamination risk; however, we have demonstrated that foliar application may pose a significant contamination risk that might lead to growth of pathogens in a portion of the onions. This research project focused on the risk of dry bulb onion contamination when poor quality water is used for crop protection sprays (pesticides or clay) or for overhead or drip irrigation applied at the end of the growing season. We conducted a total of four field trials (two in Oregon and two in Washington) to characterize the risks associated with these practices. A cocktail of rifampicin-resistant *Escherichia coli* strains served as a surrogate for *Salmonella* behavior in the field setting. Onions were sampled throughout a 30-day curing period and *E. coli* was enumerated using a combination of standard plating, most probable number, and enrichment techniques for effective quantification and detection.

Oregon field trials investigated contamination of red 'Red Wing' and white 'Cometa' onions following application of crop protection sprays (clay and pesticide) with 3 log MPN/100 mL of *E. coli*. The crop protection sprays resulted in the majority of onions being contaminated immediately after application, with contamination levels being slightly higher in the 2023 field season (maximum: 275 MPN/onion bulb) compared to 2022 (maximum: 150 MPN/onion bulb). *E. coli* die-off in 2022 was rapid, with no detectable *E. coli* on any of the onions sampled from day 2 to day 16 of curing (0/320). A single onion (1/80) tested positive on enrichment at the end of field curing. *E. coli* levels and prevalence also decreased with curing time in 2023; however, *E. coli* was detected on a larger number of onions on day 7 (11%; 17/160) which could have been impacted by an uncharacteristic precipitation event that morning. A similar percentage (1%; 3/320) of onions at the end of curing were positive for *E. coli* on enrichment. There was no meaningful difference between the two crop protection sprays nor between the two varieties of onions tested.

The 2022 Washington field trials investigated contamination of yellow 'Calibra' onions due to drip and overhead irrigation with 3 log MPN/100 mL of *E. coli*. Overhead irrigation results in contamination of nearly every onion (38/40) at levels between 2 and 13 MPN/onion bulb. *E. coli* levels on contaminated bulbs remained consistent through 7 days of curing while the percentage of contaminated onions decreased significantly (2/40). No overhead irrigated onions (0/40) tested positive at the end of field curing. In contrast, drip irrigation with equally contaminated water led to the delayed contamination of only a small percentage of onions (13%; 5/40 at 24 hrs). *E. coli* was not detected on any of the drip irrigated onions that were sampled at any other timepoints (day 0, 7, 15 or 28).

The 2023 Washington field trial was split, with a drip irrigation portion focused on risks related to an extreme contamination event (5 log MPN/100 mL) and an overhead irrigation portion probing differences in contamination and survival of different onion varieties (yellow: 'Calibra' and 'Ovation', red: 'Red Wing', white: 'Cometa'). Increasing the contamination level in the drip irrigation water led to a significant increase in the number of contaminated onions (27/40 at 24 hrs) as well as the level of contamination (as high as 1020 MPN/onion bulb). A significant precipitation event had occurred the day prior to inoculation which may have contributed to more efficient movement of *E. coli* through the soil, as suggested by high prevalence and contamination levels immediately after irrigation. A single onion (1/80) from the drip irrigation trial tested positive for *E. coli* at the end of the 2023 field curing. Overhead irrigation in 2023 resulted in a slightly elevated level of initial contamination of onions on nearly all onions (97.5%; 157/160) immediately after application. Die-off rates were similar to those in 2022; however, 2% (7/320) of onions had viable *E. coli* remaining at the end of field curing.

Overall, this study demonstrated the efficacy of typical field curing practices to mitigate risks associated with contaminated water used for irrigation purposes and for the preparation of crop protection sprays that may be used late in the growing season. We saw no evidence of growth of *E. coli* in any of the onions sampled, and the overall health and quality of the bulbs was good. A very low percentage (<3%) of onions tested positive for *E. coli* at the end of field curing, which could indicate risk for crop going directly to retail without post-harvest storage.

Background

The western states of California, Idaho, Oregon, and Washington account for >80% of US dry bulb onion production. These states share many production, storage and shipping practices, and members of our team have been actively engaged with the onion industry on produce safety research and outreach activities since the advent of the Food Safety Modernization Act (FSMA). During the summer of 2020, a large *Salmonella* Newport outbreak in the US and Canada was epidemiologically linked to red onions produced in central California. Despite an intensive investigation, the outbreak strain was not recovered from onions nor environmental samples from implicated packing facilities. A second large-scale salmonellosis outbreak was linked to red onion consumption in 2021, forcing the dry bulb onion industry to reevaluate their food safety management practices.

This research project was focused on dry bulb onion production practices, with specific emphasis on common production, harvest, and post-harvest practices used in the western region that may contribute to the contamination and/or enhance survival of *Salmonella.* Through discussions with representatives of the western onion industry, we identified specific water application practices that had not been evaluated for their impact on the safety of dry bulb onions. This project specifically focused on three unique forms of direct water application that occur during the production of dry bulb onions: overhead irrigation, drip irrigation, and crop protection sprays. Overhead irrigation is commonly used during dry bulb onion production in the Columbia Basin of Washington, western Oregon, and California, whereas drip irrigation is used almost exclusively in Treasure Valley (OR-ID border). The application of a variety of crop protection sprays, including pesticides and clay (kaolin), is a common strategy used throughout the western region to protect the quality of dry bulb onions. Dry bulb onions may receive twelve to fifteen crop protection sprays through the growing season. During the extended in-field curing phase, kaolin clay is commonly applied to red and white onions to prevent sunburn, but it is not required for yellow onions. Clay is applied by sprayer in a mixture with water from one to three times during the in-field curing phase. Clay protects the onion from UV damage, which may also protect contaminating pathogens from this same stress.

There was a need for a comprehensive assessment of onion industry growing, curing, harvesting and storage practices, and how these practices could impact contamination, pathogen survival (or growth), with consideration of varietal and seasonal effects. This project was designed and executed to provide data to support the risk evaluation of these practices.

