CENTER for PRODUCE SAFETY

CPS 2016 RFP
FINAL PROJECT REPORT

Project Title
Listeria monocytogenes growth and survival on peaches and nectarines as influenced by stone fruit packing house operations, storage and transportation conditions

Project Period
January 1, 2017 – December 31, 2017

Principal Investigator
Mary Anne Amalaradjou
University of Connecticut
Department of Animal Science
U4040, 3636 Horsebarn Road Extn.
Storrs, CT 06269
T: 860-486-6620
E: mary_anne.amalaradjou@uconn.edu

Objectives
To investigate the survival and growth of Listeria monocytogenes at high (5 log CFU/fruit) and low inoculation (3 log CFU/fruit) levels on yellow flesh peaches (var. Autumn Flame) and nectarines (var. August Fire) as influenced by stone fruit post-harvest processing, cooling, storage and transportation conditions:

1. *Evaluate the effect of unloading and staging conditions at the stone fruit packing facility (18–20 or 28–30°C [ambient cool and warm season temperatures], 40–50% RH [ambient], 1–18 h storage).*
2. *Evaluate the effect of fruit waxing (mineral oil– and vegetable oil–based fruit finish) and fungicide application (Fludioxonil and Propiconazole) at the stone fruit packing facility (18–20 or 28–30°C [ambient cool and warm season temperatures], 40–50% RH [ambient], 1–6 h storage).*
3. *Evaluate the effect of cooling, storage, and transportation conditions (1–2°C, 85–95% RH, 1–4 weeks storage) at the packing facility.*

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FINAL REPORT

Abstract

The recent recall of stone fruits (peaches, nectarines, plums, and pluots) due to potential *Listeria monocytogenes* contamination, and the isolation of highly related *Listeria* isolates from human subjects highlight the risk for pathogen transmission through stone fruit consumption. Pathogen presence on the fruit surface indicates that inadequate hygienic conditions were employed during harvesting, processing, storage and transportation. Additionally, surface contamination of fruits also provides a risk potential for cross-contamination of the product. These factors highlight the need for quality control in stone fruits intended for fresh consumption. To develop effective food safety practices, it is essential to determine the critical factors during stone fruit processing that influence *Listeria* growth and survival. Therefore, this study evaluated the ability of *Listeria* to persist on peaches and nectarines under simulated stone fruit processing conditions. The survival and growth of *Listeria monocytogenes* was investigated at high (5 log CFU/fruit) and low (3 log CFU/fruit) inoculation levels on yellow flesh peach and nectarine varieties, as influenced by stone fruit loading and staging, waxing and fungicide application, and cooling, storage and transportation conditions. The study results indicated that current stone fruit handling conditions do not favor *Listeria* growth. However, once fruit is contaminated, *Listeria* can survive on the fruit surface in significant numbers under current processing conditions. It is expected that elucidation of *Listeria* behavior under stone fruit processing conditions will help identify processes within the packinghouse continuum that can be targeted towards the development of comprehensive preventive controls for foodborne pathogens, including *Listeria monocytogenes*.

Background

Over the last decade, inclusion of fresh produce in the American diet has been steadily increasing due to heightened consumer awareness of the associated health benefits. Unfortunately, this increase in fresh produce consumption has been associated with a concomitant increase in fresh produce–associated foodborne outbreaks. In this regard, fruits including cantaloupe and other melons have been implicated in listeriosis outbreaks. More specifically, the recent recall of stone fruits (peaches, nectarines, plums and pluots) due to potential *Listeria monocytogenes* contamination, and the isolation of highly related *Listeria* isolates from human subjects highlight the risk for pathogen transmission through stone fruit consumption. Pathogen presence on the fruit surface indicates that inadequate hygienic conditions were employed during harvesting, processing, storage and transportation. Additionally, surface contamination of fruits also provides a risk potential for cross-contamination of the product. This highlights the need for quality control in stone fruits intended for fresh consumption. To develop effective food safety practices, it is essential to determine the critical factors during stone fruit processing that influence *Listeria* growth and survival.

Research Methods

The overall objective of this proposal was to investigate the survival and growth of *Listeria monocytogenes* at high (5 log CFU/fruit) and low (3 log CFU/fruit) inoculation levels on yellow flesh peach and nectarine varieties as influenced by stone fruit post-harvest processing, cooling, storage and transportation conditions. In order to simulate post-harvest handling of stone fruits, *Listeria* growth and survival on peach and nectarine varieties was evaluated at three different steps in the process: unloading and staging conditions at the stone fruit packing

facility (Objective 1); fruit waxing and fungicide application at the stone fruit packing facility (Objective 2); and cooling, storage and transportation conditions at the packing facility (Objective 3). The temperature, relative humidity, and time factors to be used in the study were determined based on the *Food Safety Guidelines for Fresh, Whole Stone Fruit Produced in California's San Joaquin Valley* (CFFA, 2015) and discussions with the California Fresh Fruit Association.

