

# Characterization and mitigation of bacteriological risks associated with packing fresh-market citrus

*OR*

## What You Should Know about Dump Tanks and Flooders

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# Acknowledgments

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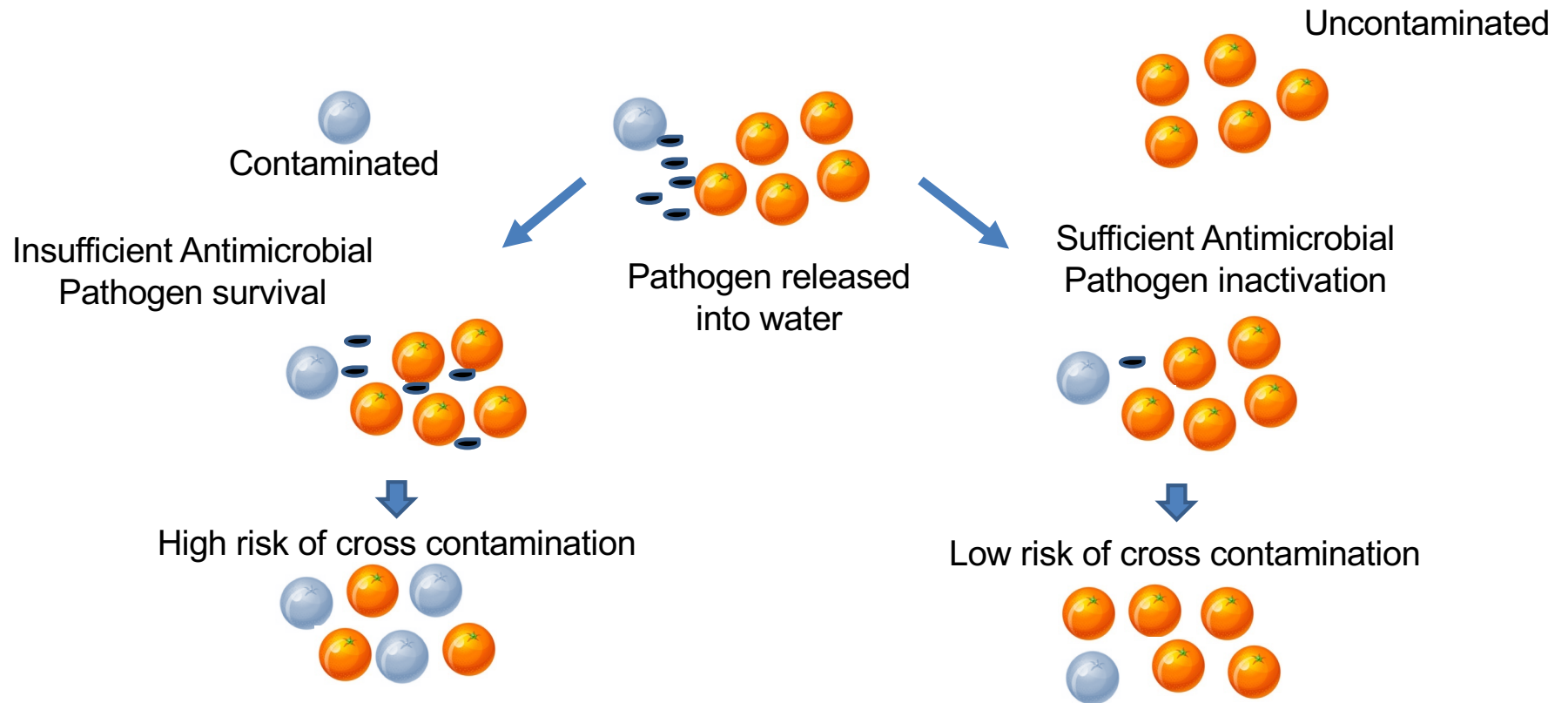
Luxin Wang, Ph.D.

Members of the Citrus Industry

# Critical need for data in support of “Adequate Sanitary Quality”



# Aqueous mediated cross contamination



**"Adequate Sanitary Quality"**

# Pre-Storage Lemon Packline



Receipt



- Chlorinated dump tank
- Sizer
- Pre-grade



3% soda ash tank



- Spray potable water
- Brush rollers



Imazalil + PAA Recirculating



Storage wax



- Storage
- 52°F (11°C);
- 24 h to 120 days

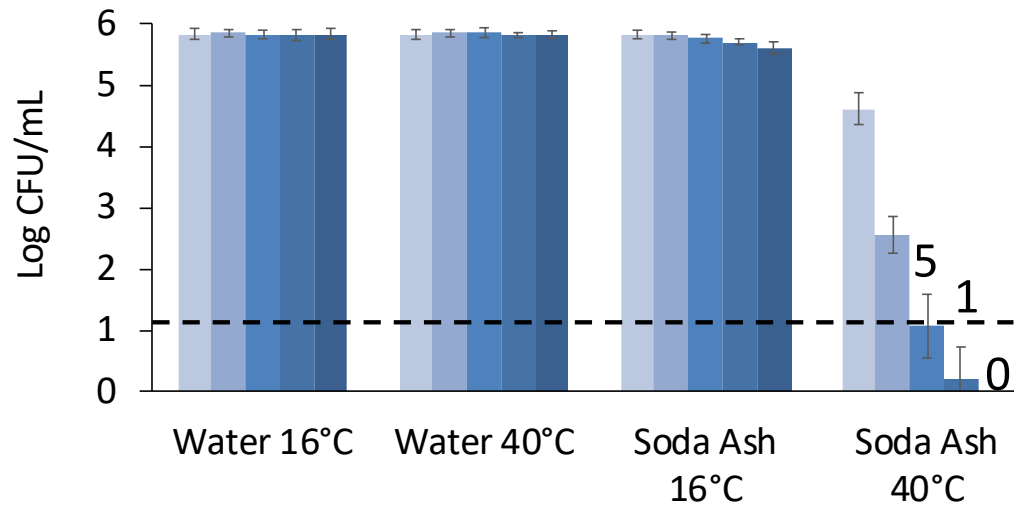
# Citrus Industry Use Survey

- Soda Ash
  - 1 to 3%
  - 60 to 115°F (16 to 46°C)
  - pH 9.5 to 13
  - Exposure
    - Seconds to minutes
  - Use
    - Week to months
- Imazalil
  - 100 to 500 ppm
  - 60 to 135°F (16 to 57°C)
  - Exposure
    - Seconds
  - Use
    - Day to weeks
- Peracetic Acid (PAA)

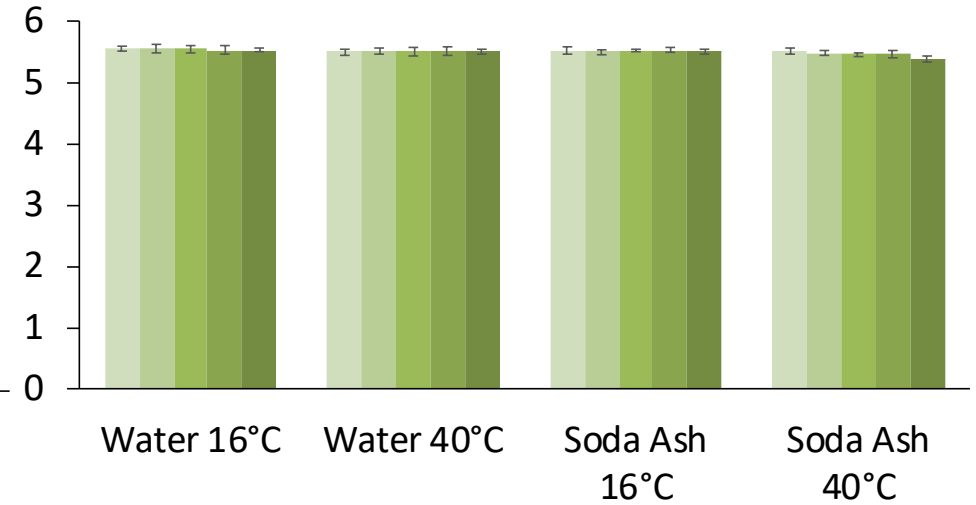
# Survival of *Salmonella* and *Listeria* in water (pH 7.90) and 3% soda ash (pH 11.30) at 16 or 40°C (60/104°F)



*Salmonella* (n=6)



*Listeria* (n=6)



