CENTER for PRODUCE SAFETY

## CPS 2020 RFP
## FINAL PROJECT REPORT

### Project Title
Evaluating food safety challenges of blueberry harvesting

### Project Period
January 1, 2021 – December 31, 2022 (extended to January 31, 2023)


### Principal Investigator
Jinru Chen, PhD
University of Georgia
Department of Food Science and Technology
1109 Experiment St.
Griffin GA 30223-1797
T: 770-412-4738
E: jchen@uga.edu

### Co-Principal Investigator
Wei Qiang Yang, PhD
Oregon State University
North Willamette Research and Extension Center
Aurora, OR 97068
T: 971-801-0386
E: wei.yang@oregonstate.edu


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### Objectives

1. *Collect information about cleaning and sanitation practices for harvest containers and mechanical harvesters among blueberry growers/packers through an anonymous survey in several U.S. states.*
2. *Validate the efficacies of selected key cleaning and sanitation practices in decontaminating harvest containers and mechanical harvesters in the fields and/or packing facilities.*
3. *Evaluate, in a laboratory setting, whether identified key industry cleaning/sanitizing practices can effectively remove microbial buildups and biofilm mass on materials used to manufacture harvest containers and mechanical harvesters.*
4. *Transfer the knowledge gained from the project to berry growers/packers and promote best industry practices for broad adoption.*


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CPS Campaign for Research

## FINAL REPORT

### Abstract
As a superfood with many health benefits, fresh blueberries, like other fresh produce, can be a potential vehicle for transmitting gastrointestinal diseases and pose a risk to public health. We hypothesized before the project that blueberry growers/packers have adopted different practices to clean and sanitize harvesting containers, flats/crates, and machine harvesters, and these practices do not have equal efficacies in decontaminating harvesting equipment and utensils. This project collected information on the harvesting and sanitation practices currently used by blueberry growers/packers through an electronic survey in different states. Also, the efficacies of current industry clean/sanitation practices were verified by measuring the microbial load on the surface of blueberry harvest containers and machine harvesters before and after the cleaning/sanitation treatments. The knowledge gained from the project is being disseminated through local, regional, and international conferences.

### Background
Blueberry is recognized as a “superfood” that has been driving global demand. However, fresh blueberries, like any other fresh fruits and vegetables, can be a potential vehicle for transmitting gastrointestinal diseases and pose a risk to public health. Fresh fruits can become contaminated with pathogenic and spoilage microorganisms in the field, during harvesting, processing, distribution, storage, and preparation (Beuchat, 1996).

Mature blueberries are harvested by handpicking or mechanical harvesters (Quansah et al., 2019). Berries for the fresh market are usually picked by hand, while mechanically harvested berries are typically frozen or processed for year-round sale. Although processing and freezing preserve berry taste and nutritional value for a longer period, more than half of blueberries are packed for the fresh market due to high market value and consumer.

Berry growers often require hand pickers to wash their hands before entering the field. Although not required by the FDA, some berry customers/clients or third-party companies may require berry hand pickers wear gloves. Blueberry hand pickers usually place picked fruits into plastic buckets. Workers then take full plastic buckets to inspection/weighing stations at the edge of fields where blueberries are either packed directly into clamshells or dumped into flats/crates which are transported to a packinghouse.

Mechanical harvesting not only improves harvesting efficiency but also reduces microbial contamination of blueberries due to reduced contact with berry handlers. However, mechanical harvesting also has food safety concerns as bacterial cells from berries and their production environment can attach to the surface of mechanical harvesters (Mehra et al., 2013). Colonized microorganisms on harvester surfaces have the probability to survive and even form biofilms which are a collective of one or more species of microorganisms that can grow on many different surfaces. Microorganisms that can form biofilms include both spoilage and pathogenic bacteria, yeasts, and molds. Once microbial cells attach to and form biofilms on the fruit contact surface of a mechanical harvester, they are not easily removed (Gazula et al., 2019). According to Pagedar et al. (2010), the hydrophobic nature of some areas of mechanical harvesters may increase the possibility of biofilm retention. Biofilms formed over time are likely to assist bacterial cells to evade sanitizing treatment (Carmichael et al., 1998). Thus, establishing an effective routine to clean and sanitize mechanical harvesters is important to prevent biofilm development on berry contact surfaces of mechanical harvesters (Di Ciccio et al., 2015).

