RESEARCH IN PROCESS

Characterization and Mitigation of Bacteriological Risks Associated with Packing Fresh-Market Citrus

SUMMARY

Green and blue molds (*Penicillium digitatum* and *Penicillium italicum*) result in significant losses of citrus fruit during storage and shipping. To reduce these losses, fungicides (e.g. sodium bicarbonate, imazalil) are applied to citrus fruit in tanks, sprays, or waxes during packing. These fungicides are not bactericidal thus their recirculation and reuse provide an opportunity for cross contamination among fruit and on equipment surfaces. The use of common water sanitizers, such as chlorine and peroxyacetic acid (PAA), to control bacterial loads must consider fungicide compatibility. Under laboratory conditions the minimum free chlorine concentrations at higher pH levels for sodium bicarbonate solutions and minimum PAA concentrations in imazalil solutions will be determined for rapid elimination of *Salmonella* and *Listeria monocytogenes*.

OBJECTIVES

The overall goal of the proposed research is to improve the ability of the citrus industry to successfully apply validated preventive controls that prevent the spread of bacterial foodborne pathogens in recirculating fungicide applications used by the packinghouse industry.

1. Determine the potential for *Salmonella* and *Listeria monocytogenes* to survive in float tanks, drench systems and systems where sodium bicarbonate and imazalil are recirculated/reused.
2. Determine minimum levels of compatible sanitizers that control, reduce, or eliminate *Salmonella* and *L. monocytogenes* in recirculated/reused sodium bicarbonate and imazalil solutions.
3. Determine the potential for survival or growth of *Salmonella* and *L. monocytogenes* on citrus fruit from harvest to pre-shipping storage under typical and sub-optimal conditions.

METHODS

Rifampin-resistant cocktails of *Salmonella* (3 strains) or *L. monocytogenes* (6 strains) were inoculated (~5 log CFU/ml) into water with and without imazalil (300 ppm) and with and without 0, 10, 15, and 20 ppm of PAA. Mixtures were stored at either 16 or 40°C for up to 5 min. At each time point 1 ml of the sample was added to 9 ml of D/E neutralizer broth and the surviving population was determined by plate count on tryptic soy agar with rifampin (TSAR). The remaining sample was incubated at 37°C for 48 h, and the presence of the pathogens in the enrichment was confirmed by streaking onto CHROMagar Listeria or CHROMagar Salmonella and incubation at 37°C for 24 h. Concentrations of PAA in water and imazalil (300 ppm) were measured over 120 min using a Reflectoquant Peracetic Acid Test.

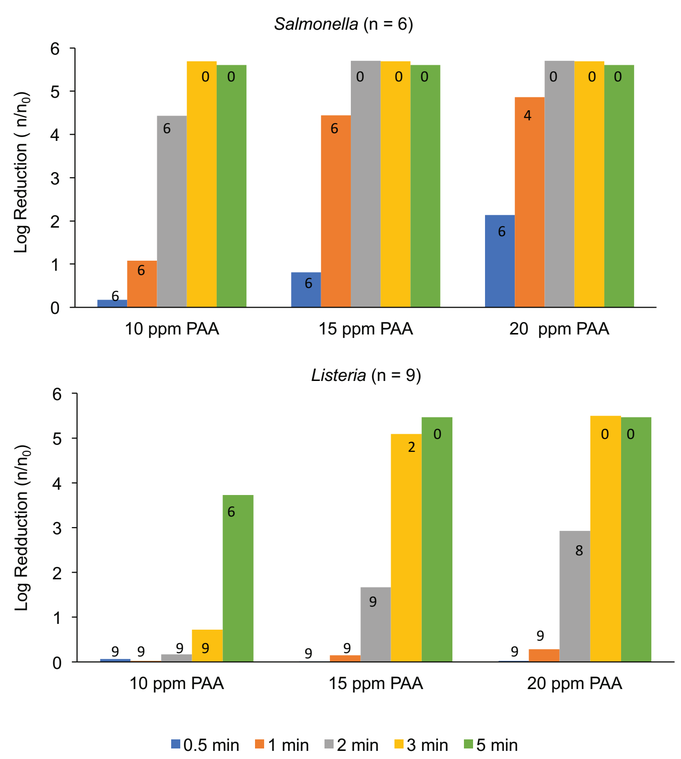


Figure 1. Effect of PAA concentration and exposure time on the inactivation of *Salmonella* and *Listeria* in 300 ppm imazalil held at 16°C. The number of positive tubes out of 6 or 9 D/E broth enrichments (48 hours at 37°C) are shown.

RESULTS TO DATE

The *Salmonella* cocktail was more sensitive to PAA than the *Listeria* cocktail. At 16°C a ≥5 log reduction of *Salmonella* or *Listeria* was observed in ≥2 min or ≥3 min, respectively, at 15 or 20 ppm of PAA (Fig. 1). Imazalil may be applied to citrus at cool ambient temperatures or as heated solutions. Greater survival of either *Salmonella* or *Listeria* was observed at 16°C compared to 40°C (Fig. 2). At 40°C ≥5 log reductions of *Salmonella* and *Listeria* were observed within 0.5 and 1 min, respectively. Increasing the imazalil concentration from 100 to 500 ppm did not impact the efficacy of 20 ppm PAA (data not shown). Concentrations of PAA declined after ~5 min in water or 300 ppm imazalil held at 23°C; declines in PAA concentration were generally slowed in imazalil preparations (Fig. 3).