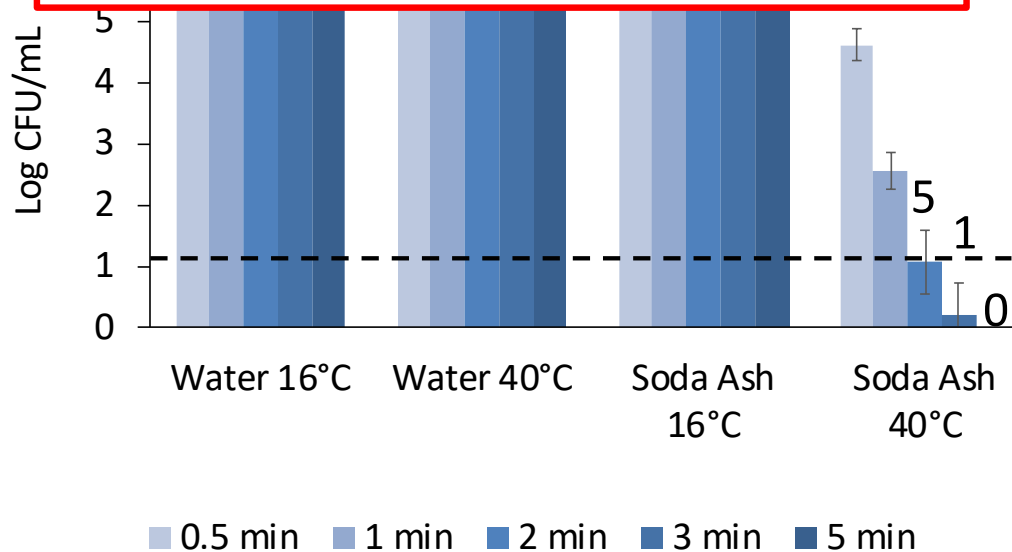
0.5 min 1 min 2 min 3 min 5 min

0.5 min 1 min 2 min 3 min 5 min

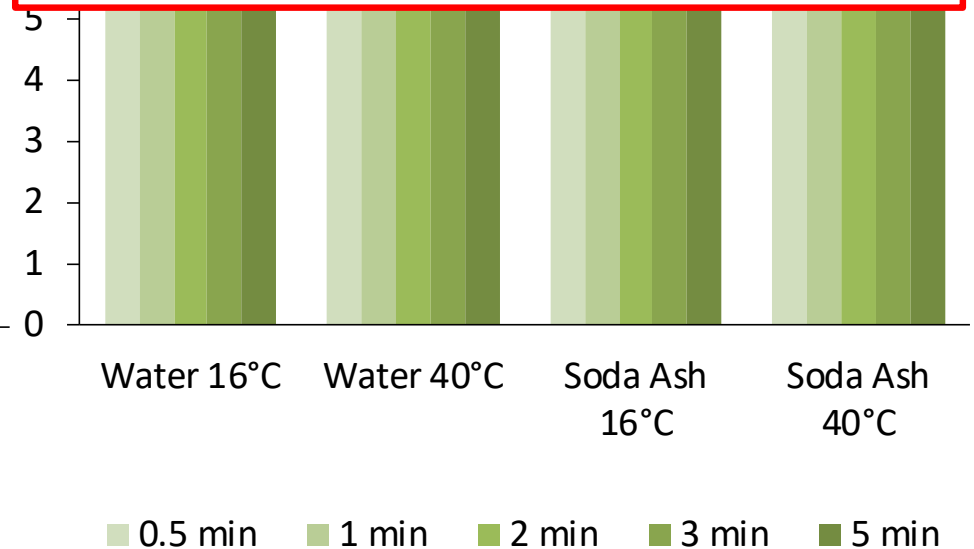
# Survival of *Salmonella* and *Listeria* in water (pH 7.90) and 3% soda ash (pH 11.30) at 16 or 40°C (60/104°F)



≥5 log reduction of *Salmonella*  
in ≥3 min at 40°C/104°F

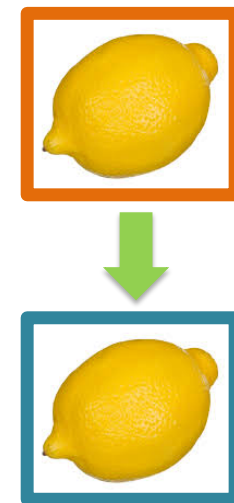
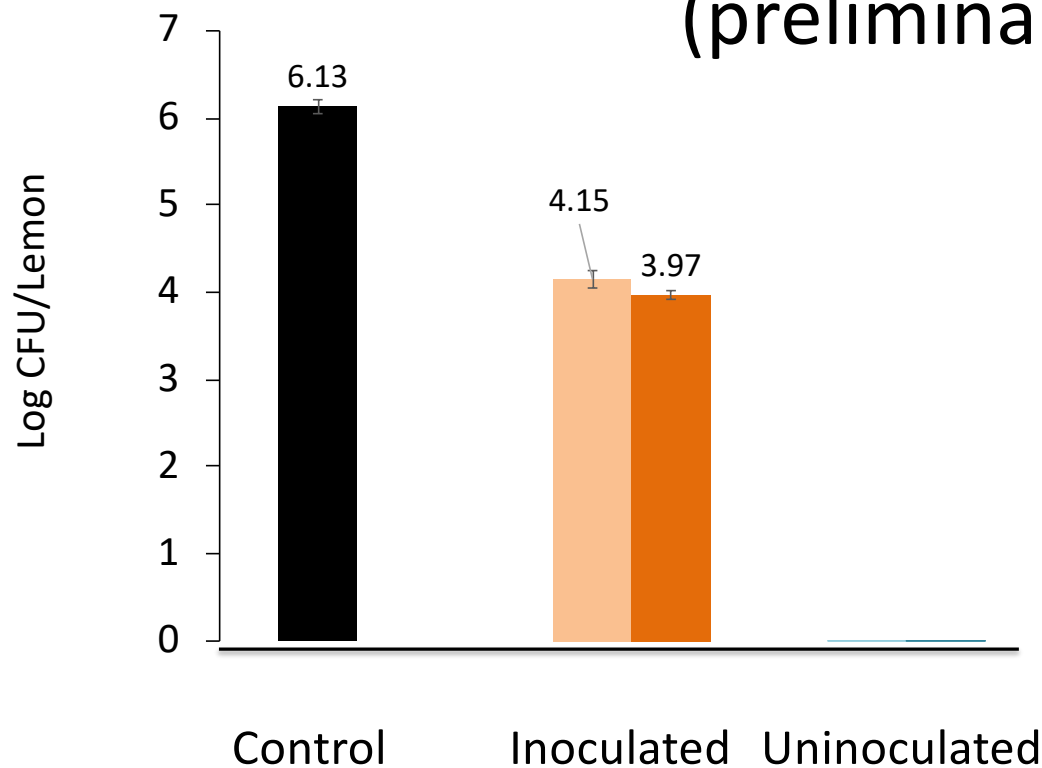


No reduction of *Listeria*  
in 5 min at 40°C/104°F

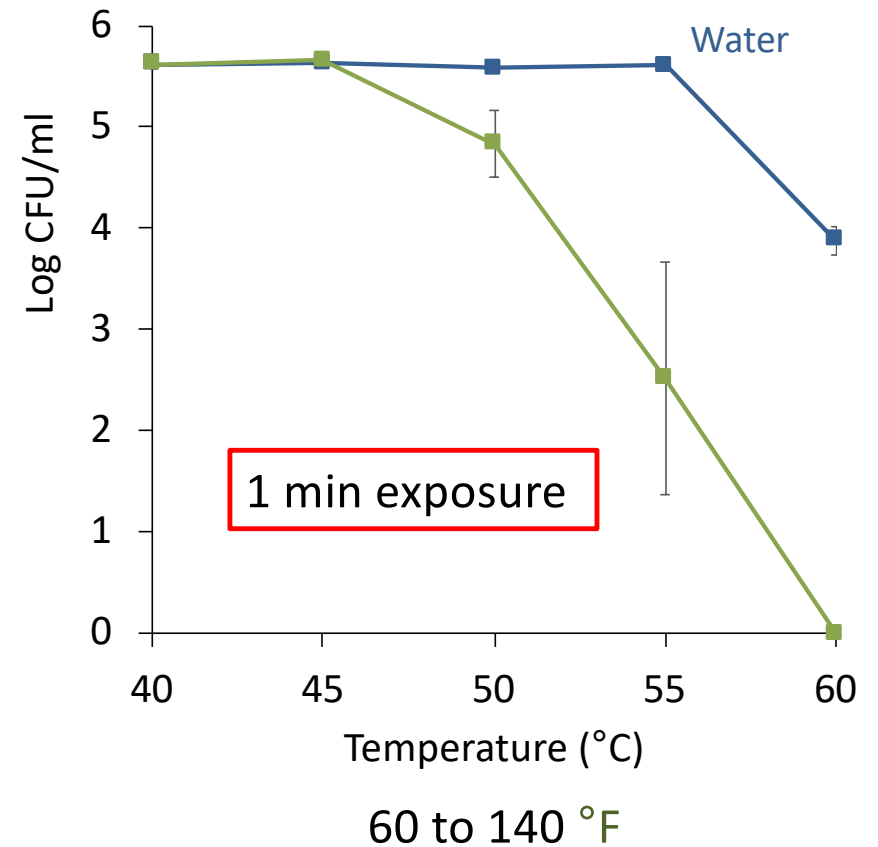
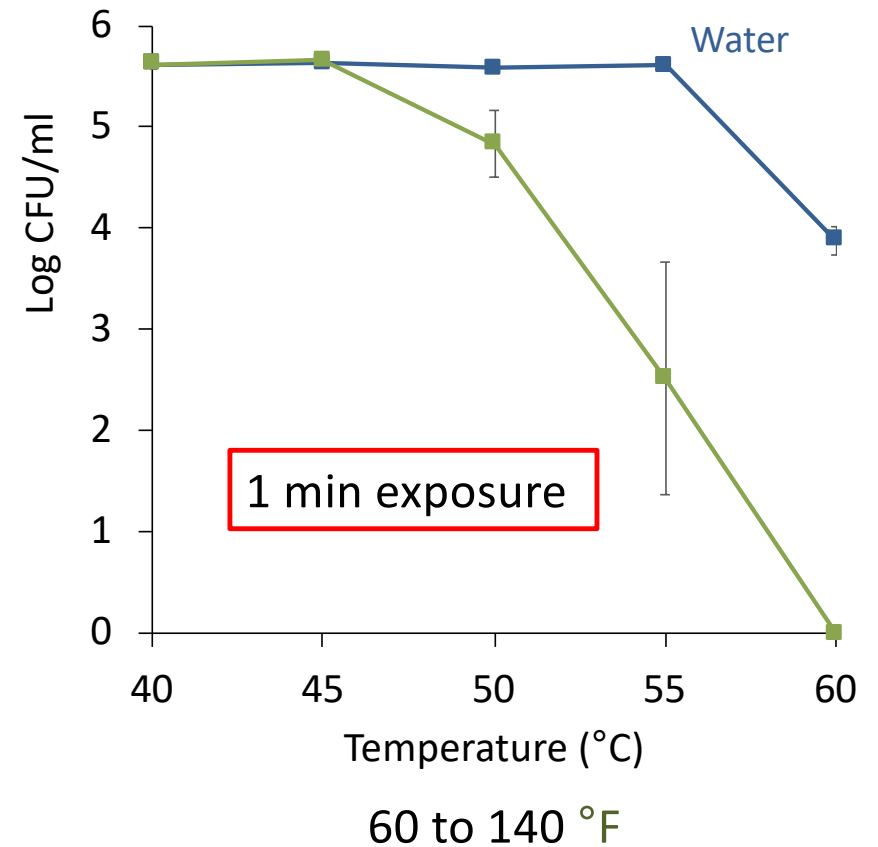
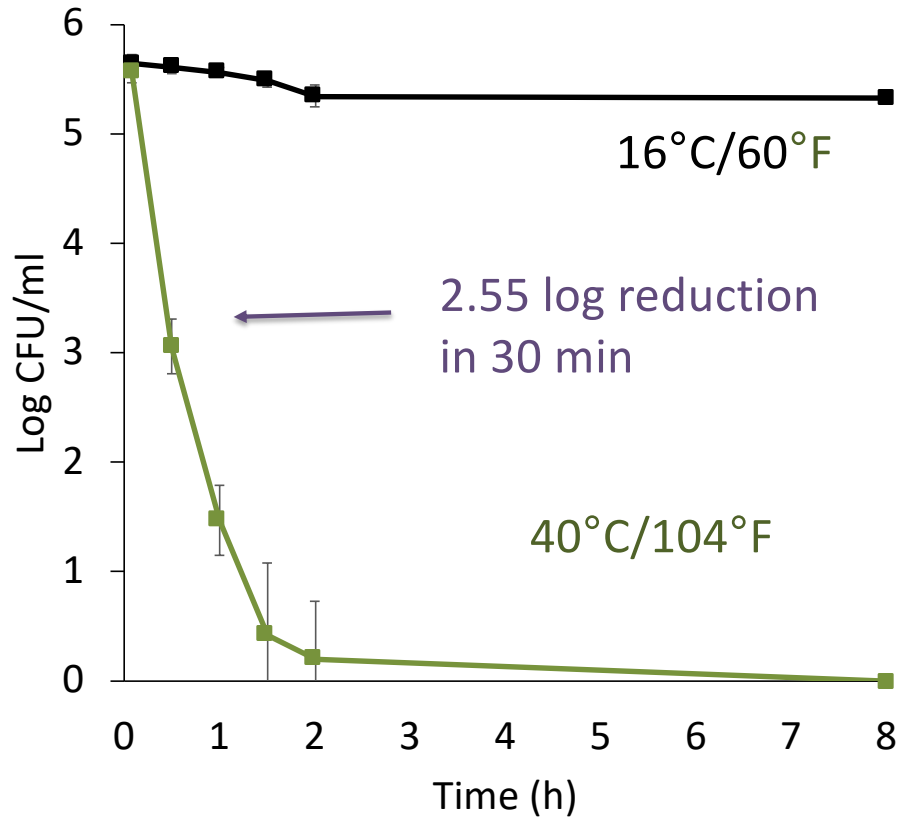