> **Hypothesis:** Unique physiological differences and production practices among red, white, and yellow dry bulb onion varieties impact risk of contamination, multiplication, and survival of *Salmonella* and *Escherichia coli* (surrogate) during production, harvest, and in-field curing.

We proposed two specific research objectives that were tested in field trials at experiment stations in two of the dominant western dry bulb onion growing regions. The Malheur Experiment Station in Treasure Valley (OR-ID border) is the leading research site for dry bulb onions in the US, with demonstrated experience in establishing and maintaining complex field trials, including produce safety research involving inoculations with bacteria and pesticide applications. The Franklin County Extension field site in the Columbia Basin (WA) has been conducting research on dry bulb onions for the last 16 years under the same management and is the primary field site for onion research in the Columbia Basin. Given the similarity in dry bulb onion production practices in the western US, results from these experiment stations should be broadly applicable throughout the region.

Objective 1. **Quantify *E. coli* contamination rates, die-off, and potential growth in onions due to application of crop protection sprays, including clay application to prevent sunburn.**

> This objective was completed at the Malheur Experiment Station in Ontario, Oregon, using red (Red Wing) and white (Cometa) onions. Water used to prepare crop protection sprays (pesticide: Pristine®; clay: Surround®) was inoculated to deliver approximately 2-3 log CFU/onion. Contaminated applications were applied on a single day at the end of the growing season. This field trial was repeated over two growing seasons (2022 and 2023) to account for variability in environmental conditions.

Objective 2. **Quantify *E. coli* contamination rates, die-off, and potential growth in onions due to drip and overhead irrigation with contaminated water.**

> This objective was completed at the Franklin County Extension field site in Washington state. Contaminated irrigation events (overhead and drip) occurred on the last day of irrigation. In the first year of the field trial, onion contamination was compared between drip and overhead irrigation applications using water contaminated with 3 log CFU/100 ml. In 2023, the trials were split to target different research objectives. Contamination rates related to drip irrigation were evaluated using water inoculated at 100X higher *E. coli* concentration (5 log CFU/100 ml). Contamination rates related to overhead irrigation were compared across four different onion cultivars (yellow: Calibra and Ovation; red: Red Wing; white: Cometa) while targeting the same water inoculation level as the 2022 trial.

Research Methods and Results

Methods:

Bacterial strains: Generic *E. coli* strains TVS 353, TVS 354, and TVS 355 previously isolated from agricultural samples and adapted to be resistant to rifampicin (Dr. Trevor Suslow, University of California, Davis) were used for all field studies. Individual strains were revived from −80°C storage by streaking onto tryptic soy agar supplemented with rifampicin (TSAR; 50 μg/mL) with incubation at 37°C for 24-48 hours. Isolates were transferred to tryptic soy broth (TSB) and incubated at 37°C for 24 hours. Culture aliquots of 1 mL were spread onto TSAR in large format petri dishes (150 mm) and incubated at 37°C for 24 hours to create a bacterial lawn. Lawns were harvested by applying 8 mL of 0.1% peptone water to the agar surface, scraped with a sterile cell spreader, and the liquid was transferred to a sterile 50-mL conical tube. Equal volumes of individual strains were mixed to create a cocktail (~10 log CFU/ mL). The cocktail was diluted in water on farm to achieve the targeted final concentration of *E. coli* for each objective.

Objective 1. **Quantify *E. coli* contamination rates, die-off, and potential growth in onions due to application of crop protection sprays, including clay application to prevent sunburn.**

Field trials: Red (variety 'Red Wing') and white (variety 'Cometa') onions (eight plots per color) were planted at the Malheur Experiment Station in 2022 and 2023. Seeding rates were 160,000-180,000 per acre. Onions were grown using typical planting and production practices (fertilizer, pesticide management, etc.) for the region. Irrigation during growing was accomplished by subsurface drip using well water. Irrigation was ceased when 50% of the onion tops had fallen (mid-August). Crop protection sprays (pesticide: Pristine®; clay: Surround®) were mixed using well water inoculated with a generic *E. coli* cocktail (described above) and applied by a certified pesticide applicator using a CO_2-powered backpack sprayer. Water samples were collected from the sprayers by placement of sterilized jars throughout the plots. Water samples were enumerated using the IDEXX Colilert with QuantiTray 2000 to verify inoculation level. Pesticide selection was determined by Co-PI Reitz in conjunction with preliminary studies indicating that Pristine® supported the growth of *E. coli* and *Salmonella* in solution. Four plots of each onion variety were inoculated by pesticide application and four plots were inoculated by clay solution. Crop sprays were applied at recommended dosages which resulted in the application of 2-4 ml per plant. Onions remained undisturbed in the field for 7 days after spray application, were then lifted to the surface of the soil and continued field curing for a total of 28-29 days. Temperature and humidity data were collected from the AgriMet weather station that is maintained on site at the Malheur Experiment Station (latitude 43.9777; longitude -117.01527).

Onion sample collection and analysis: Onions were randomly sampled from each replicated plot within each treatment group on the initial day of contamination (day 0) and throughout the 30-day curing period. During the 2022 field trial, onion samples (10 per plot) were sampled at 0, 6, and 24 hours after spray application. On days 2, 7 (lifting), and 16, 10 onions per plot (40 per treatment per day) were collected and analyzed. At harvest (day 28-29), 20 onion samples per plot were sampled. During the 2023 trial, onion samples were collected on day 0, 1, 7 (10 onions per plot), and 28-29 (20 onions/plot). *E. coli* levels on each onion were determined using a combination of standard plate counting, most-probable number, and enrichment methods. All media contained 50 μg/ml of rifampicin to selectively detect the inoculum used in this study.