Peaches and nectarines: Unripened, unwaxed fruits (yellow flesh peaches, var. Autumn Flame; nectarines, var. August Fire) were procured immediately after harvest from Gerawan Farming (Reedley, California). Upon receipt, fruits were visually inspected for defects (bruises, moldy growth, breaks in peel), and any defective fruit was discarded. All fruits were maintained at 4°C with 90% relative humidity (RH) until use. A day before each experiment, the required number of fruits were transferred to room temperature (20 or 30°C) for tempering prior to use.

Fruit inoculation: A five-strain cocktail of *L. monocytogenes* consisting of produce isolates (LM1, LM2, LM3 – apple isolates) and human isolates (Scott A, LM 19115) was used for the study. Each strain was cultured separately in 10 ml of sterile brain heart infusion (BHI) broth and nalidixic acid (NA; 50 µg/ml) at 37°C for 24 h with agitation (100 rpm). Cultures were transferred for two consecutive 24-h periods onto BHI agar plates containing NA to produce a bacterial lawn. To prepare the inoculum, growth from the bacterial lawn was transferred to 0.1% buffered peptone water (BPW) to an absorbance of 0.2%. The approximate bacterial count in each culture was determined spectrophotometrically. Equal portions from each of the five strains were combined to make the pathogen cocktail. The bacterial population in the five-strain mixture was determined by plating 0.1-ml portions of appropriate dilutions on modified Oxford media with NA, followed by incubation at 37°C for 48 h. Appropriate dilutions of the five-strain mixture in BPW was used to obtain the desired level of inoculum. Fruits were individually spot-inoculated with the bacterial cocktail (7 log or 5 log CFU/fruit) by placing 50 µl around the stem end. After inoculation, fruits were held for 24 h at room temperature in a biosafety hood to allow the inoculum to dry.

Objective 1. Evaluate the effect of unloading and staging conditions at the packing facility: Following *Listeria* inoculation and drying, stone fruits were placed in sterile bins and stored at 18–20 or 28–30°C (ambient cool and warm season temperatures) and 40–50% RH (ambient humidity) for 1 to 18 h to simulate stone fruit handling during transportation to and staging at the packing facility. At designated times during the storage (0, 2, 6, 12, 18 h) fruits were sampled for microbiological analysis, and the entire experiment was repeated three times (total of 12 peaches per time point for each treatment). The peaches were individually transferred to sterile stomacher bags containing 100 ml of BPW, each fruit was hand rubbed for 2 min, and BPW was processed as described below. A similar experimental set-up was used to evaluate *Listeria* persistence on nectarines.

Objective 2. Evaluate the effect of fruit waxing and fungicide application at the packing facility: Peaches or nectarines were inoculated and dried before they were used to investigate the effect of mineral oil–based (PrimaFresh®220) and vegetable oil–based (PrimaFresh®55EU) fruit finish containing fungicides (Fludioxonil [PacRite®FDL] or Propiconazole [Mentor®]) on *Listeria* survival. The two fungicides were prepared according to manufacturer instructions for fruit spraying: 4 oz. per 100 gallons for Propiconazole, and 16 fl. oz. per 100 gallons for Fludioxonil. Inoculated fruits were sprayed with one of the four different wax-fungicide combinations using a gravity-feed dual action air-nozzle sprayer at 28–30°C or 18–20°C (ambient warm and cool season temperatures) and 40–50% RH (ambient humidity). Each fruit was sprayed with one pull each to the stem and calyx ends, and three pulls to coat the rest of the fruit surface. Following

waxing, fruits were packed in cardboard fruit boxes to simulate packinghouse practices, and then held at 28–30°C or 18–20°C and 40–50% RH for up to 6 h. Surviving *Listeria* populations on peaches (n = 12/treatment/time for each variety) were enumerated during fruit holding. A similar experimental set-up was employed to evaluate *Listeria* persistence on nectarines.

Objective 3. Evaluate the effect of cooling, storage and distribution conditions: Following inoculation and waxing (as described above), peaches/nectarines were placed into sterile cardboard boxes that were then cooled and stored at 1–2°C and 85–90% RH for up to 4 weeks to simulate packing, storage, and distribution conditions. Surviving *Listeria* populations on fruits (n = 12/treatment/time for each variety) were enumerated at different times during the four-week storage period (0, 1, 5, 7, 14, 21, 28 d). A similar experimental set-up was employed to enumerate *Listeria* populations on nectarines.

Research Results

Standardization of inoculum drying time on fruits:
Peaches and nectarines (n = 15, 3/trial) were spot inoculated with 7 or 5 log CFU/fruit by placing 50 µl of a five-strain mix around the stem end. After inoculation, fruits were held at room temperature in a biosafety hood for 1–24 h to allow for the inoculum to dry. Following drying, *Listeria* populations on inoculated fruits were enumerated at different times to ascertain initial pathogen load and optimum drying times. Results of this experiment demonstrated that approximately 5.2 and 3.1 log CFU of *Listeria* was recovered from the peach surface up until 24 h following fruit inoculation and drying. Similar results were obtained with nectarines. Hence, for all experiments in objectives 1–3, fruits were dried for 24 h following surface inoculation.