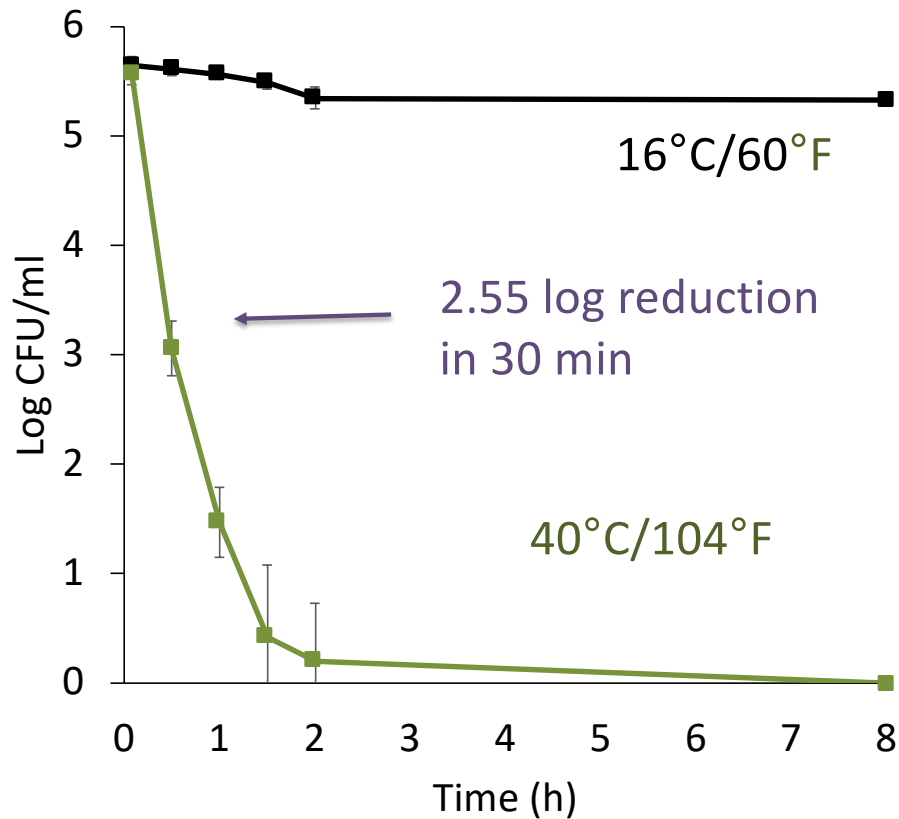
# Transfer of *L. monocytogenes* from inoculated to uninoculated lemon: **3% soda ash** at 40°C/104°F (preliminary data)



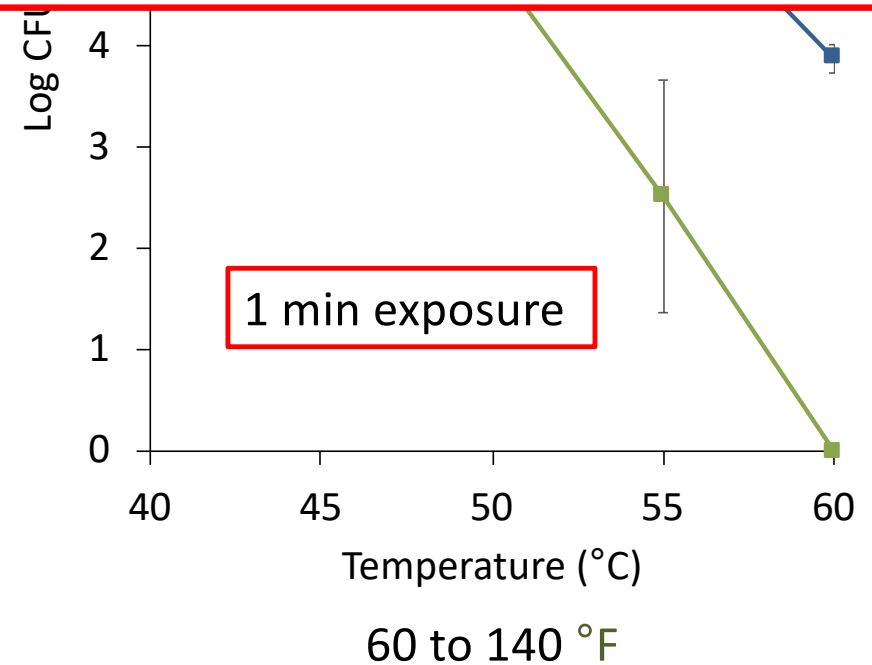
# Survival of *Listeria* in 3% soda ash pH 11.30 (n=6)



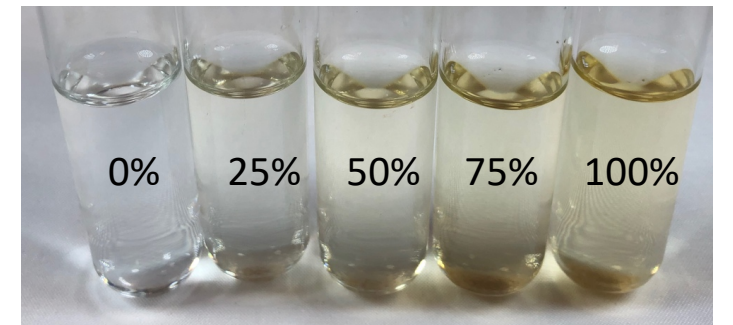
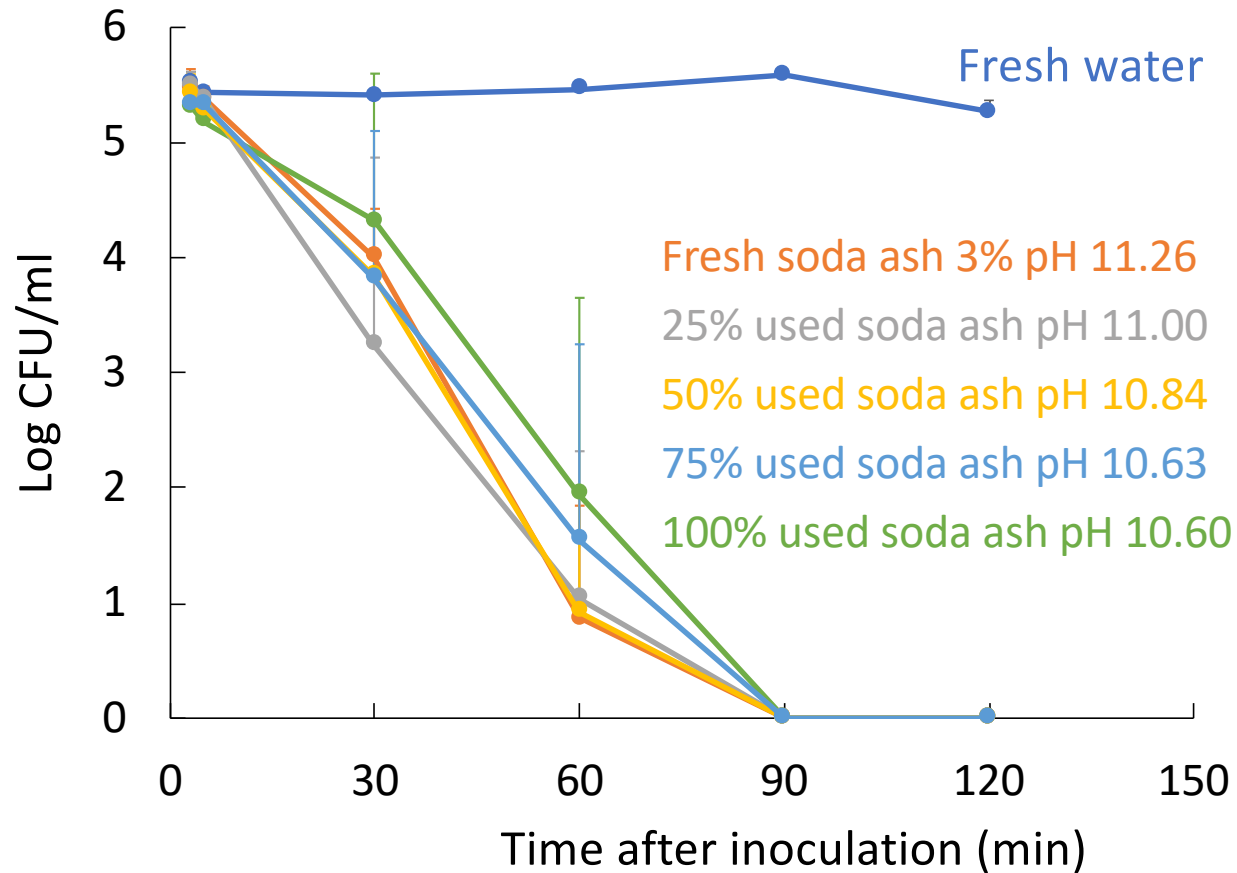
# Survival of *Listeria* in 3% soda ash pH 11.30 (n=6)