Supporting preliminary laboratory efforts: Preliminary laboratory studies were conducted to evaluate various crop protection sprays on the survival of *E. coli* and *Salmonella* in water at ambient temperature. Chemicals evaluated were Badge SC®, Luna® Tranquility, Mankocide®, Pristine®, Tanos®, Quadris®, and Roval®. The intention was to identify a crop protection spray that represented the highest risk for contamination. The rifampicin-resistant *E. coli* cocktail and a cocktail of *Salmonella* strains associated with the two onion outbreaks were selected for pesticide testing. Chemicals were tested at their recommended dosage and inoculated at 5 log CFU/mL. Populations were monitored after incubation at 25°C for up to 48 hrs.

Objective 2. **Quantify *E. coli* contamination rates, die-off, and potential growth in onions due to drip or overhead application of contaminated irrigation water.**

Field trials: In April 2022, yellow onions (variety 'Calibra'; 8 plots) were planted at the Franklin County Extension Field Site in Pasco, Washington. Seeding rate was 160,000-180,000 per acre. Onions were grown using typical planting and production practices (fertilizer, pesticide management, etc.) for the region. Irrigation was accomplished by overhead irrigation using well water. When 50% of the onion tops had fallen, a final irrigation session was performed using water inoculated with the generic *E. coli* cocktail at 3 log CFU/100 ml (described above). Four plots were treated with contaminated drip irrigation water and four with contaminated overhead irrigation water. The contaminated irrigation event (drip or overhead) delivered approximately 0.4 acre-inch. Water samples were collected throughout the contaminated irrigation event and enumerated using the IDEXX Colilert with QuantiTray 2000 to verify inoculation level. Onions remained undisturbed in the field for 7 days after spray application, were then lifted to the surface of the soil and continued field curing for a total of 29 days. Temperature and humidity data were collected from nearby WSU AgWeather Net station.

In April 2023, yellow onions (variety 'Calibra'; 4 plots) were planted to conduct a similar drip irrigation trial using a higher contamination level (5 log CFU/100 mL). In addition, four plots of four varieties (yellow: 'Calibra' and 'Ovation'; red: 'Red Wing'; white: 'Cometa') were planted for the overhead irrigation trial. Irrigation contamination levels for the overhead irrigation were targeted to deliver 3 log CFU/100 mL (identical to 2022).

Onion sample collection and analysis: Onions were randomly sampled on day 0, 1, 2, 7 (lifting), 16 (10 per plot), and 28 (20/plot). *E. coli* levels on each onion determined using a combination of standard plate counting, most-probable number, and enrichment methods. All media contained 50 μg/ml of rifampicin to selectively detect the inoculum used in this study.

Statistical analyses: Combined results of the MPN, plating, and enrichment methods were used to calculate cell density of rifampicin-resistant *E. coli* on onion bulbs using the following equation:

MPN/onion = $P/\sqrt{NT}$ where *P* is the number of positive aliquots, *N* is total mL of sample in all negative aliquots, and *T* is total mL of sample (200 mL for onion bulb samples; 100 mL for leaf samples). Additional data were collected for each of the onion bulbs analyzed, including mass and diameter, as well as whole and cross-sectional photos.

For prevalence assessments, onion samples were considered positive for rifampicin-resistant *E. coli* if they were confirmed positive by any analysis method (e.g., plating, MPN, or enrichment). Onions samples were counted as negative if *E. coli* was not confirmed by any method. Fisher's exact tests (GraphPad, La Jolla, CA) were used to compare prevalence of *E. coli* on onions contaminated by different irrigation methods, at different stages of curing, and/or for differences between cultivars.

Results:

Objective 1. **Quantify *E. coli* contamination rates, die-off, and potential growth in onions due to application of crop protection sprays, including clay application to prevent sunburn.**

Pesticide and spray applications were estimated to deliver approximately 2-4 mL of contaminated water to each onion plant. Water testing results indicated that the average quantity of *E. coli* delivered to each plant (leaves and bulbs) was approximately 300 MPN/plant in 2022 and 670 MPN/plant in 2023.

Contaminated pesticide and clay applications resulted in a majority of the onions having detectable levels of *E. coli* immediately after treatment (day 0) in both years (Figure 1). The clay application on the white variety of onions had the lowest prevalence (55% and 45%) in both years. Conversely, the pesticide application on the red variety resulted in the highest prevalence on day 0 in both years (90% and 92.5%). Similarly, those same treatments had the lowest and highest cell densities enumerated on individual onions bulbs in both years (Figure 2).

In 2022, the prevalence of *E. coli* on both varieties receiving both contaminated crop sprays decreased sharply during the first 24 hours (Figure 1 – top panel). *E. coli* was not detected on any onion bulbs analyzed from days 2 to 16 (total of 480 onion samples). After 29 days of field curing, a single onion (red/pesticide) out of 320 samples tested positive for *E. coli* on enrichment. Similarly, *E. coli* quantity decreased sharply during the first 24 hrs (Figure 2).

In 2023, initial prevalence was comparable to 2022 (Figure 1); however, the cell density of *E. coli* on onion bulbs immediately after spray was higher (Figure 2). *E. coli* prevalence did consistently decrease throughout the field curing period in 2023; however, survival at low levels over time was substantially improved compared with 2022. At the end of field curing, three onions (1: red/pesticide; 2: white/clay) out of 320 tested positive for *E. coli* (Figure 2).

Weather data associated with the field trials is shown in Figure 3. It is worth noting that there were a few uncharacteristic precipitation events during field curing in 2023, including a few days of low temperature with a substantial rain event on day 7 and another smaller event later in curing. It is possible that this increase in moisture on the plants supported longer-term survival of *E. coli* on the onion bulbs.