Objective 1. The effect of unloading and staging conditions at the stone fruit packing facility:
As shown in Fig. 1 (peaches) and Fig. 2 (nectarines), holding of artificially contaminated fruit under the simulated stone fruit unloading and staging conditions had no significant effect on *Listeria* survival. More specifically, both at warm and cool ambient temperatures, no significant reduction in *Listeria* population was observed, with approximately 5.7 and 3.8 log CFU/fruit being recovered by the end of the storage period (18 h) for the high and low inoculum, respectively (Fig. 1). Similar results were obtained with experiments performed on nectarines. Irrespective of the holding temperature, RH and inoculum load, *Listeria* was able to survive equally well on peaches and nectarines. However, these holding conditions did not favor any significant increase in pathogen populations.

Objective 2. The effect of fruit waxing and fungicide application at the stone fruit packing facility:
To simulate waxing and fungicide application at the stone fruit packinghouse, two commonly used waxes, namely PF220 (mineral oil–based) and PF55EU (vegetable oil–based), were employed in combination with two widely used fungicides, namely Propiconazole and Fludioxonil. Following inoculation, drying and wax-fungicide application, fruits were stored at ambient temperature and humidity for 6 h. As observed with objective 1, irrespective of the type of wax and fungicide applied, *Listeria* was able to survive in significant numbers on peaches (Fig. 3, 4) and nectarines (Fig. 5, 6). More specifically, no significant reductions in pathogen populations were observed throughout the duration of holding. Further, *Listeria* survival was observed with both high and low inoculum on fruits. Moreover, environmental conditions (ambient warm vs cool temperature) did not deter *Listeria* survival. Approximately 5.4–5.6 and 3.3–3.7 log CFU of *Listeria* were recovered from peaches and nectarines at warm and cool temperatures at the end of the holding period. Although no reductions in *Listeria* populations were observed, it is critical to note that stone fruit waxing and fungicide application under

ambient warm and cool temperatures did not favor *Listeria* growth, as evidenced by no increase in pathogen population.

Objective 3. The effect of cooling, storage and distribution conditions:
Following fungicidal treatment and waxing, both peaches and nectarines were packed in cardboard boxes and stored under 1–2°C, as in the packinghouse. As previously identified by several researchers, *Listeria* is a psychrotropic bacteria capable of surviving under low temperatures. In corroboration with these findings, results obtained from objective 3 demonstrate the ability of *Listeria* to survive on fruits held at 1–2°C. Over the 4-week storage period, irrespective of the inoculum load and wax-fungicide treatment, no significant reductions in *Listeria* populations were observed on either peaches or nectarines. At the end of storage (4 weeks) approximately 5.3–5.5 and 3.5–3.9 log CFU of *Listeria* were recovered from treated peaches and nectarines at high and low inoculum level, respectively (Fig. 7, 8). This result indicates that although low temperature may deter pathogen growth, it does not inhibit *Listeria* survival and persistence on peaches and nectarines.

Outcomes and Accomplishments

The absence of practical technologies that provide a necessary kill step for pathogens on fresh produce such as peaches and nectarines provides a unique challenge to the stone fruit industry. Furthermore, the recent recall of stone fruits due to potential *Listeria* contamination has highlighted the need to generate risk reduction knowledge towards the development of preventive controls for foodborne pathogens. In order to develop such preventive controls, it is critical to understand the effect of stone fruit processing and transportation conditions on pathogen growth and survival. This comprehensive investigation was undertaken to understand the influence of various stone fruit processing conditions on *Listeria* survival on peaches and nectarines. Since environmental conditions including ambient temperature and humidity, and packing practices such as waxing and fungicide application, and storage influence pathogen survival, a combination of these factors relevant to the stone fruit packing industry were simulated in the present study. Results of the study indicated that current stone fruit handling and storage practices do not favor *Listeria* growth; however, these conditions do not deter *Listeria* survival. Therefore, under current stone fruit handling and packing conditions, *Listeria* can survive in significant numbers on peaches and nectarines and thereby compromise the microbiological safety of these stone fruits. Further, the results indicated that commercial waxes and fungicides employed in the stone fruit industry do not exhibit any inhibitory effect on *Listeria* survival. In conclusion, the findings of this study demonstrate the need for development and implementation of preventive controls at the stone fruit packinghouse to prevent *Listeria* contamination and deter pathogen persistance.

Summary of Findings and Recommendations

1. Currently employed stone fruit handling and storage conditions at the packinghouse did not favor or support *Listeria* growth. No increase in *Listeria* populations was observed under any of the three stages simulated in the study.
2. *Listeria* can survive on peaches and nectarines in significant numbers during stone fruit staging, waxing and fungicide application, and cold storage for extended periods of time.
3. Initial pathogen load (population during contamination) had no effect on pathogen survival. Significant *Listeria* populations were recovered from fruit surfaces at both high and low inoculum levels under all three objectives.