BENEFITS TO THE INDUSTRY

Commercial citrus packinghouses need to characterize potential public health risks of standard practices and apply appropriate science-based mitigation strategies (preventive controls) to current standard practices. The project is intended to generate data to validate the efficacy of practices used in citrus packinghouses to prevent the spread of microorganisms among citrus fruit, and can be used to support individual packinghouse food safety plans.

Figure 2. Effect of temperature on the inactivation of *Salmonella* and *Listeria* in water with or without imazalil (IMZ, 300 ppm; n = 6). The number of positive tubes out of 6 D/E broth enrichments (48 hours at 37°C) are shown. The limit of detection is indicated by a dashed line (1.3 log CFU/ml).

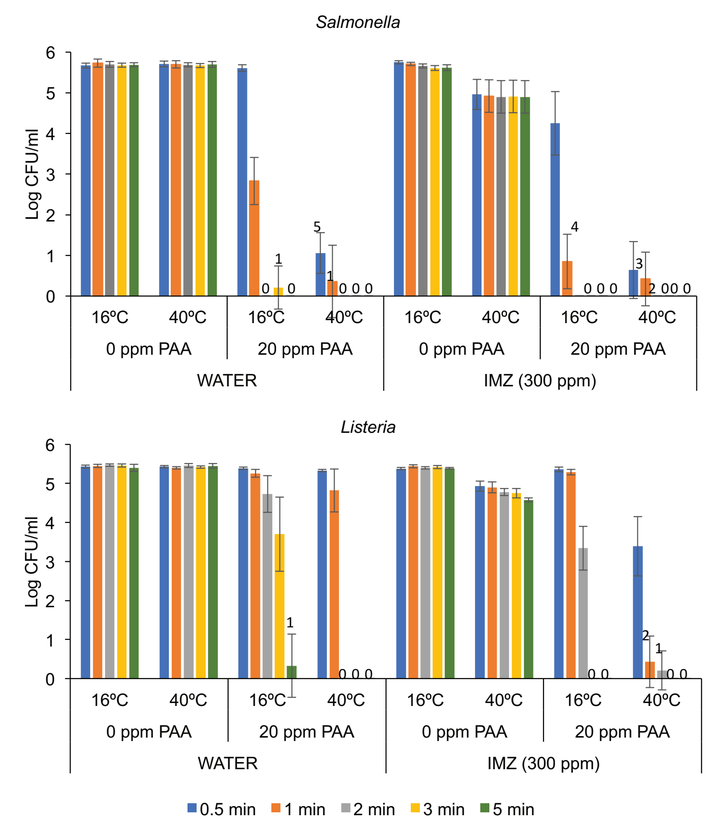
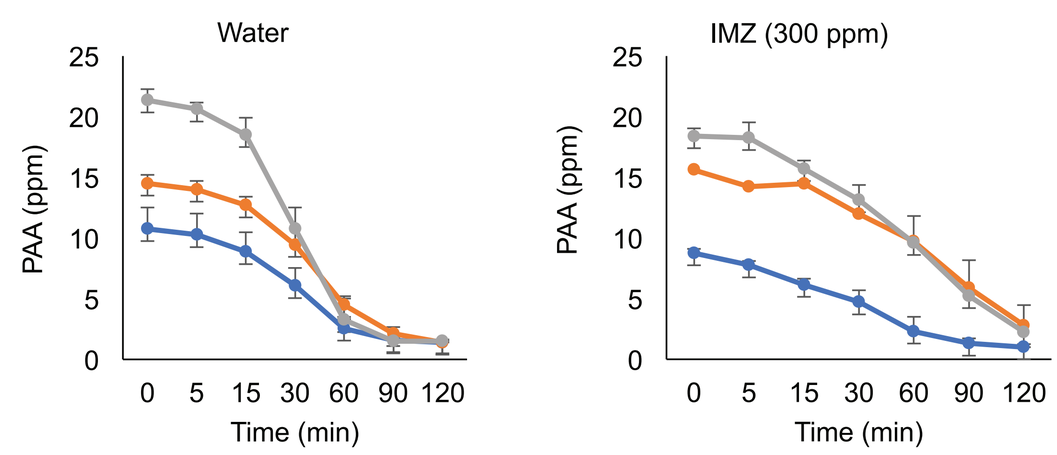


Figure 3. Concentration of PAA in water and imazalil (300 ppm) held at 23°C.



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ACKNOWLEDGEMENTS

We thank Anne-Laure Moyne, Vanessa Lieberman, and Ethan Morgan for their technical assistance, and Jim Cranny, California Citrus Quality Council, Mohsen Sales, DECCO U.S. Postharvest, Inc. and many other members of the citrus industry for materials, expert opinion, packinghouse access, and general support.

LENGTH OF FUNDING

January 1, 2017 – December 31, 2018

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