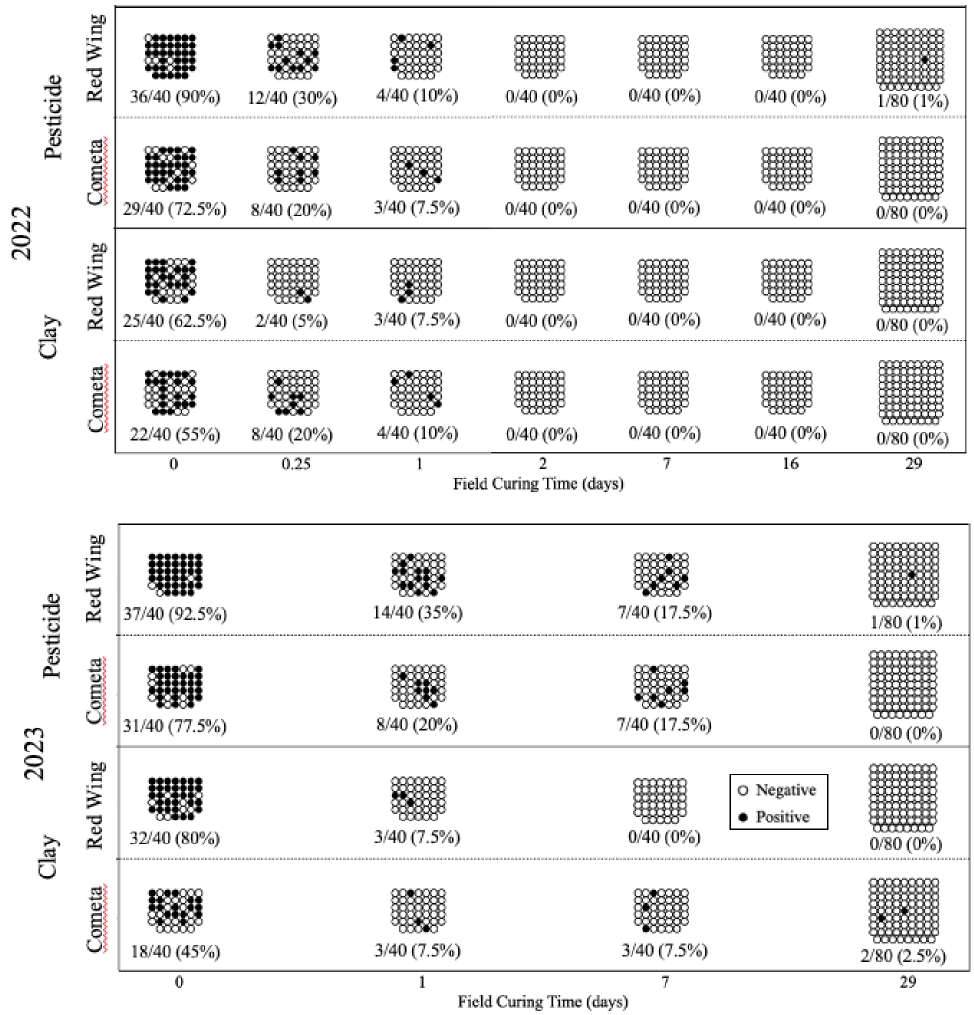


Figure 1. Prevalence of *Escherichia coli* detection on red (cultivar 'Red Wing') and white (cultivar 'Cometa') onions treated with crop protection sprays prepared with contaminated water during 2022 and 2023 field trials conducted at the Malheur County Experiment Station in Ontario, OR. Black closed circles indicate onion bulbs that were confirmed positive for *E. coli* by at least one analysis method (plating, most probable number, and/or enrichment).

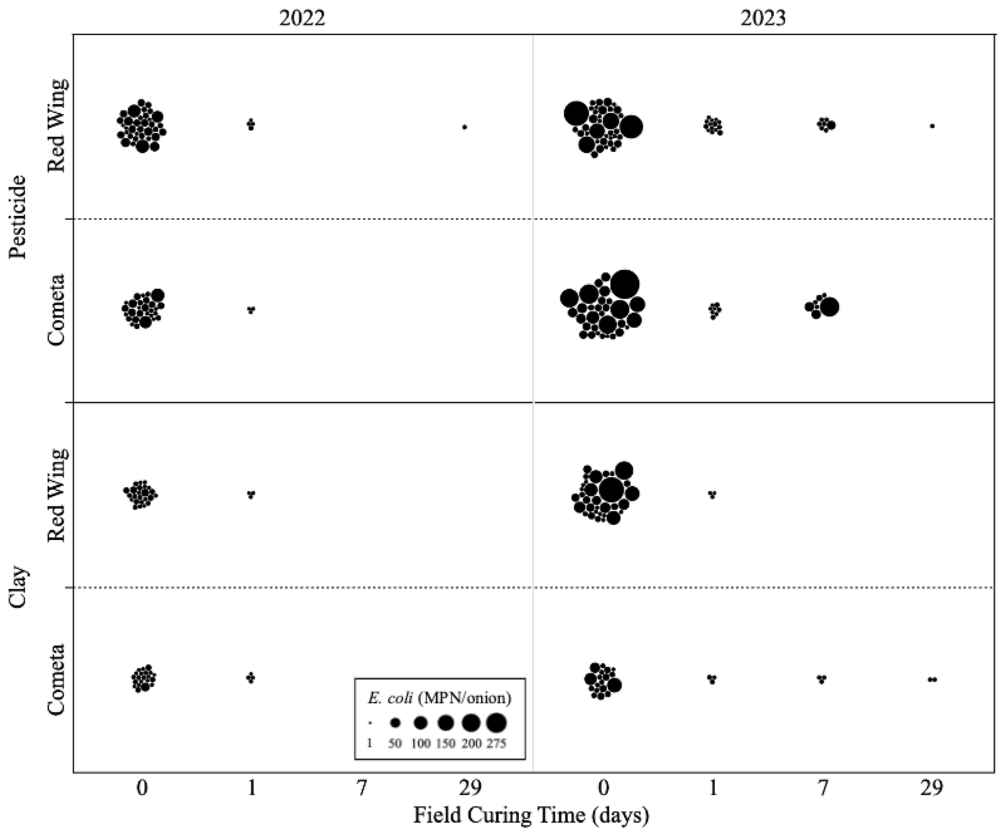
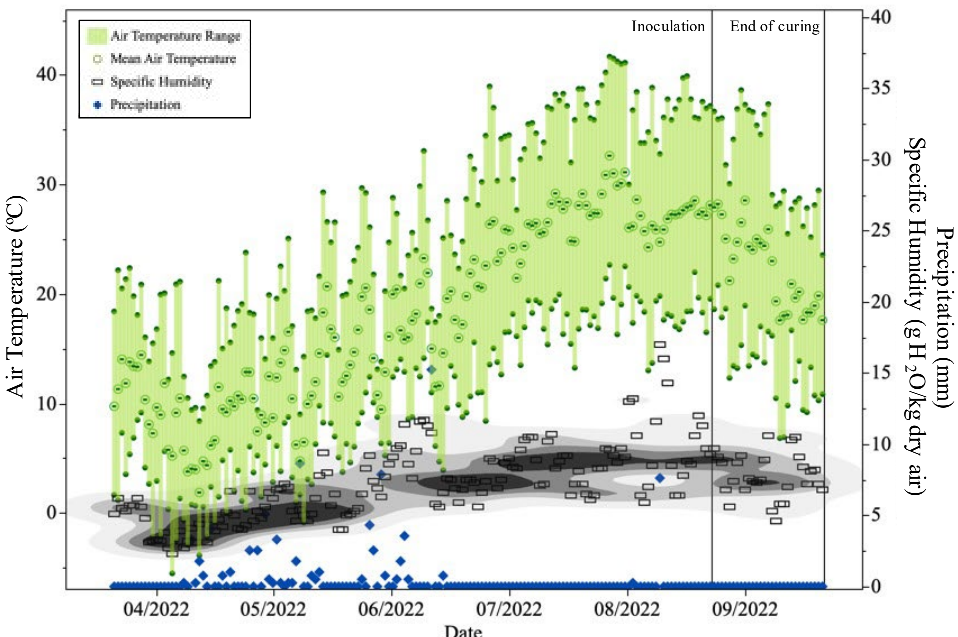