Stackable berry flats/lugs facilitate the effective transfer of harvested berries from the field to packinghouse operations. However, contaminated and unsanitary flats/lugs might be important sources of microbial contamination for blueberries for both fresh and processed markets. Information about how often the flats/lugs were washed, how they were washed, and whether cleaners or sanitizers were used during the washing process was not ascertained.

## Research Methods and Results

***Objective I.*** *Collect information about cleaning and sanitation practices for harvest containers and mechanical harvesters among blueberry growers/packers through an anonymous survey in several U.S. states.*

### Methods

*Survey and questionnaire*

A survey questionnaire with 22 questions was posted on a Google survey site. The survey link was circulated among blueberry growers in different states of the U.S. Responses to the survey questionnaire were summarized by the project team.

### Results

Over 70 respondents from 13 different states of the U.S. have responded to the cleaning and sanitation survey (**Figure 1**). Survey results revealed that about 3% of respondants had never sanitized their harvest containers, while 75% sanitized with varying frequencies (**Figure 2**). Similarly, about 2% of the survey respondents had never sanitized their machine harvesters, with the others either sanitized the harvesters after each use, once a day, or once a week (**Figure 3**).

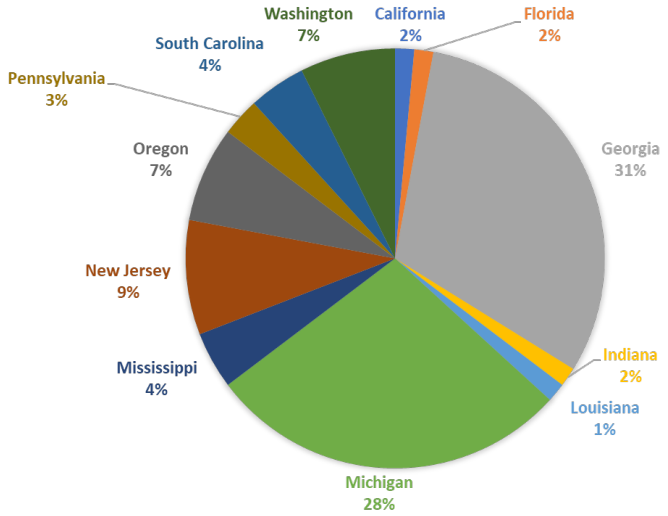


**Figure 1. Distribution of survey respondants**

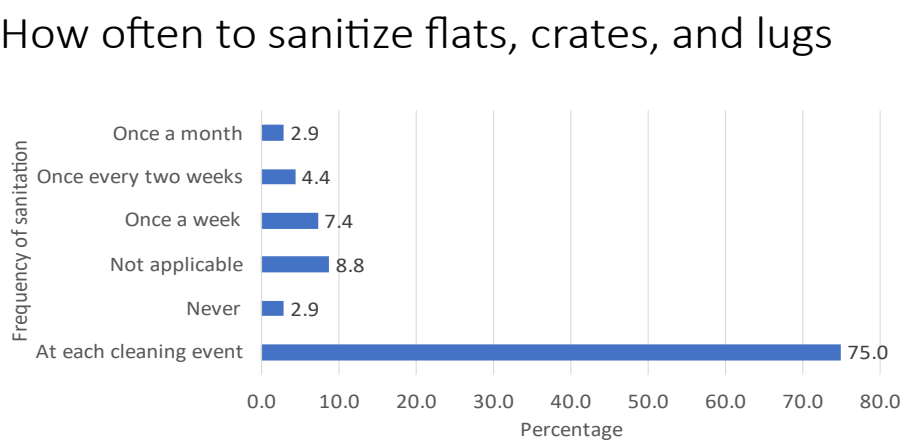


**Figure 2. Practices in sanitizing harvest containers**

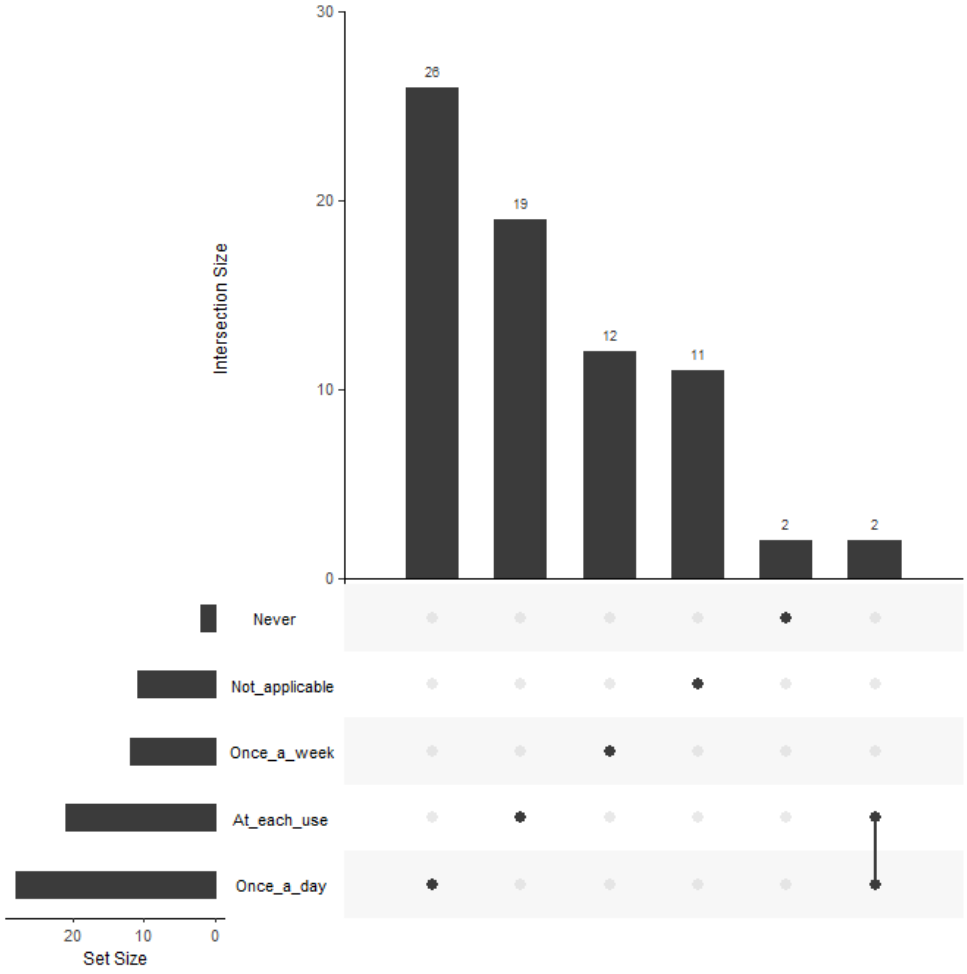