4. The most commonly employed fungicide and wax treatments did not exert any inhibitory effect on *Listeria* survival on peaches and nectarines.
5. Current stone fruit storage and transportation conditions did not deter pathogen survival or persistence (no reduction in *Listeria* population was observed).

Recommendations

Results from this study provide a comprehensive understanding of how various stone fruit processing conditions influence *Listeria* survival and growth. Specifically:

- None of the three stages—namely staging, waxing-fungicide application, and cold storage—support or favor *Listeria* growth on stone fruits.
- Although there was no growth, *Listeria* survived in significant numbers on peaches and nectarines under these packinghouse conditions.
- To promote the microbial safety of stone fruits, it is crucial to prevent fruit contamination that can occur anywhere from harvesting to packing and storage.
- In addition to preventing contamination, there is a need to incorporate antimicrobial interventions to inhibit *Listeria monocytogenes* survival at each stage of the packing process.

Collaboration and support: We appreciate the support from CFFA and Gerawan Farming Inc. (George Nikolich) for hosting the PI and for helping us understand the stone fruit production and packing process. We also thank George Nikolich, Gerawan Farming, for providing the fruits and for coordinating fruit procurements throughout the study. We thank Ms. Michelle Smith, Pace International, for providing us with the fungicides and wax coatings used in the study.

APPENDICES

Publications and Presentations

Presentations:

Amalaradjou MA. 2017. *Listeria monocytogenes* growth and survival on peaches and nectarines as influenced by stone fruit packinghouse operations, storage and transportation conditions. CPS Research Symposium, Denver, CO, June 20–21.

Kuttappan D, M Muyyarikkandy, MA Amalaradjou. 2017. Influence of commercial stone fruit processing conditions on *Listeria monocytogenes* persistence on peaches and nectarines. Poster presented at New England Vegetable & Fruit Conference, Manchester, NH, Dec 12–14.

Kuttappan D, M Muyyarikkandy, MA Amalaradjou. *Listeria monocytogenes* persistence on peaches and nectarines under commercial stone fruit processing conditions. *Abstract submitted for* IFT 2018 Annual Meeting & Food Expo, Chicago, IL, July 15–18, 2018.

Publications:

We anticipate submitting a paper on the effect of commercial stone fruit packinghouse conditions on *Listeria* survival on peaches and nectarines for publication in a peer-reviewed journal by December 2018.

Budget Summary

Funds utilized:	
Personnel (Salaries and Fringe benefits):	27,136.22
Supplies:	48,388.92
Travel:	2,103.76
Other costs:	6,666.02
Indirect costs:	2,028.18
Total Expenses:	$86,323.10

Remaining project funds will be used for travel and publication costs in year 2.