**≥5 log reduction of *Listeria*  
in soda ash in 1 min at 60°C/140°F**



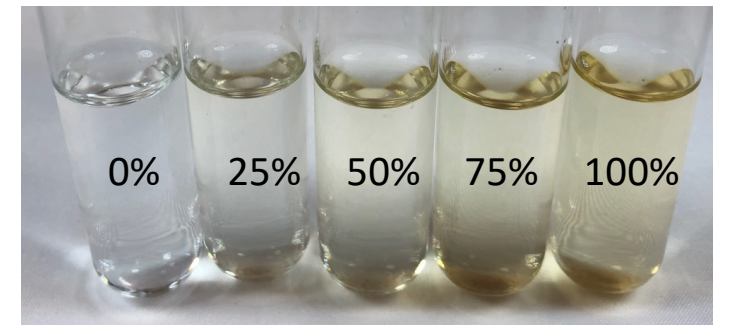
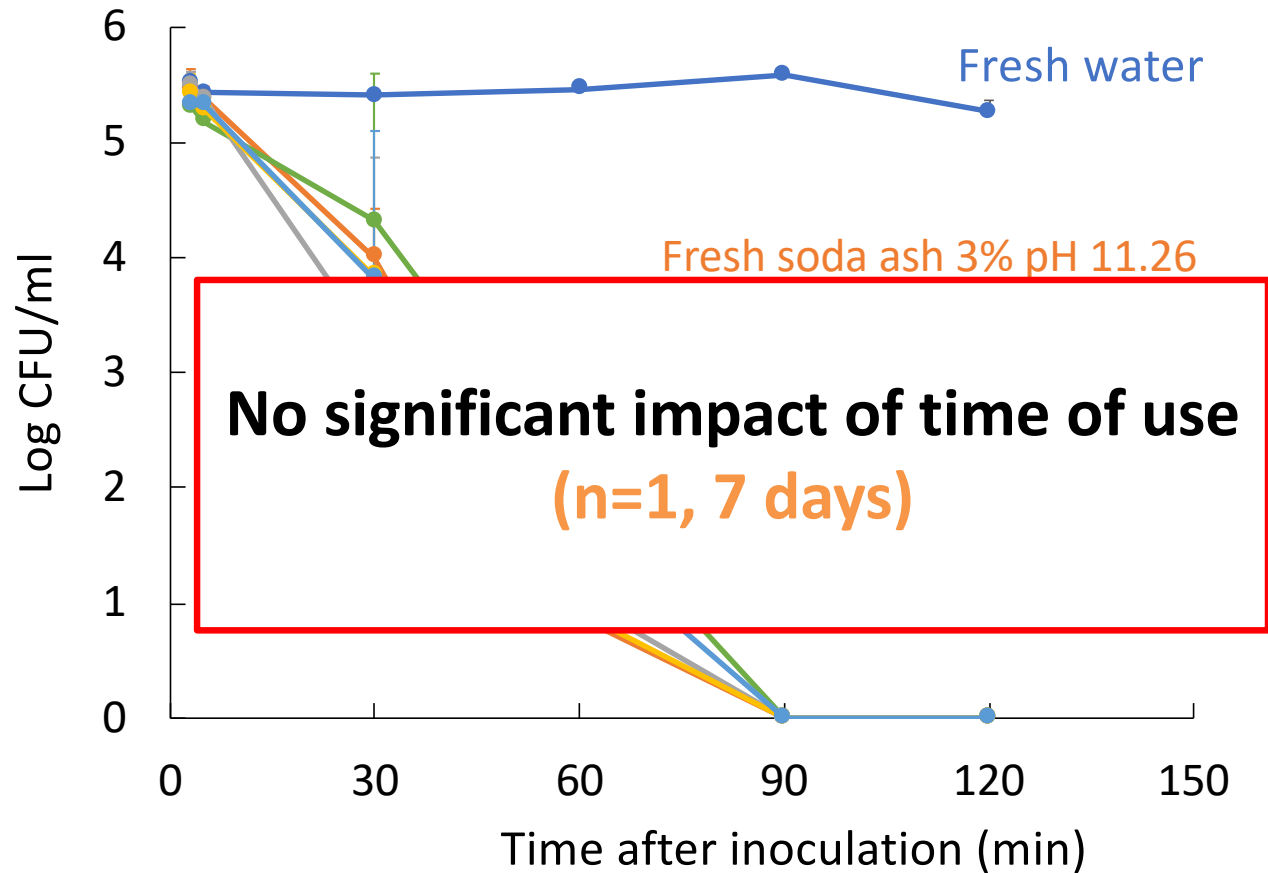
# Inactivation of *Listeria* in used **3% soda ash** 40°C/104°F



Soda ash turbidity

Fresh → Used (7 days)

# Inactivation of *Listeria* in used **3% soda ash** 40°C/104°F



Soda ash turbidity

Fresh → Used (7 days)

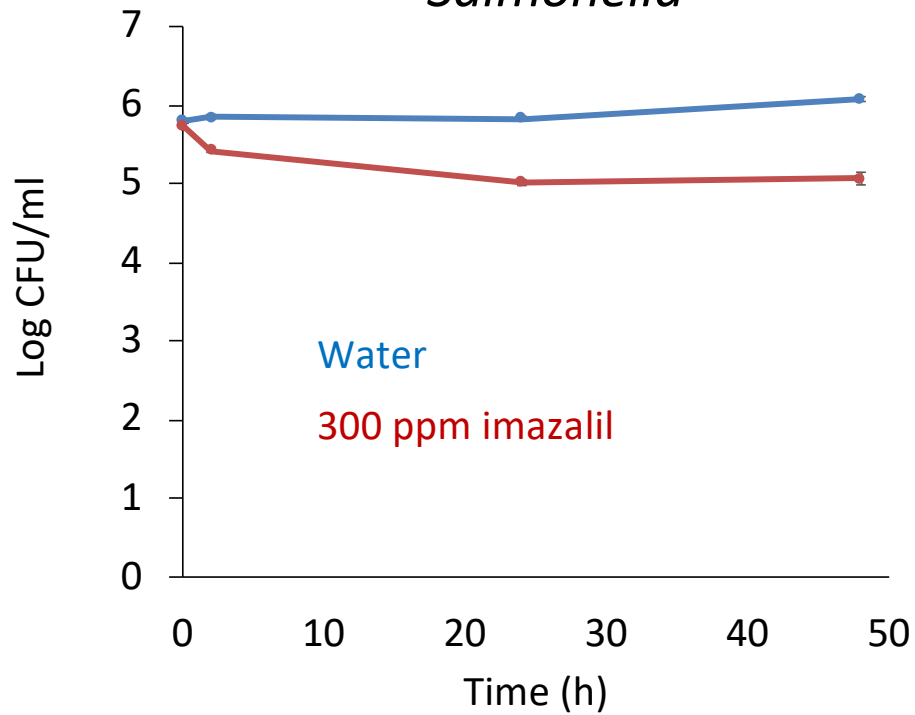
# Key Outcomes

- Soda ash
  - Cross contamination is possible under normal simulated use
  - Current practice of nightly heating soda ash
    - Rapidly reduces populations of *L. monocytogenes*
  - No significant difference in impact on *L. monocytogenes* with extended use material

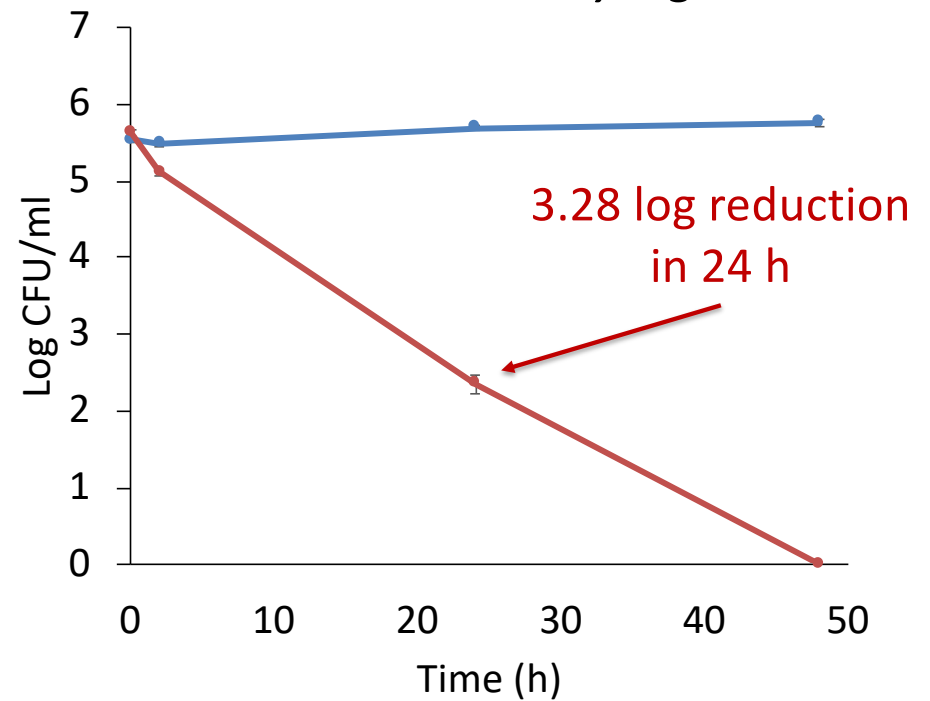
# **Imazalil with Peroxyacetic Acid (PAA)**