Figure 2. Quantity of *Escherichia coli* detected on individual red (cultivar 'Red Wing') and white (cultivar 'Cometa') onion bulbs treated with crop protection sprays prepared with contaminated water during 2022 and 2023 field trials conducted at the Malheur County Experiment Station in Ontario, OR. On days 0, 1, and 7, a total of 40 onions were analyzed per day for each treatment. The sample size was increased to 80 onions per treatment on day 29. Onion bulb samples with no detectable *E. coli* are not displayed in the figure.

A) 2022 Field Trial



B) 2023 Field Trial

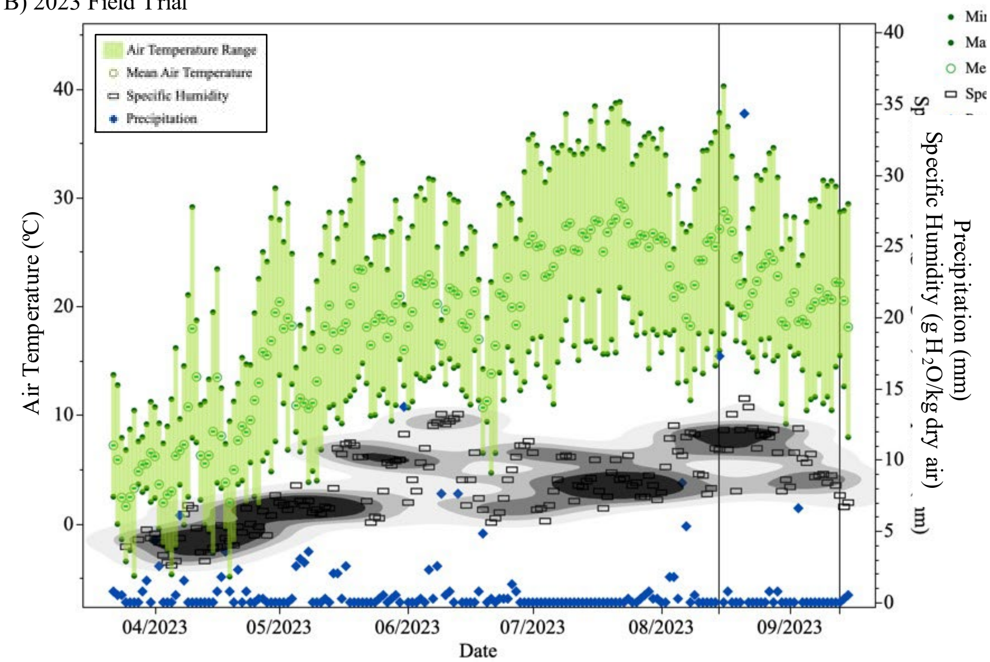


Figure 3. Air temperature (range and mean), specific humidity, and precipitation in Ontario, Oregon, during the onion growing and curing periods of the A) 2022 and B) 2023 field trials. Weather data was downloaded from United States Bureau of Reclamation AgriMet Database for the Ontario, OR site location.

Objective 2. **Quantify *E. coli* contamination rates, die-off, and potential growth in onions due to drip or overhead application of contaminated irrigation water.**

Drip irrigation:

The 2022 field trial using contaminated water for drip irrigation delivered on average, 4000 MPN/100 mL over the course of a 0.40 acre-inch irrigation event. The 2022 irrigation was applied to a dry field and *E. coli* was not detected on any of the onions (0/40) immediately following the irrigation period (Figure 4A). After 24 hours, 13% (5/40) of these onions had detectable levels of *E. coli* ranging from 1.4 to 11 MPN/onion bulb (Figure 4B). *E. coli* was not detected on any of the onions tested on any of the subsequent sampling days (7, 15, 28) during the 2022 growing season.