Figures 1–8

Fig. 1

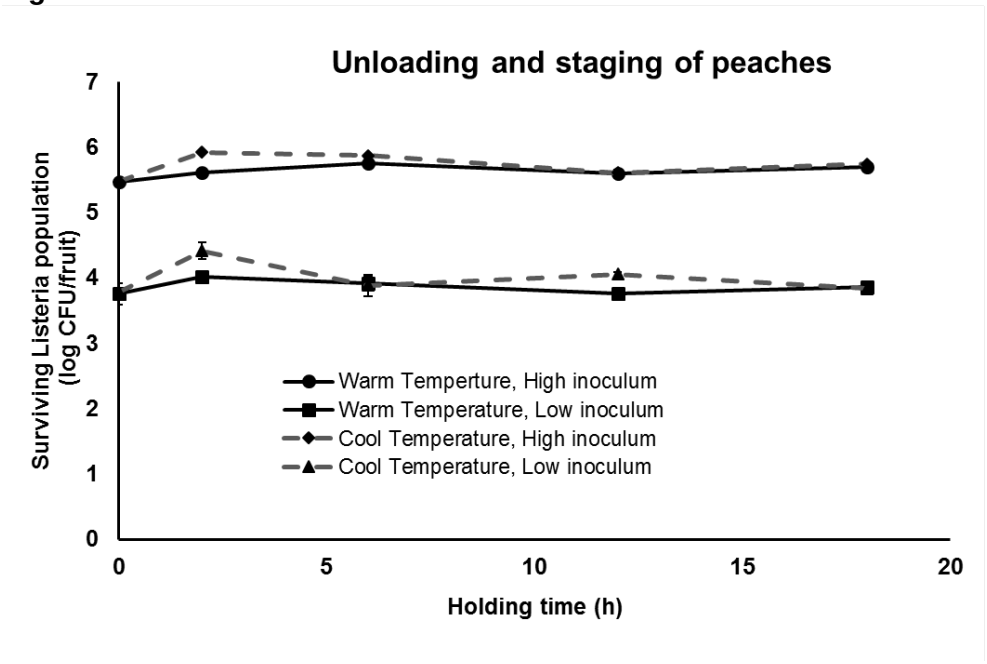


Fig. 2

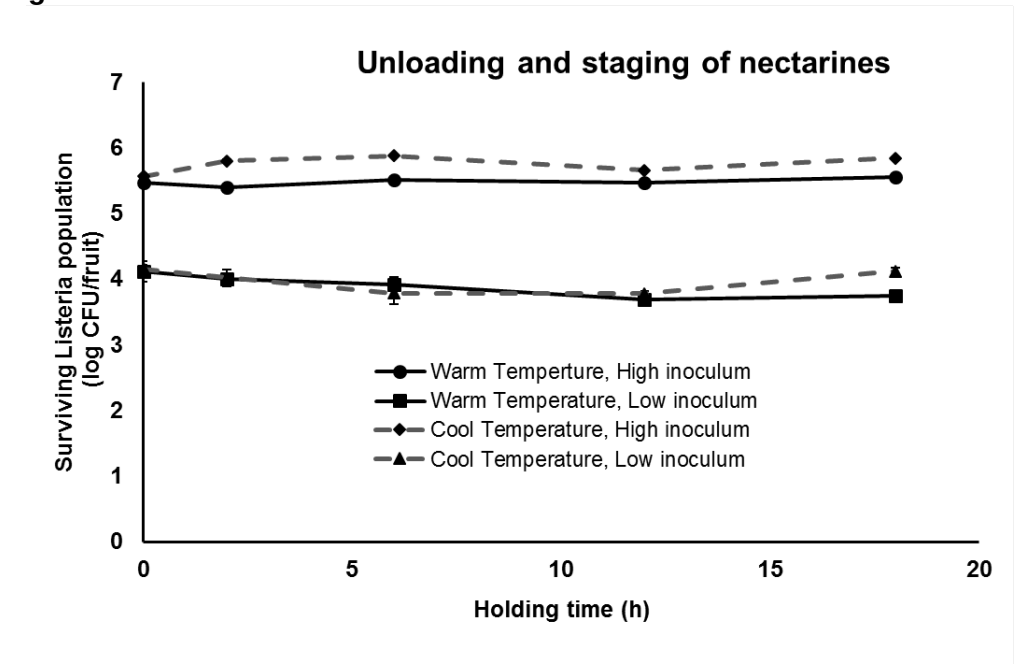
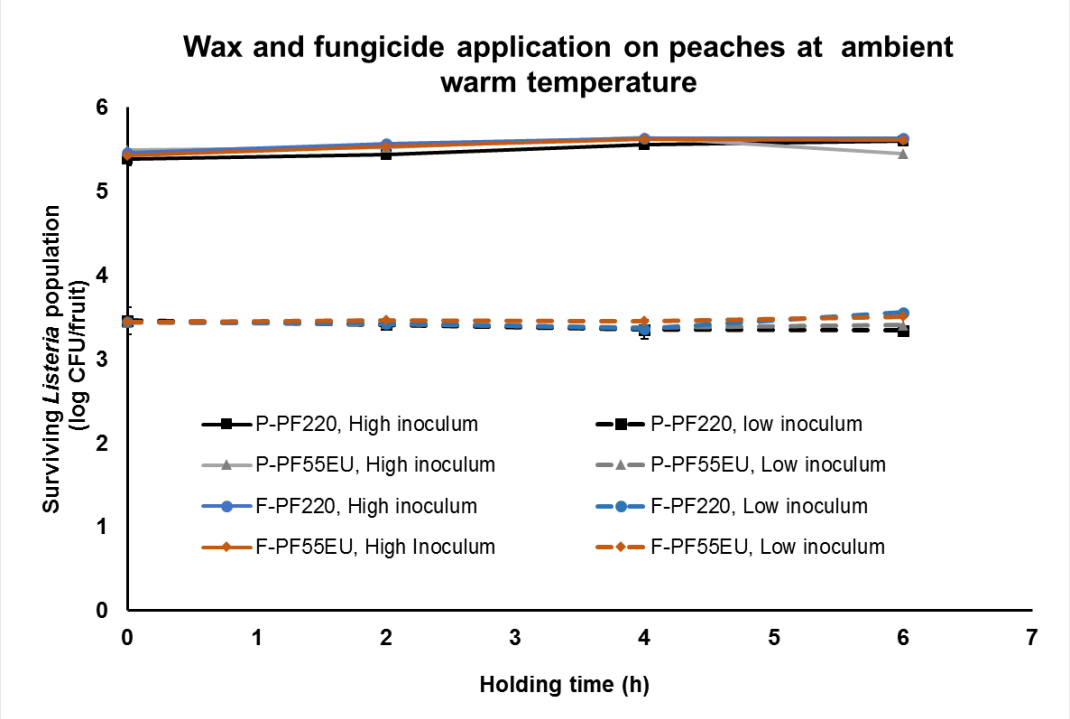


Fig. 3



Legend
P-PF220: Propiconazole & mineral oil–based wax
P-PF55EU: Propiconazole & vegetable oil–based wax
F-PF220: Fludioxonil & mineral oil–based wax
F-PF55EU: Fludioxonil & vegetable oil–based wax