**Figure 3. Practices in sanitizing harvesters.**

***Objective II.*** *Validate the efficacies of selected key cleaning and sanitation practices in decontaminating harvest containers and mechanical harvesters in the fields and/or packing facilities.*

## Methods

*Harvest container sample collection*

Four fresh blueberry packing facilities, two in Georgia and two in Oregon, participated in the study. Each facility was randomly visited twice on two separate packing days during the 2021 harvest season. Ten independent swab samples (100 $cm^2$ each), each from used berry lugs, cleaned/sanitized lugs, used handpicking buckets, or cleaned/sanitized buckets were collected in Georgia (n = 160). In Oregon, swab samples of used and cleaned/sanitized flats were collected along with those from used and cleaned/sanitized picking buckets (n = 160). A sterile environmental sampling sponge (Nelson and Jamerson, Marshfield, WI) moistened with Dey-Engley neutralizing broth (Becton Dickinson, Sparks, MD) in a Whirl-Pak® bag (Nasco, Fort Atkinson, WI) was used to swab the 100 $cm^2$ area, using a template from 3M™ (St. Paul, MN), on the surface of berry harvesting containers with 10 horizontal and 10 vertical strikes.

*Machine harvester sample collection*

Four or six machine harvesters in Georgia and Oregon, respectively, were each sampled twice on two different harvest days in the summer of 2022. Areas within a 100 $cm^2$ window at nine different sites (upper and lower side walls, upper and lower beating bars, catcher plates, horizontal and vertical conveyors, lugs, and filling flap) of the top loaders (n = 9) were sampled, whereas seven different sites (excluding vertical conveyor and filling flap) of the bottom loaders (n = 2) were sampled.