For the 2023 field trial, the contamination level of the water was increased by a factor of nearly 100X (230,000 MPN/100 mL) to evaluate an extreme single contaminated irrigation event at the end of the growing season. This irrigation event also corresponded with a rare precipitation event in Pasco, WA (Figure 5B) and fields were already muddy when the water was applied. This combination of circumstances resulted in the immediate transfer and detection of *E. coli* on the onion bulbs, with 63% (25/40) of onions testing positive after the irrigation session (Figure 4A). This prevalence increased slightly (68%) at the 24-hour timepoint. *E. coli* levels on onions ranged from 1 MPN to 1100 MPN per onion bulb (Figure 4B). Prevalence decreased significantly after 7 days of field curing (18%; 7/40); however, the range of *E. coli* cell density on the bulbs remained consistent. At the end of field curing, a single onion (1/80) was confirmed to have a low level of surviving *E. coli* (2 MPN/onion).

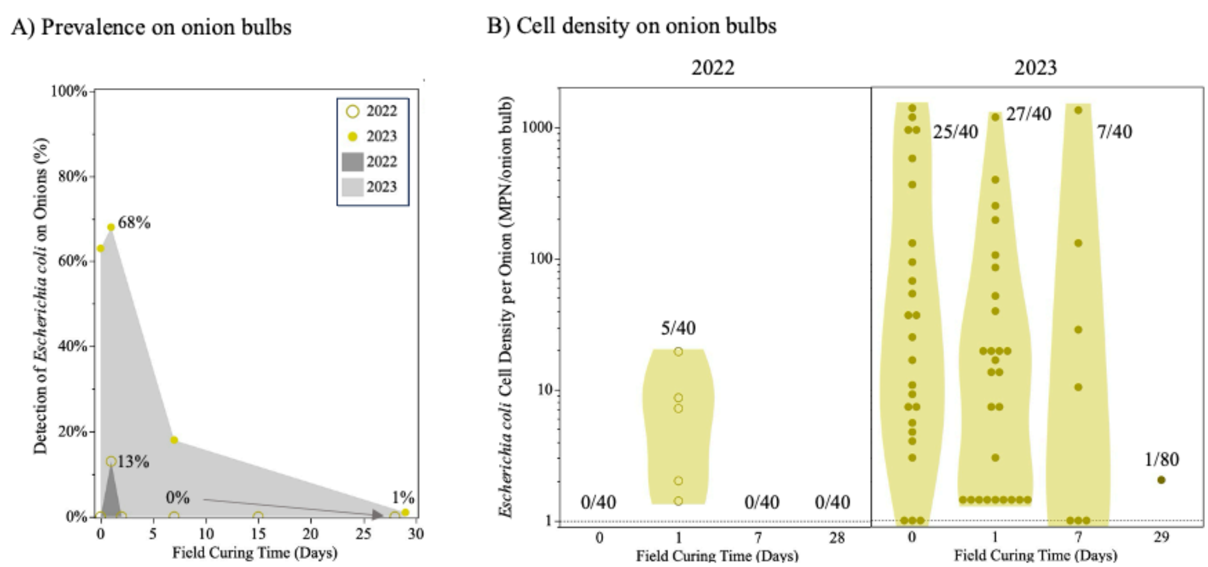
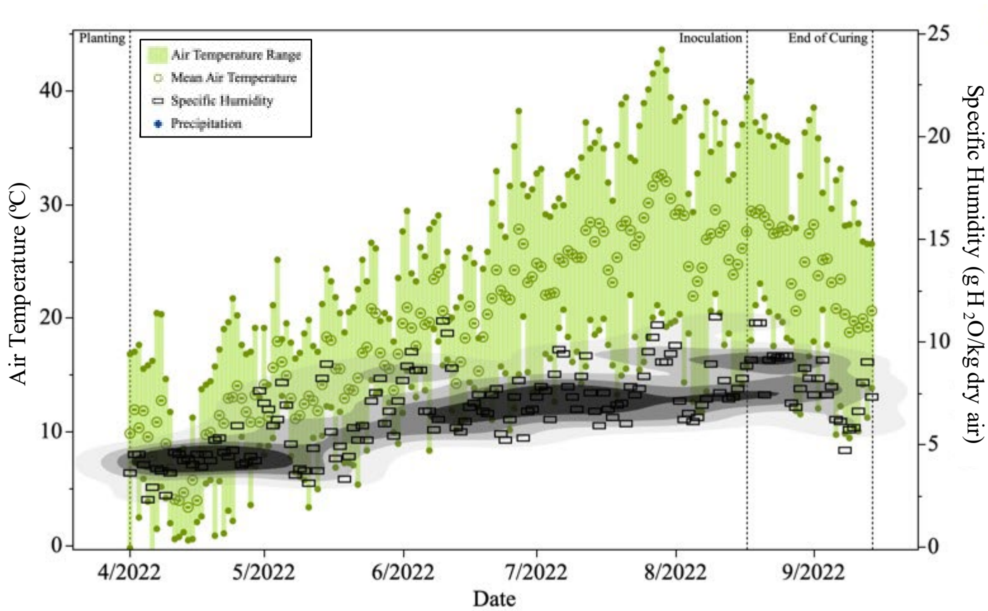


Figure 4. Contamination rates and survival of *Escherichia coli* on Calibra onion bulbs after drip irrigation (0.4 acre-inch) with inoculated water during 2022 and 2023 field trials in Pasco, Washington. Average estimated *E. coli* cell density in irrigation water was 4000 MPN/100 mL in 2022 and 230,000 MPN/100 mL in 2023. A) Percentage of onion bulbs with detectable (>1 MPN/bulb) levels of rifampicin-resistant *E. coli* through the field curing period following a single 0.4-acre-inch drip irrigation application (day 0). B) Cell density of *E. coli* recovered from onion bulbs throughout field curing during the 2022 and 2023 field trials. Dashed line indicates detection limit (1 MPN/onion bulb).