Fig. 4

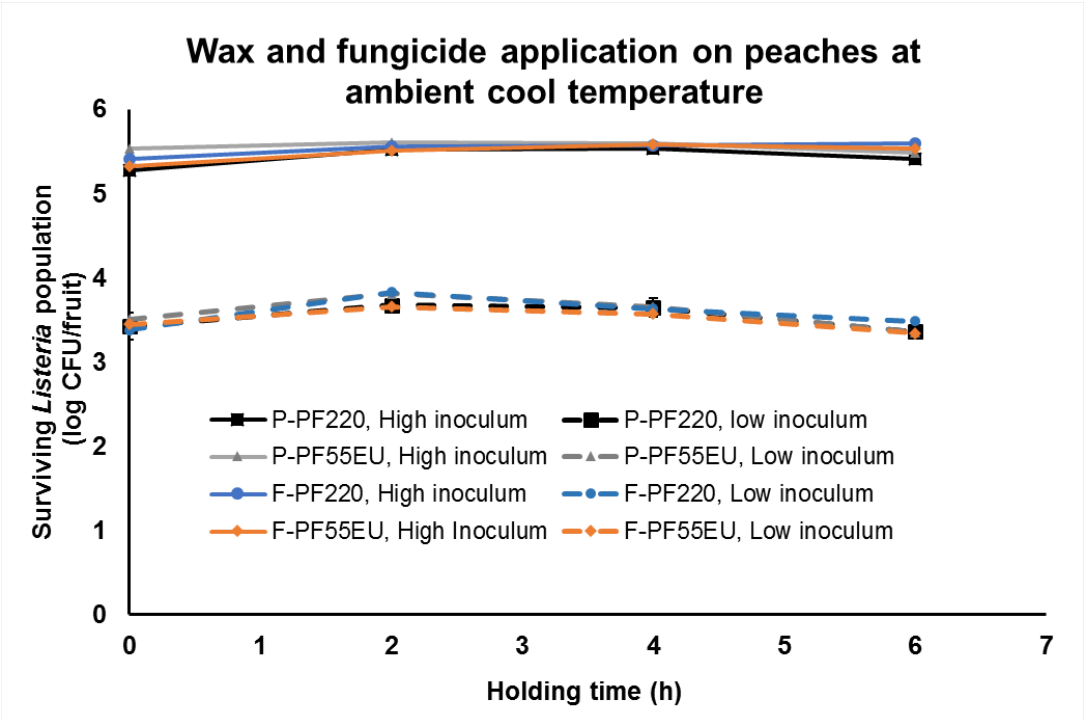


Fig. 5

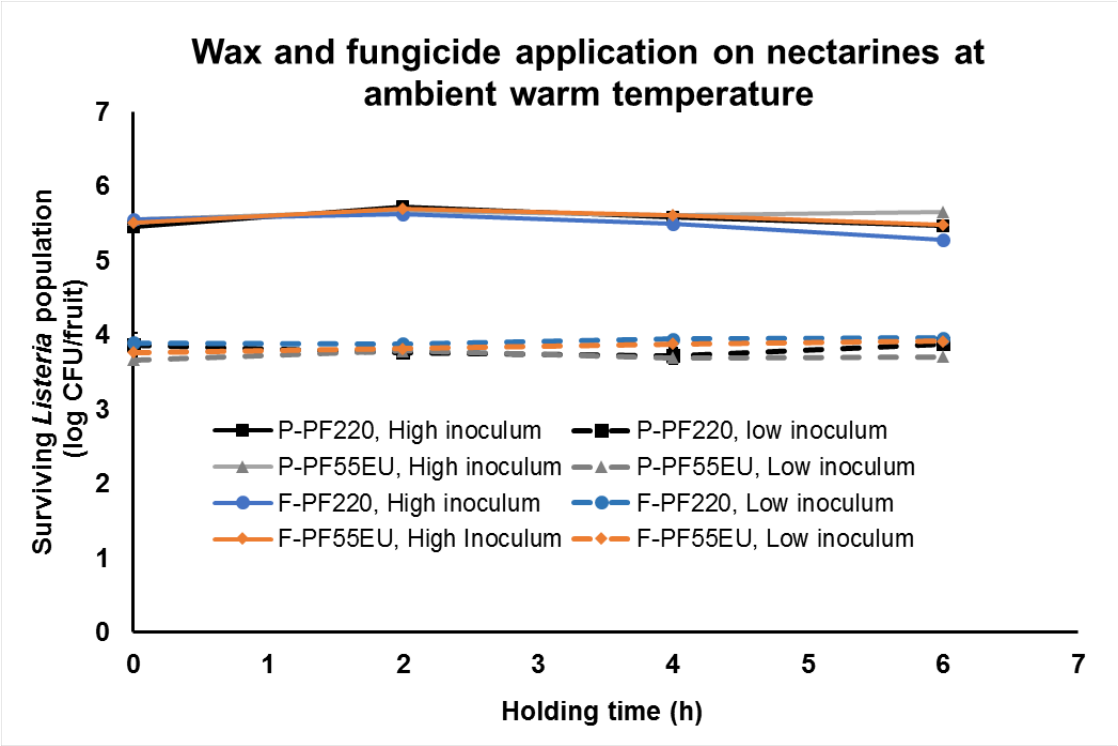

Wax and fungicide application on nectarines at ambient warm temperature
Surviving Listeria population (log CFU/fruit)
Holding time (h)
P-PF220, High inoculum
P-PF220, low inoculum
P-PF55EU, High inoculum
P-PF55EU, Low inoculum
F-PF220, High inoculum
F-PF220, Low inoculum
F-PF55EU, High Inoculum