*Sample handling and transportation*

Samples from irregular or non-flat surfaces were collected within the 100 $cm^2$ window using the same sampling technique. Detailed measurements of these surfaces were taken to calculate the actual surface areas swabbed. The swab samples were stored in portable coolers at 4°C after collection and during transportation. For samples collected in Oregon, sampling sponges were hand massaged for 1 min after the samples are collected, mixed with appropriate concentration of glycerol, and stored at -20°C before being transported by air to our laboratory in Griffin, Georgia in an insulated polystyrene foam container (Polar Tech 266C Thermo chill insulated carton with foam shipper, Genoa IL) with 5 lb. of dry ice.

*Microbiological analysis*

Sponge rinsates were thawed at 4°C before microbiological analysis. Counts of total aerobes (TA) and total coliforms (TC) were determined on tryptic soy agar and MacConkey agar, respectively, at 37°C for 24 h; those of total yeasts and molds (YM) on acidified potato dextrose agar (pH 3.5) at 25°C for 72 h. Presumptive colonies of fecal coliforms and enterococci were selected on MacConkey agar at 44.5°C and Enterococcus agar at 37°C with 24 h of incubation. Presumptive fecal coliform colonies were confirmed by growth on triple sugar iron slants at 37°C for 24 h, as well as in EC broth (bio-WORLD, Dublin, OH) with Durham Tube (6 x 50 mm, Kimble Chase®, Vineland, NJ) at 44.5°C for 48 h. Salt tolerance of enterococci was confirmed in brain heart infusion broth with 6.5% sodium chlorite at 37°C for 24 h.

*Statistical analysis*

Values of microbial counts from different production fields/packing facilities, from different types of harvesting containers or different machine harvesters, and at different sampling times (before and after cleaning and sanitation) were fit into a general linear model with a split-plot arrangement Statistical Analysis Software University Edition (SAS, Institute, Cary, NC). The analysis was performed using the Fisher's least significant difference test. The percentage of samples with confirmed fecal coliform and *Enterococcus* presence in the total number of samples collected from different sample sites, facilities, types of containers, and sampling times were calculated.

## Results

On average, the used lugs (UL) and used buckets (UB) from the two GA facilities had significantly ($P < 0.05$) higher mean TA and YM counts, but not TC counts, than their respective cleaned/sanitized lugs or buckets (i.e., CL or CB, respectively) (**Table 1**). The used picking buckets from the two OR facilities had significantly higher mean TA and YM, as well as TC counts, than the cleaned buckets (**Table 2**); however, the used flats (UF) only had significantly higher YM counts than the cleaned flats (CF). About 1.3% of GA samples tested positive for fecal coliforms and 3.8% of the samples tested positive for enterococci (**Table 3**). For samples from OR, 3.8% were positive for fecal coliforms and 10.6% were positive for enterococci.

Results of the harvester surface survey revealed that the horizontal and vertical conveyors, as well as the catch plates, carried significantly higher microbial loads. About 7.8% of surface samples collected in GA tested positive for fecal coliforms and 14.1% were positive for enterococci (**Table 4**). Among samples collected in OR, 5.6% were positive for fecal coliforms and 10.2% were positive for enterococci.

**Table 1** Mean populations of total aerobes, total yeasts and molds, and total coliforms from samples collected from different types of harvest containers during two visits to individual fresh blueberry facilities in Georgia.

| | | Total aerobes | Total yeasts and molds | Total coliforms |
|---|---|---|---|---|
| | | Log CFU/cm$^2$ | | |
| Facility | G1 (n = 60) | 2.39$^A$ | 2.56$^A$ | 0.17$^A$ |
| | G2 (n = 100) | 1.40$^B$ | 1.57$^B$ | 0.14$^A$ |
| Container | UB (n = 40) | 1.76$^B$ | 2.67$^A$ | 0.10$^A$ |
| | CB (n = 40) | 1.08$^C$ | 0.67$^C$ | 0.12$^A$ |
| | UL (n = 40) | 2.42$^A$ | 2.62$^A$ | 0.15$^A$ |
| | CL (n = 40) | 1.88$^B$ | 1.80$^B$ | 0.24$^A$ |
| Visit | One (n = 80) | 2.20$^A$ | 2.14$^A$ | 0.22$^A$ |
| | Two (n = 80) | 1.34$^B$ | 1.74$^B$ | 0.08$^A$ |

**Table 2** Mean populations of total aerobes, total yeasts and molds, and total coliforms from samples collected from different types of harvest containers during two visits to individual fresh blueberry facilities in Oregon.