# Survival in 300 ppm imazalil at 16°C (60°F)

*Salmonella*

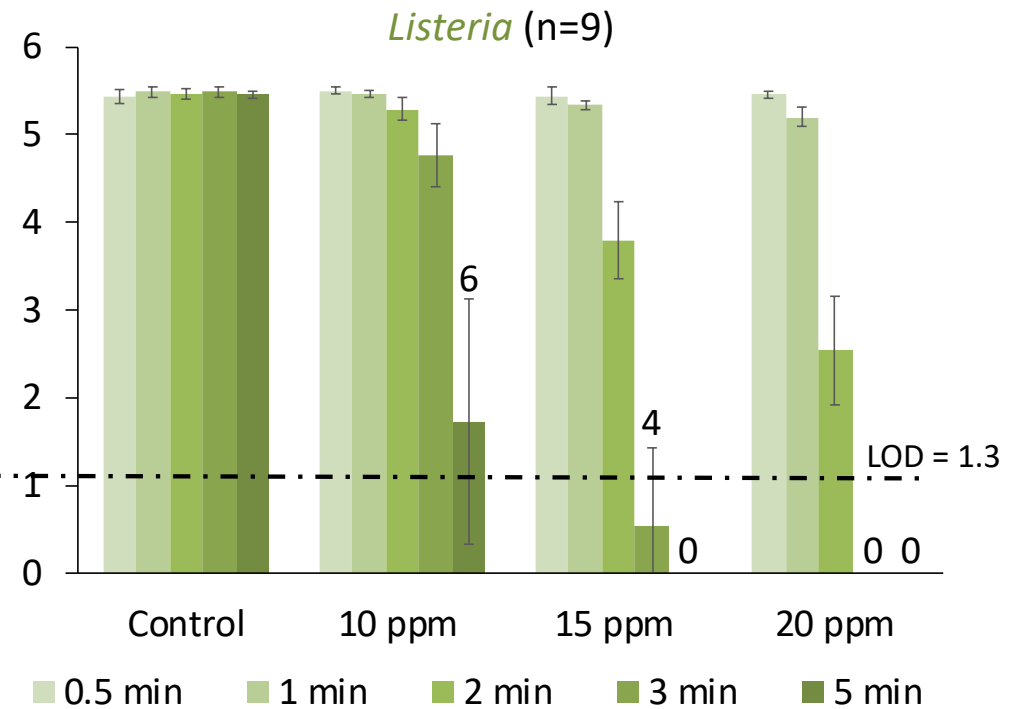
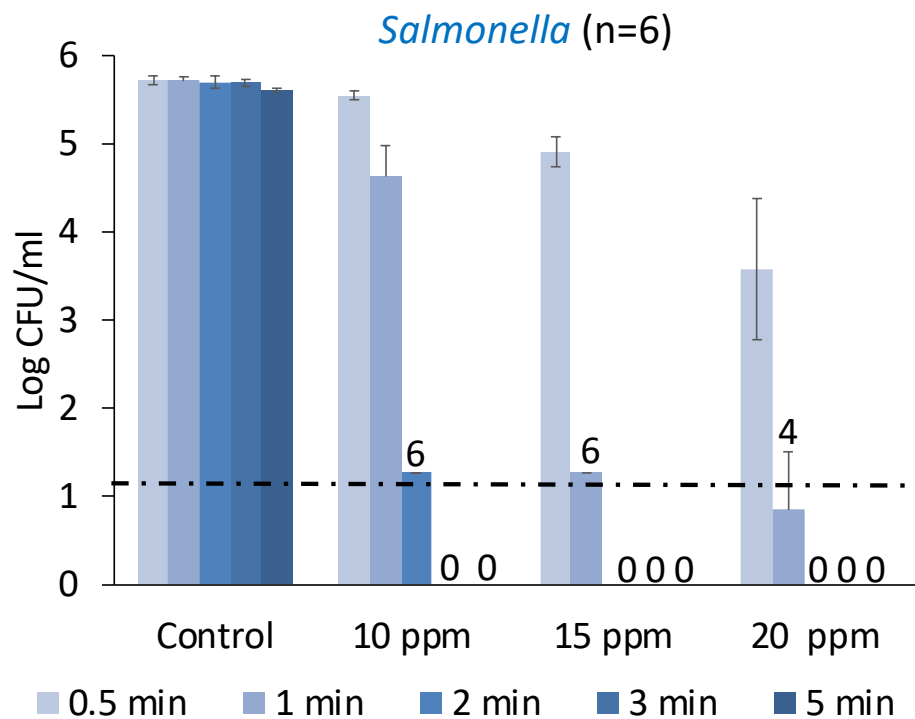


*Listeria monocytogenes*





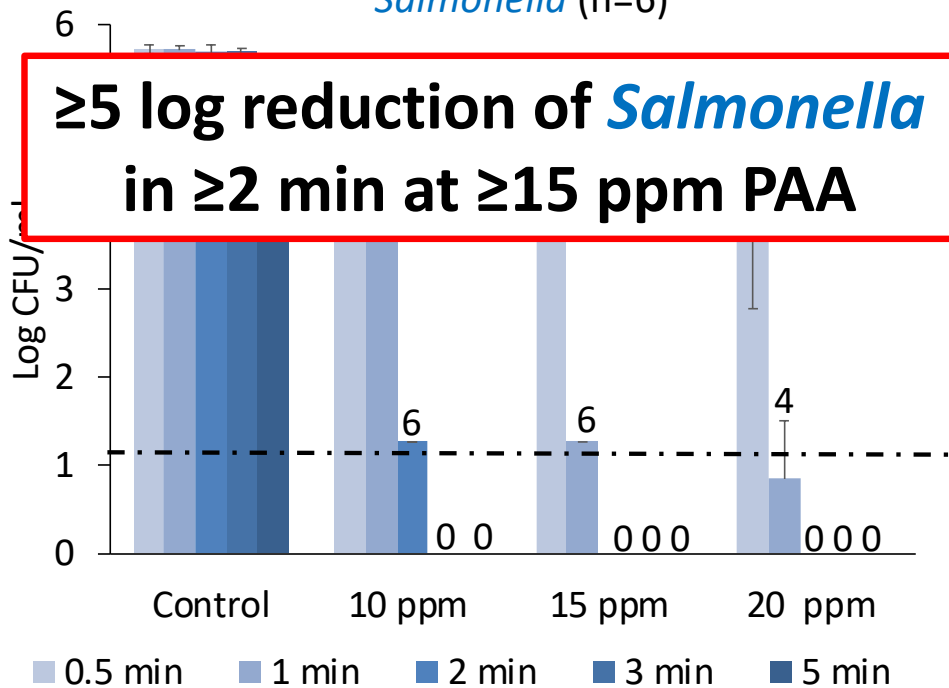
# Inactivation of *Salmonella* and *Listeria* in 300 ppm imazalil with PAA at 16°C (60°F)



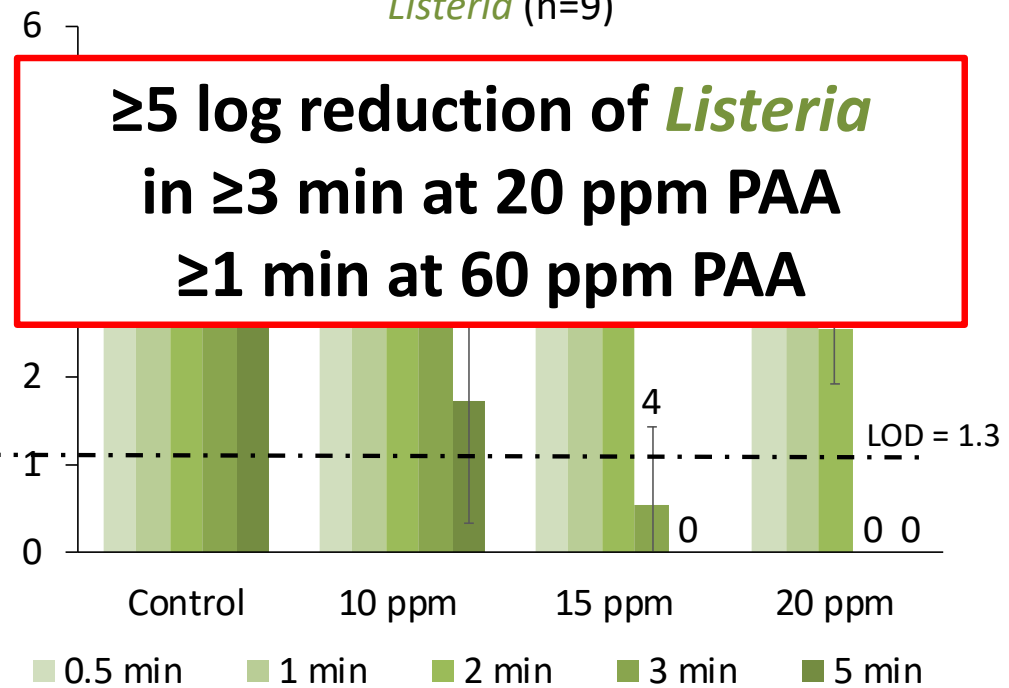
# Inactivation of *Salmonella* and *Listeria* in 300 ppm imazalil with PAA at 16°C (60°F)



*Salmonella* (n=6)