F-PF55EU, Low inoculum

Fig. 6

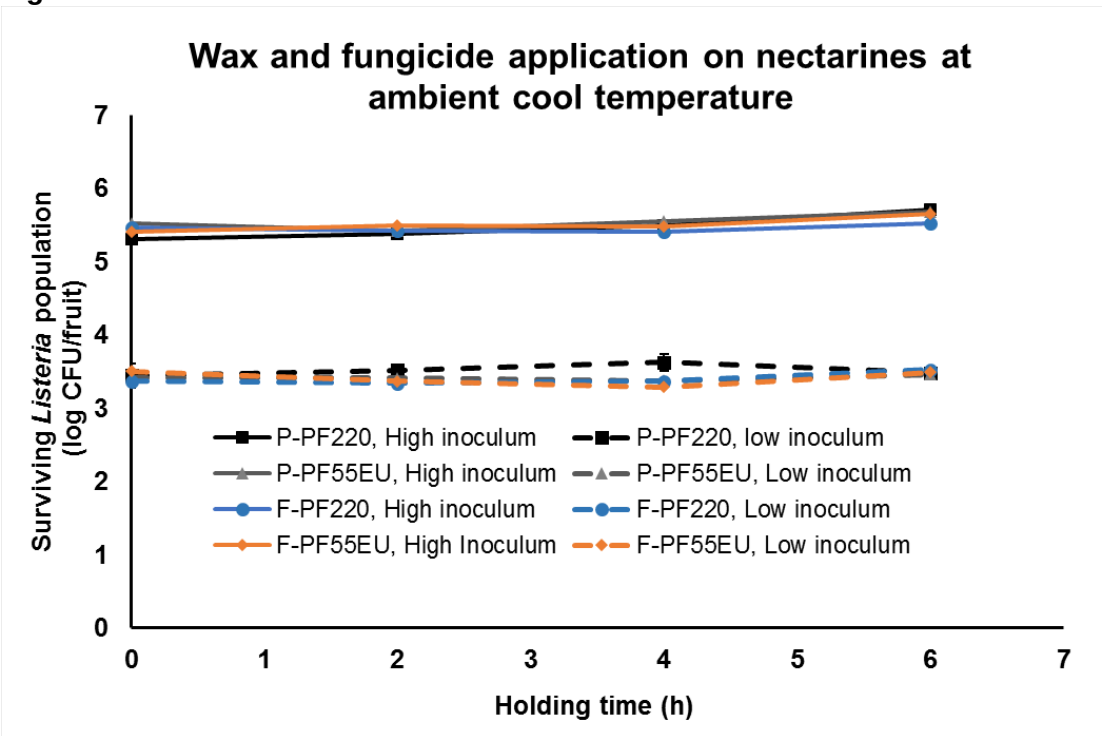

Wax and fungicide application on nectarines at ambient cool temperature
Surviving Listeria population (log CFU/fruit)
Holding time (h)
P-PF220, High inoculum
P-PF220, low inoculum
P-PF55EU, High inoculum
P-PF55EU, Low inoculum
F-PF220, High inoculum
F-PF220, Low inoculum
F-PF55EU, High Inoculum
F-PF55EU, Low inoculum