| | | Total aerobes | Total yeasts and molds | Total coliforms |
|---|---|---|---|---|
| | | | Log CFU/cm$^2$ | |
| Facility | O1 (n = 80) | 1.93$^{A}$ | 2.21$^{A}$ | 0.09$^{A}$ |
| | O2 (n = 80) | 1.27$^{B}$ | 1.88$^{B}$ | 0.07$^{A}$ |
| Container | UB (n = 40) | 2.22$^{A}$ | 3.06$^{A}$ | 0.205$^{A}$ |
| | CB (n = 40) | 1.54$^{B}$ | 1.17$^{D}$ | 0.04$^{B}$ |
| | UF (n = 40) | 1.35$^{BC}$ | 2.52$^{B}$ | <0.06$^{B}$ |
| | CF (n = 40) | 1.28$^{C}$ | 1.42$^{C}$ | 0.06$^{B}$ |
| Visit | One (n = 80) | 1.81$^{A}$ | 2.25$^{A}$ | 0.09$^{A}$ |
| | Two (n = 80) | 1.39$^{B}$ | 1.83$^{B}$ | 0.07$^{A}$ |

**Table 3** Number and percentage of samples positive to total coliforms, fecal coliforms, and enterococci from harvester containers.

| | Total coliforms | | | Fecal coliforms | | | Enterococci | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. of positive | Sample size | % positive | No. of positive | Sample size | % positive | No. of positive | Sample size | % positive |
| **Facility in GA** | | | | | | | | | |
| G1 | 5 | 40 | 12.5 | 1 | 40 | 2.5 | 1 | 40 | 2.5 |
| G2 | 5 | 120 | 4.2 | 1 | 120 | 0.8 | 5 | 120 | 4.2 |
| Total | 10 | 160 | 6.3 | 2 | 160 | 1.3 | 6 | 160 | 3.8 |
| **Facility in OR** | | | | | | | | | |
| O1 | 13 | 80 | 16.3 | 5 | 80 | 6.3 | 12 | 80 | 15.0 |
| O2 | 11 | 80 | 13.8 | 1 | 80 | 1.3 | 5 | 80 | 6.3 |
| Total | 24 | 160 | 15.0 | 6 | 160 | 3.8 | 17 | 160 | 10.6 |

**Table 4** Number and percentage of samples positive to total coliforms, fecal coliforms, and enterococci from machine harvesters.

| Source | Total coliforms | | | Fecal coliform | | | Enterococci | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. of positive | Sample size | % positive | No of positive | Sample size | % positive | No. of positive | Sample size | % positive |
| **Facilities in GA** | | | | | | | | | |
| Farm 1 | 29 | 72 | 40.3 | 6 | 72 | 8.3 | 10 | 72 | 13.9 |
| Farm 2 | 14 | 56 | 25.0 | 4 | 56 | 7.1 | 8 | 56 | 14.3 |
| Total | 43 | 128 | 33.6 | 10 | 128 | 7.8 | 18 | 128 | 14.1 |
| **Facilities in OR** | | | | | | | | | |
| Farm 1 | 17 | 108 | 15.7 | 9 | 108 | 8.3 | 15 | 108 | 13.9 |
| Farm 2 | 17 | 108 | 15.7 | 3 | 108 | 2.8 | 7 | 108 | 6.5 |
| Total | 34 | 216 | 15.7 | 12 | 216 | 5.6 | 22 | 216 | 10.2 |

***Objective III.*** *Evaluate, in a laboratory setting, whether identified key industry cleaning/ sanitizing practices can effectively remove microbial buildups and biofilm mass on materials used to manufacture harvest containers and mechanical harvesters.*

## Methods

Different colors (orange, ivory, red, medium blue, royal blue, green, or yellow-colored) of high-density polyethylene, the material used to manufacture blueberry harvest containers and picking buckets, were tested for their ability in supporting bacterial accumulation and biofilm formation by 5 different groups of *E. coli* strains isolated from fruits, blueberry packing lines, or machine harvesters. Furthermore, the efficacies of cleaning/sanitizing treatments (using soap, sodium hypochlorite, or water; with soaking or no soaking; with manual or machine washing) in removing the biofilms on selected materials were determined.

## Results

Results of the biofilm formation assay showed that the color of HPDE coupons did not significantly ($P > 0.05$) affect microbial buildups and the amount of biofilm mass accumulated on bacterial contact surfaces (**Table 5**). Results of the sanitation study showed that soaked coupons had less biofilm mass than unsoaked coupons; simulated manual washing removed less biofilm mass than simulated machine washing; treatment with hand soap removed more biofilms than the treatment with sodium hypochlorite.