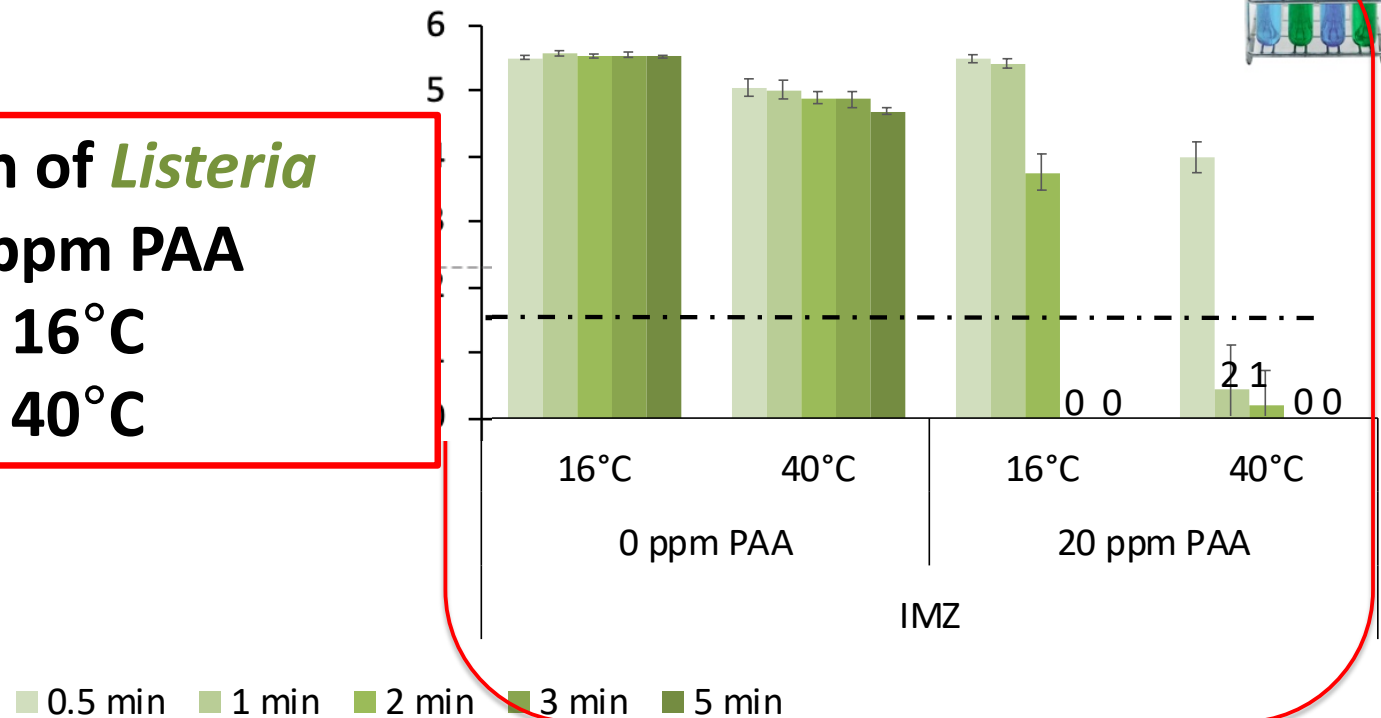
*Listeria* (n=9)



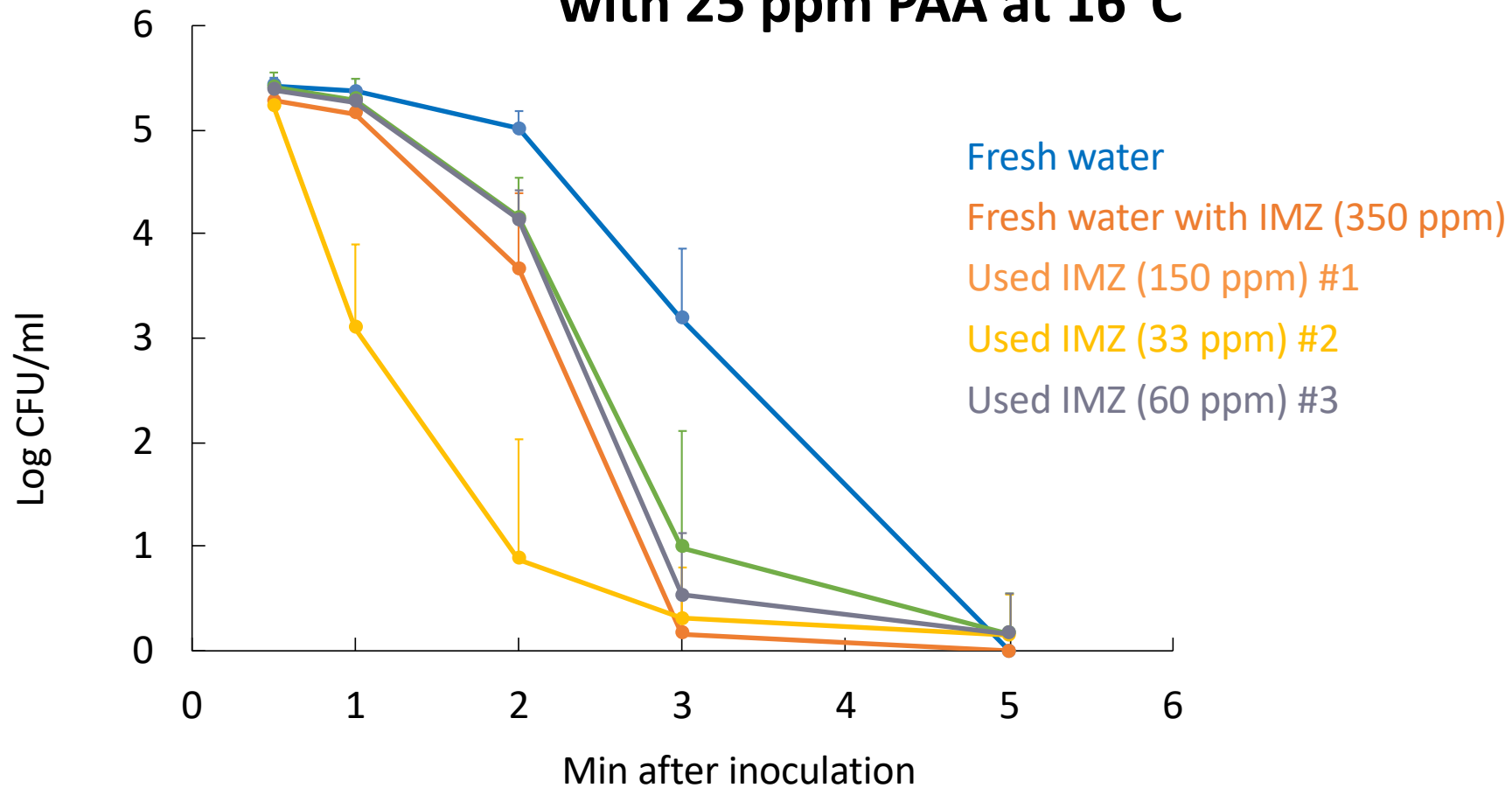
# Inactivation of *Listeria* in 300 ppm imazalil with PAA at 16 and 40°C (60 and 104°F) n=6



**≥5 log reduction of *Listeria*  
in IMZ at 20 ppm PAA  
≥3 min at 16°C  
≥1 min at 40°C**



## Survival of *Listeria* in fresh and used imazalil with 25 ppm PAA at 16°C



# Key Outcomes

- Imazalil with  $\geq 20$  ppm PAA
  - Significantly reduces
    - Both *Listeria monocytogenes* and *Salmonella*
    - Across temperatures of use
    - In fresh and end-of-use material

# Dump tanks and recirculating systems

- $\geq 5$  log reduction of *Listeria* in 3% soda ash:
  - $\geq 1$  min at 60°C
- $\geq 5$  log reduction of *Listeria* in imazalil
  - 20 ppm PAA:  $\geq 3$  min at 16°C or  $\geq 1$  min at 40°C
- $> 5$  log reduction of *Listeria* in 3% SBC (not shown)
  - 18 ppm free chlorine:  $\geq 20$  s at pH 8.0 and 9.5

# “Typical” Pre-Storage Lemon Packline



Receipt



- Chlorinated dump tank
- Sizer
- Pregrade



3% soda ash tank  
(sodium carbonate)



- Potable water
  - Spray
  - Brush rollers



Imazalil + PAA Recirculating



Storage wax



- Storage
  - 52°F (11°C);
  - 24 h-120 days

## Pre-Storage Line

**Receive Lemons**

**Bin Dump**-chlorinated water

**Popv Sizer**-remove small fruits

**P**

**Soak Ta**

**Washing**

**Fungicid**  
ove

**Storage**

fungicide sprayed over rotating brushes

**Color Sorting/Box Fill**

**Storage**; 52°F (11°C); 24 h-120 days



## Packing Line

**Box Dump Rinse**

**Rot Removal**-by hand on PVC rollers

**Washing**-SBC+chlorine (recirculating flood)

PVC rolls

nges

IMZ+PAA  
es

ng brushes

**Packing**

**Storage/Cooling**; 40-42°F

**Shipping**

What is the impact of all of these steps?



What is the impact?

What are you harvesting?

Multiple Products

What time of year?

Long Season

What is the weather?

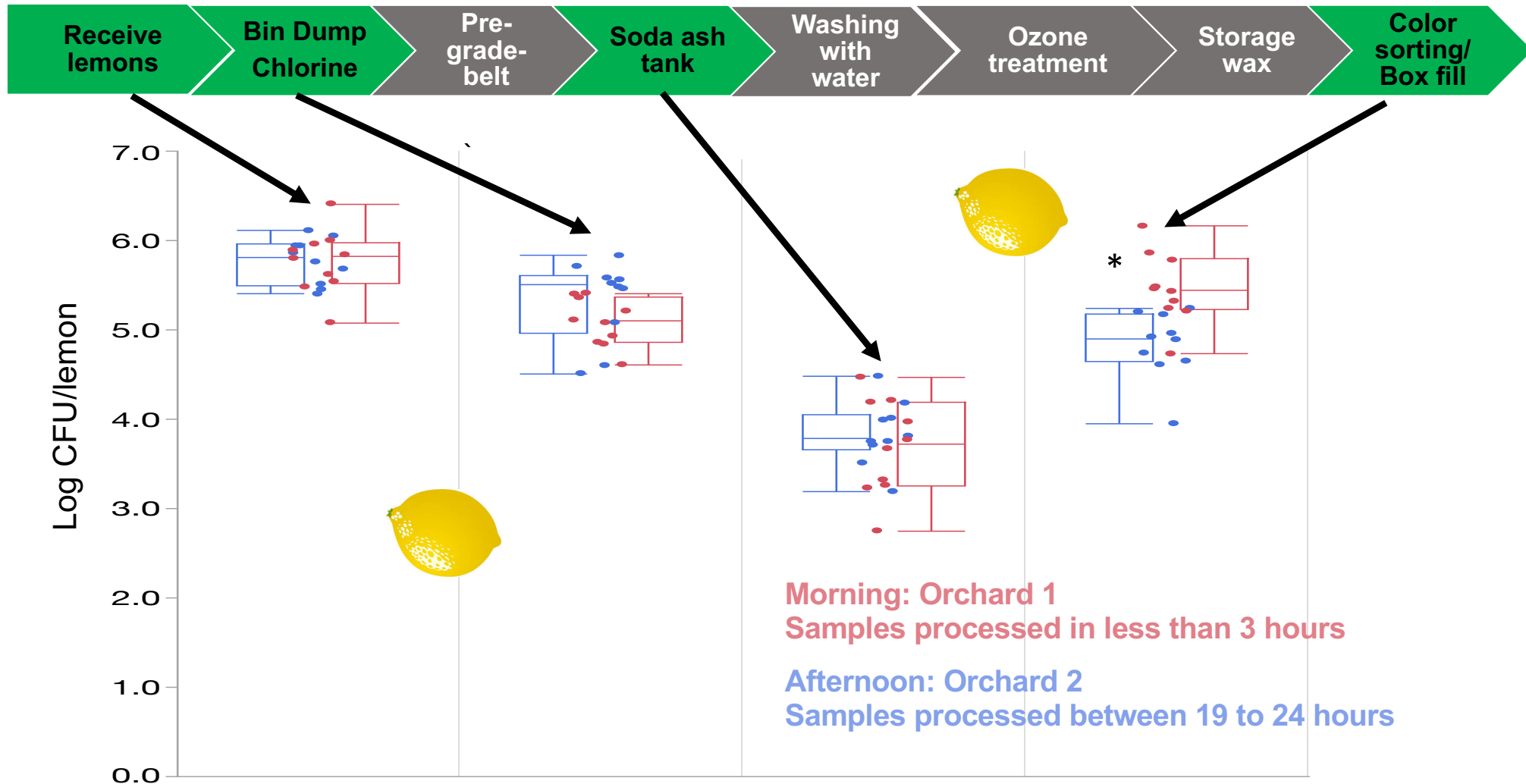
Packinghouse layout?