Fig.7

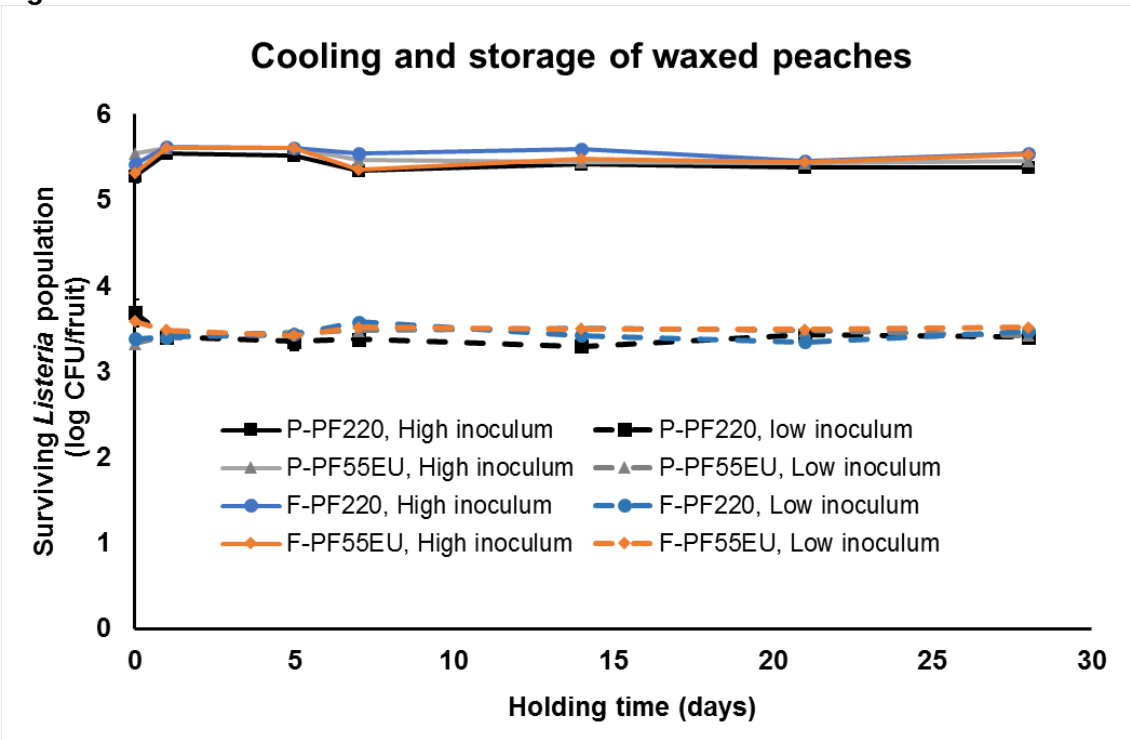
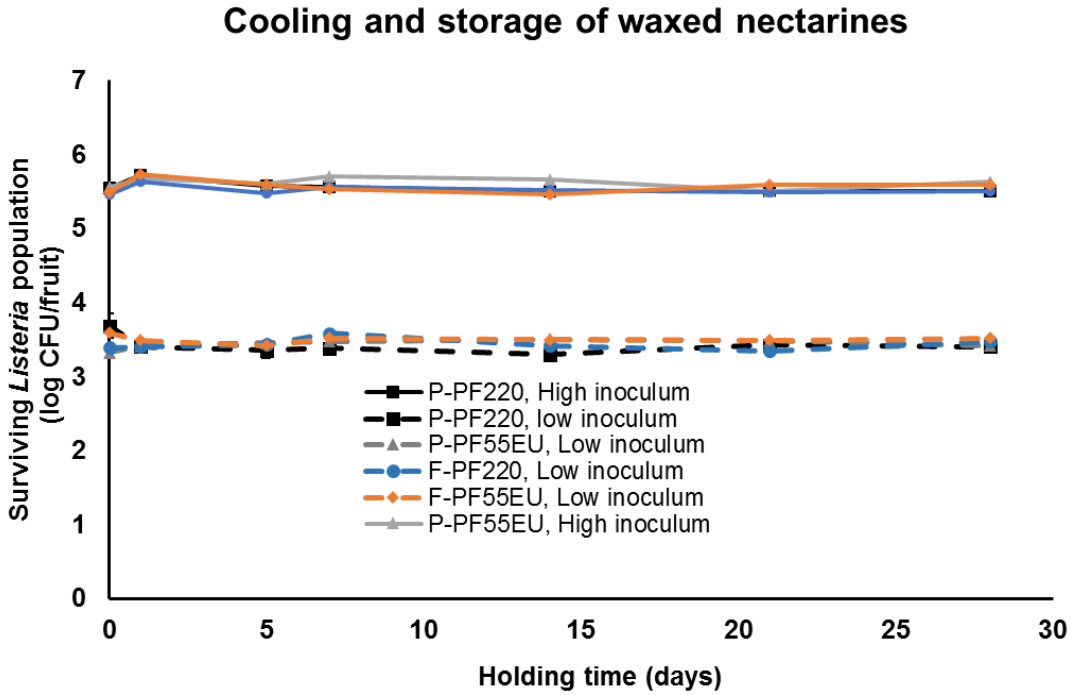

Cooling and storage of waxed peaches
Surviving *Listeria* population (log CFU/fruit)
Holding time (days)
P-PF220, High inoculum
P-PF220, low inoculum
P-PF55EU, High inoculum
P-PF55EU, Low inoculum
F-PF220, High inoculum
F-PF220, Low inoculum
F-PF55EU, High inoculum
F-PF55EU, Low inoculum

Fig. 8


Cooling and storage of waxed nectarines
Surviving *Listeria* population (log CFU/fruit)
Holding time (days)
P-PF220, High inoculum
P-PF220, low inoculum
P-PF55EU, Low inoculum
F-PF220, Low inoculum
F-PF55EU, Low inoculum
P-PF55EU, High inoculum