A) 2022 Field Trial



B) 2023 Field Trial

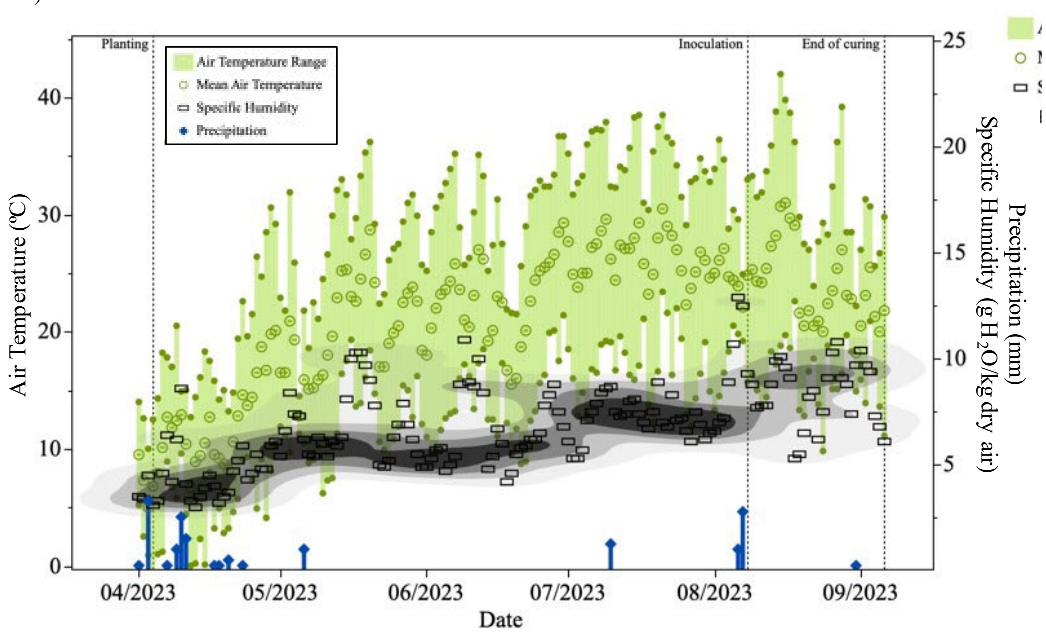


Figure 5. Air temperature (range and mean), specific humidity, and precipitation in Pasco, WA, during the onion growing and curing periods of the A) 2022 and B) 2023 field trials. Weather data was downloaded from Washington State University's AgWeatherNet for the Pasco, WA site location.

Overhead irrigation:

Contaminated overhead irrigation trials in 2022 and 2023 delivered approximately 1000 MPN/100 mL throughout a 0.4 acre-inch irrigation event. In both years, this resulted in the immediate contamination of nearly all (97.5%) of onions across all test plots (Figure 6A). Prevalence decreased significantly during the first 24 hours; however, the majority of onions remained positive. By day 7 of field curing, prevalence had reduced to 15% (6/40) in 2022 and 7% (11/160) in 2023 (Figure 6A). Average cell density on individual onions decreased significantly by day 7 in 2023 (Figure 6B). *E. coli* was not detected on any of the overhead irrigated onions (0/80) at the end of curing in 2022; however, 7 out of 320 onions (2%) were confirmed as positive for *E. coli* at the end of curing in 2023. There was no meaningful difference in *E. coli* contamination or survival between onion varieties.

The weather during the 2022 field trial was ‘typical’ for the region, with no detectable precipitation throughout the entire onion growing season (Figure 5A). The growing season of 2023 had many days with recordable precipitation, including immediately prior to the contaminated irrigation event as well as a small amount of precipitation late in the curing period (Figure 5B).