**Table 5** Biofilm removal from HPDE coupons using sanitizing treatments

| | OD 550 nm |
|---|---|
| Soaking | 0.28991A |
| No soaking | 0.17992B |
| | |
| Manual | 0.27723A |
| Machine | 0.19259B |
| | |
| 100 ppm NaOCl | 0.25851A |
| Water | 0.22481AB |
| 2% soap | 0.22142B |

***Objective IV.*** *Transfer the knowledge gained from the project to berry growers/packers and promote best industry practices for broad adoption.*

One manuscript has been published in a peer-reviewed journal, and three poster presentations have been made at the annual meeting of professional conferences.

## Outcomes and Accomplishments

The research project had at least two distinct, measurable and quantifiable outcomes:

Outcome 1. Increased awareness of effective cleaning and sanitation practices for harvesting containers and equipment
**Goal**: Fill the knowledge gap on the practices of blueberry growers/packers in cleaning and sanitation of harvesting containers and mechanical harvesters
**Performance measure**: Complete a practice survey among blueberry growers/packers
**Activity**: Conduct the practice survey in four different states of the U.S. – Note: Project survey responses were obtained from 13 different states.
**Quantifiable outcome**: At least 120–160 responses to survey questionnaire – Note: Project survey received over 70 responses.

Outcome 2. Improved hygiene conditions of blueberry harvesting containers and equipment
**Goal**: Promote effective cleaning and sanitation practices for harvesting containers and equipment among blueberry growers/packers
**Performance measure**: Complete validation studies for selected cleaning and sanitation practices currently used by blueberry growers/packers in the fields/packing facilities
**Activity**: Evaluate the efficacies of the cleaning and sanitation treatment
**Quantifiable outcome**: Improved hygiene conditions of harvesting containers and mechanical harvesters as reflected by the differences in microbial loads before and after the cleaning and sanitation treatments

## Summary of Findings and Recommendations

The study collected information on growers' practices in cleaning and sanitizing blueberry harvest containers and machine harvesters. Results of the validation survey suggest that the current cleaning and sanitation practices are largely working, but there is space for further improvement. A multilingual education program based on the results of this study would help improve the food safety knowledge of personnel working in the blueberry field and packing facilities.

Growers are transitioning to the use of modified machine harvesters for harvesting fresh market blueberries due to labor and production constrains. Machine harvester manufacturers are using soft elastic polymer sheets to cover hard surfaces such as catch plates on the machine to reduce physical damage to harvested fruits. These polymer sheets are hydrophobic and likely encourage a higher level of microbial buildup on machine surfaces. This should be a potential food safety issue for the blueberry harvesting.

## APPENDICES

### Publications and Presentations

### Publication

Dai, Y., R. Holland, S. Doane, W. Q. Yang, and J. Chen. 2023. Hygiene status of blueberry harvest containers cleaned and sanitized with various approaches. *Food Bioscience* 52:102434. https://doi.org/10.1016/j.fbio.2023.102434

### Presentations

Dai, Y., R. Holland, S. Doane, W.-Q. Yang, and J. Chen. 2022. Evaluating food safety challenges of blueberry harvesting. Southeast Regional Fruit and Vegetable Conference Abstr. Book. 6-9 January, Savannah, GA.

Dai, Y., R. Holland, S. Doane, W.-Q. Yang, and J. Chen. 2022. Efficacies of cleaning and sanitizing treatments for blueberry harvest containers. Int. Assn. Food Prot. Annu. Mtg. Prog. Abstr. Book. 31 July – 3 August, Pittsburgh, PA. Poster no. P2-173.

Dai, Y., R. Holland, S. Doane, W.-Q. Yang, and J. Chen. 2023. Efficacies of cleaning and sanitizing treatments for blueberry harvest containers. Southeast Regional Fruit and Vegetable Conference Abstr. Book. 5-8 January, Savannah, GA.

### Budget Summary

This project was awarded $196,435 in research funds, and the majority of funds were spent. Remaining funds will be used for travel to the June 2023 CPS Research Symposium.

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Quansah, J., H. Gazula, R. Allen, H. Scherm, C. Li, F. Takeda, and J. Chen. 2019. Microbial quality of blueberries for the fresh market. Food Control 100:92-96. https://doi.org/10.1016/j.foodcont.2018.12.034