Varying practices

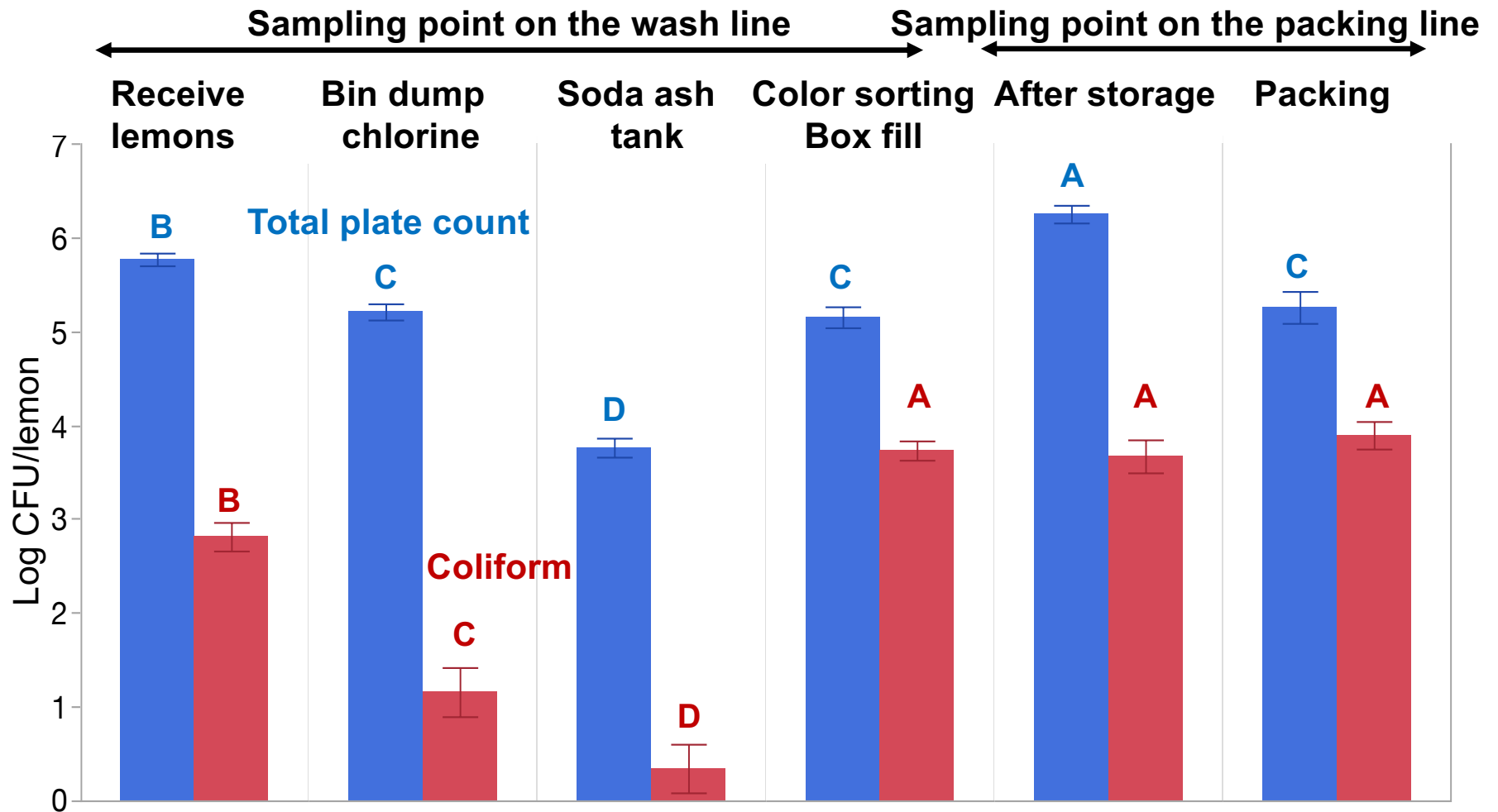
Different age and size

Production volume

## Change in lemon microbial populations in the wash line (preliminary data)



## Variation in lemon microbial population in the wash and packing lines





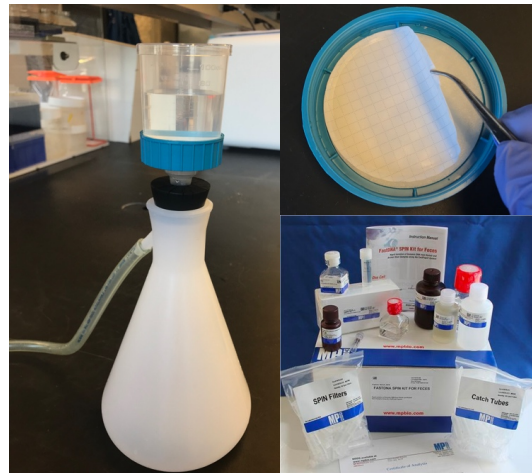
# Sequencing analysis of citrus native microflora



20 ml PBS + 0.05% Tween 20, pH7.4

30s shaking  
60s rubbing  
30s shaking

Lemon  
Processing



FastDNA Spin Kit for feces

Filtration  
DNA extraction



16S Amplicon PCR Forward Primer = 5'  
TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG  
16S Amplicon PCR Reverse Primer = 5'  
GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC

Library  
Preparation



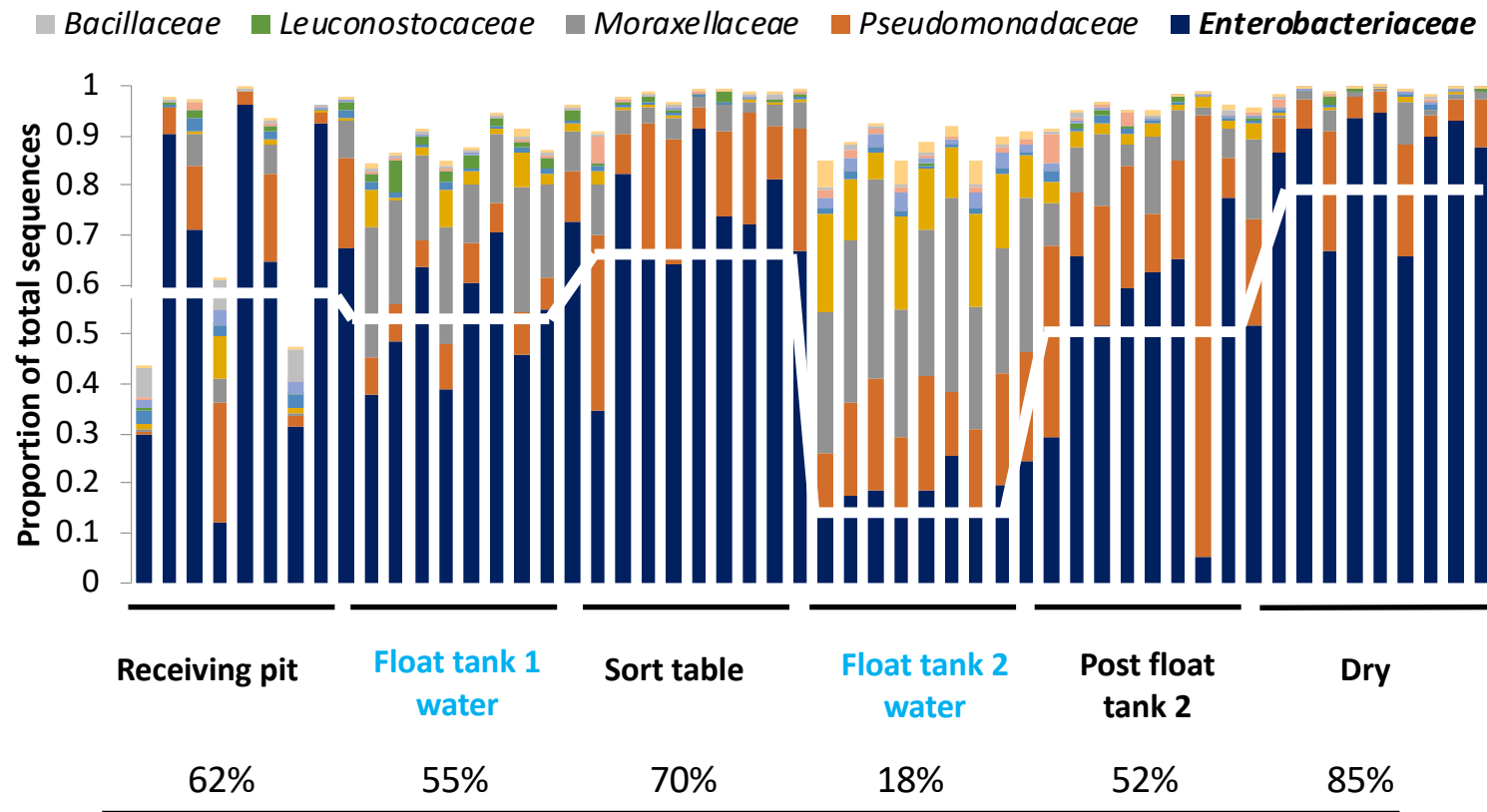
Illumina MiSeq

Sequencing

Sheng and Wang

# Anticipated Data Outcome

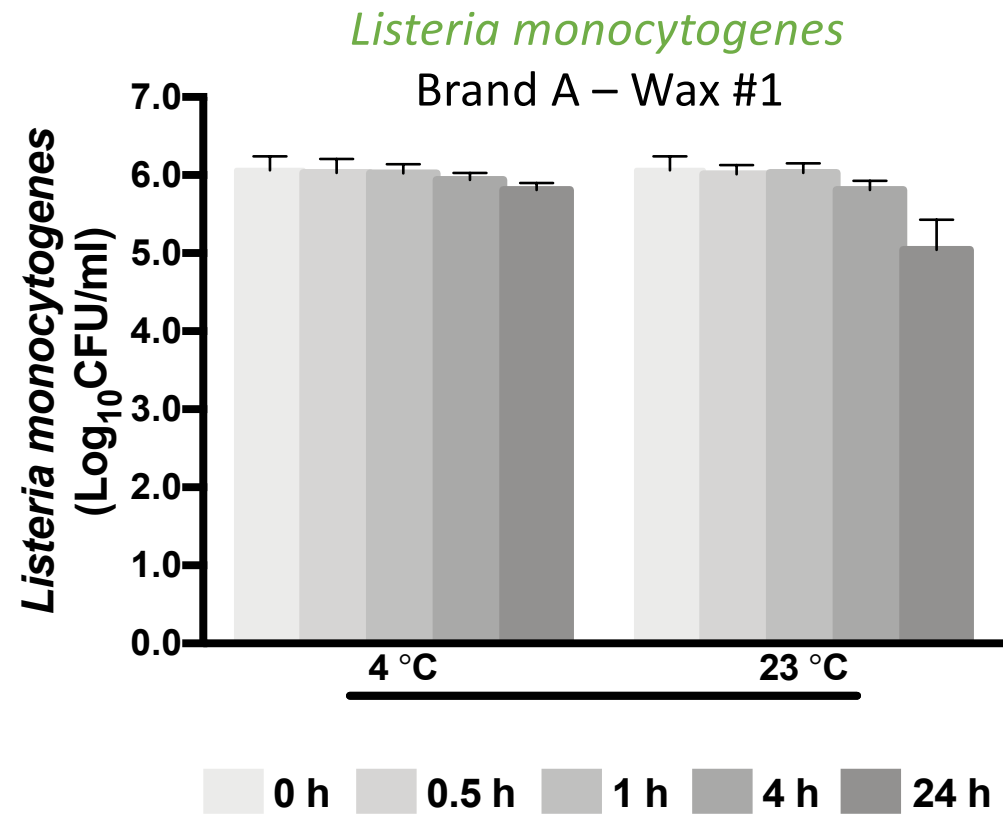
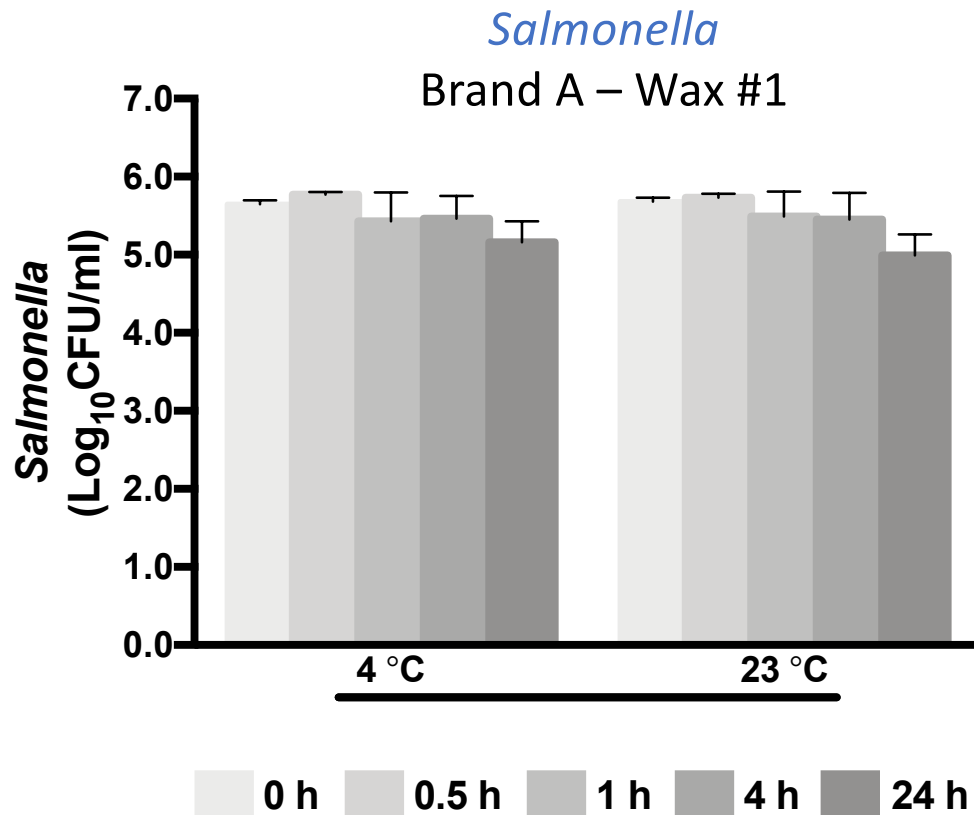
## E.g. shown for WALNUTS



Davidson and Harris, unpublished

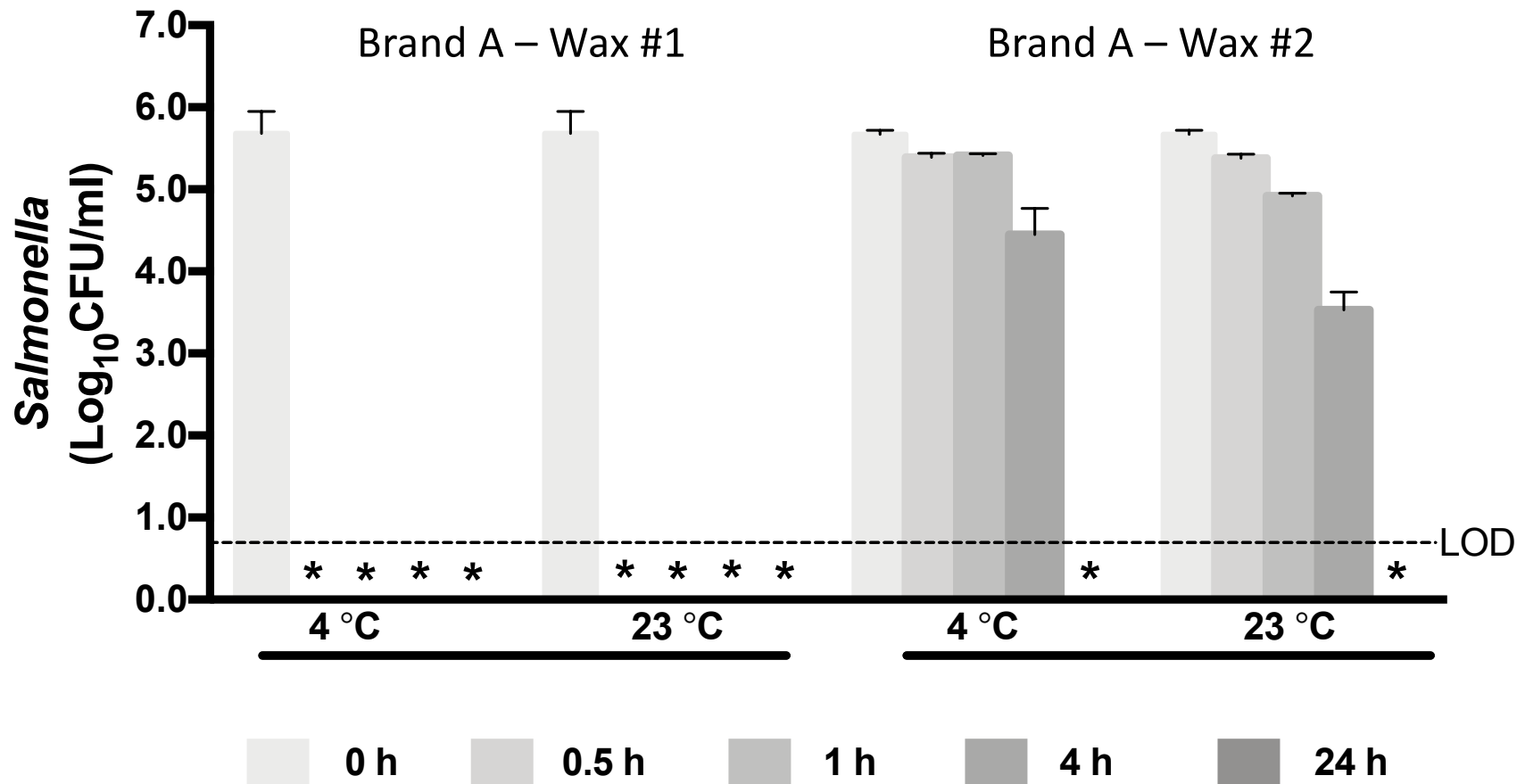
Average *Enterobacteriaceae* proportion per location

# Survival in Lemon Storage Wax



# Survival of *Salmonella* in Citrus Finishing Wax

Sheng and Wang



# Citrus Food Safety Plans

- Each packinghouse and pack line
  - Engagement
- Foundational programs
  - GMPs/Sanitation/Monitoring/Adjusting
- Water quality – dump tanks and recirculation
  - What are the controls to prevent cross contamination?
  - How are they measured?
  - Validate/verify measurements.
  - Corrective actions?