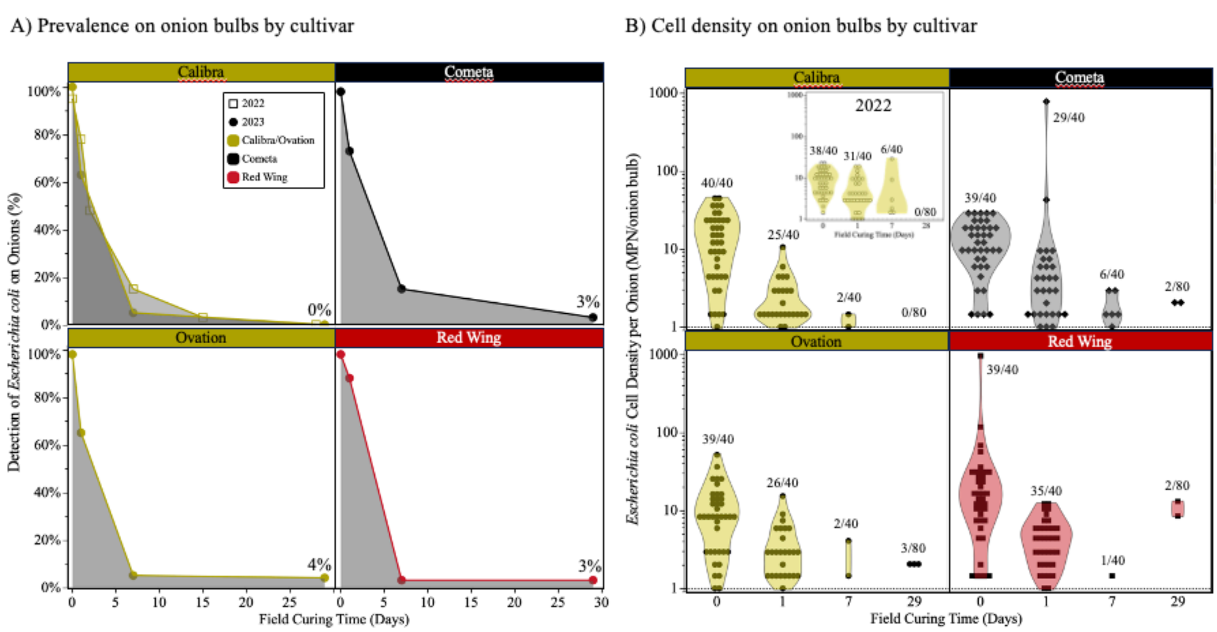


Figure 6. Contamination rates and survival of *Escherichia coli* on four cultivars (Calibra, Cometa, Ovation, and Red Wing) of onion bulbs after overhead irrigation (0.4 acre-inch) with inoculated water (1000 MPN/100 ml) during 2022 and 2023 field trials in Pasco, WA. A) Percentage of onion bulbs with detectable (>1 MPN/bulb) levels of rifampicin-resistant *E. coli* through the field curing period following a single 0.4-acre-inch overhead irrigation application (day 0). B) Cell density of *E. coli* recovered from onion bulbs throughout field curing during the 2023 trial.

Outcomes and Accomplishments

Project objectives and workplan were completed in alignment with the original proposal. The original proposal included the opportunity to adjust the study design in year 2. This adaptable approach was helpful in supporting the ability to investigate higher rates of contamination in drip irrigation as well as the expansion of varietal differences treated with contaminated overhead irrigation.

This project was a significant logistical undertaking to successfully execute concurrent field trials in two locations in different states as well as coordinating sample collection and analysis in tandem at both sites which were hundreds of miles from the host laboratory. This project was successful because of the strong relationships forged between the PIs, their respective teams, and their dedication to the onion industry as well as their belief in the importance of food safety. Ongoing and consistent communication across the different sites and within each team was critical to the success of this project. Support from the off-site co-PIs was also critical in making necessary decisions related to sample analysis and prioritization. It is estimated that 25 people provided critical labor and intellectual input to make this project a success along with countless support staff that assisted with logistics, transportation, lodging, ordering, inventory, accounting, etc.

Summary of Findings and Recommendations

Four field trials investigating three unique direct water application methods (overhead irrigation, drip irrigation, and crop protection sprays) provided further evidence of risk reduction during standard field curing practices used by onion growers in the western US. However, risk reduction does not equal risk elimination, as results also demonstrated that a small percentage of onions (<3%) could host a small number of viable *E. coli* (and by inference *Salmonella*) at the end of field curing. It is unknown whether this finding represents a reasonable risk, but this data does provide key information to support evidence-based decision-making for the industry.

Data from these field trials clearly demonstrated differences in contamination due to different types of water applications at moderately high contamination levels (3 log CFU/100 mL). Overhead irrigation with contaminated water led to the immediate contamination of nearly the entire crop (>97%). Crop protection spray applications resulted in immediate contamination of a majority of the crop (72% on average). Drip irrigation resulted in the lowest percentage of contaminated onions (13%).

E. coli was seemingly more stable on the onions during the 2023 field trials compared with 2022. The weather in these two growing regions tends to be consistently very dry; however, both locations experienced rare and significant precipitation at the end of the growing season and during field curing. The impact of these precipitation events cannot be confirmed as the reason for *E. coli*’s increased stability on the onions; however, this data prompts a consideration of these types of weather events on microbial survival.

APPENDICES

Publications and Presentations

Publications:

Two scientific manuscripts are nearing finalization and will be submitted for publication in peer-reviewed scientific journals in spring 2024. Drafts will be shared with CPS for review prior to submission.

Outreach article with project updates "*Produce Safety Research Update: Studying Pre-Harvest Contamination Risks and Managing Cross-Contamination During Post-Harvest Storage and Handling*" Onion World, May 2023.

Outreach article describing project published in October 2022 issue of Onion World titled "Field Trials Focus on Food Safety".

Supported the update and editing of the 2nd edition of "Commodity Specific Food Safety Guidelines for the Dry Bulb Onion Supply Chain" led by the National Onion Association and the International Fresh Produce Association.

Presentations:

- Virtual project site visit with Center for Produce Safety was completed on November 29, 2023.
- Graduate students Jason Racine and Sasha Nerney presented research progress update at Pacific Northwest Vegetable Association annual meeting in Kennewick, WA in November 2023.
- PI Waite-Cusic delivered research project presentation at University of Idaho virtual webinar series on April 3, 2023.
- PI Waite-Cusic delivered research project presentation at annual Western Regional Center to Enhance Food Safety in Prosser, WA on May 4, 2023.
- PI Waite-Cusic delivered poster and oral presentation at the annual Center for Produce Safety meeting in Buckhead, GA on June 20, 2023.
- PI Waite-Cusic and co-PI Harris presented a research progress update as part of Commodity Specific Food Safety Guidelines for the Dry Bulb Onion Industry Webinar hosted by the International Fresh Produce Association in October 2022.
- Graduate student Jason Racine presented research progress update at Pacific Northwest Vegetable Association annual meeting in Kennewick, WA in November 2022.
- Virtual project site visit with Center for Produce Safety was completed on December 2, 2022.
- PI Waite-Cusic presented a talk entitled "Update on Food Safety Concerns for Onions and the FSMA Water Rule" at the Idaho-Malheur County Growers Meeting on February 1, 2022 in Nampa, ID.

Budget Summary

Budget and expenditure summary is listed below based on broad categories. Overall, sufficient funds were available to complete the project; however, it did require close management and requests to pivot funds between salary, travel, and supply categories based on employee type and availability, travel/lodging needs to accommodate teams, and limited supply availability and inflationary impacts.

Total project budget: $388,905

Expenditures as of March 1, 2024: $380,664 (Salary/OPE: $196,371; Supplies: $106,608; Conferences: $1,225; Travel: $27,851; Subcontract [WSU]: $35,901; F&A Cost: $12,708)

Remaining balance: $8,241*
(*Remaining balance is allocated for travel to CPS annual meeting, publication fees, and associated F&A costs.)