



# **FOOD SAFETY ISSUES: MICROBIOLOGICAL EVALUATION OF MECHANICALLY-HARVESTED CITRUS FRUIT, 2005 - 2009**

Michelle D. Danyluk  
Citrus Mechanical Harvesting Field Day  
April 22<sup>nd</sup>, 2009  
mddanyluk@ufl.edu



**UNIVERSITY OF  
FLORIDA**  
**IFAS**

# STUDY OBJECTIVES

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- ✘ Understand the impact of mechanical harvesting on:
  1. Fruit surface microflora
  2. Corresponding juice microflora
- ✘ Different harvesting systems:
  - + OXBO 3210 pull-behind harvester (catch frame)
  - + OXBO 3220 self-propelled continuous canopy shaker
  - + OXBO Pick-Up Machine
  - + CMNP Application

# STUDY OBJECTIVES

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- ✘ Fruit collected:
  - + OXBO 3210 pull-behind harvester (catch frame)
    - ✘ Hand, ground, catch frame (CF)
  - + OXBO 3220 self-propelled continuous canopy shaker
    - ✘ Hand, ground
  - + OXBO Pick-Up Machine
    - ✘ Hand, ground, Pick-Up (PU)
  - + CMNP Application
    - ✘ Hand, ground; CMNP, No CMNP



# STUDY OBJECTIVES

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## ✘ Microbiological tests

- + Total Plate Count – enumerates most microorganisms
- + Acidophilic Plate count – enumerates microorganisms capable of growth in Acid conditions
- + Generic *Escherichia coli* enrichment – identifies if generic *E. coli* a possible fecal indicator is present
- + *Salmonella* enrichment – identifies if *Salmonella* is present
- + *Alicyclobacillus* enumeration – enumerates *Alicyclobacillus*, a juice spoilage organism

# METHODS & MATERIALS

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- ✘ Fruit per trial
  - + 25 non-defective fruit randomly selected from each of the sample groups described
  
- ✘ Fruit brought to lab for microbiological analysis
  - + 30 mL buffer; shake/rub/shake to remove microorganisms from fruit surface
  - + Plate on Plate Count Agar (for total aerobic plate count – APC) and Orange Serum Agar (for total acidophilic plate count)
  - + *Alicyclobacillus* count – Samples heat shocked 75°C for 15 min, and plated onto Ali agar
  - + Results reported in log CFU/orange or CFU/orange (Ali)

# METHODS & MATERIALS

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- ✘ Composite samples for *Salmonella* and *E. coli* analyses
  - + Due to expense and time constraints
  - + Buffer samples were composited for every five fruit
  - + VIP *Salmonella* test kit (BioControl) and E\*Colite™ test kit (Charm Sciences)
- ✘ Parallel testing for juice samples aseptically prepared from sample fruit (reported as CFU/ml juice and/or presence of pathogen or indicator organism)
- ✘ Methods detailed in: Parish et al., 2001. Proc. Fla. State. Hort. Soc. 114:174-176.



# STUDY OBJECTIVES

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- × Four year study:

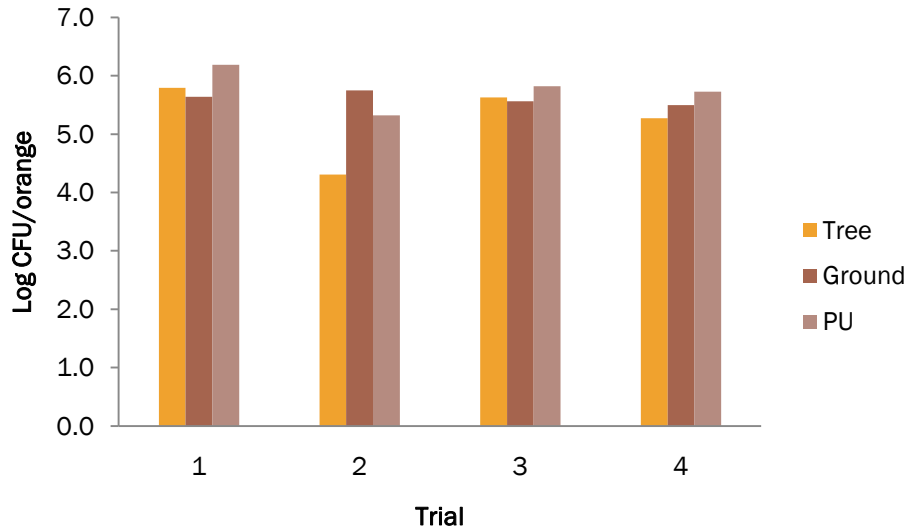
  - + 2005-2006

    - × Hand, ground, pick-up machine

    - × Fruit only

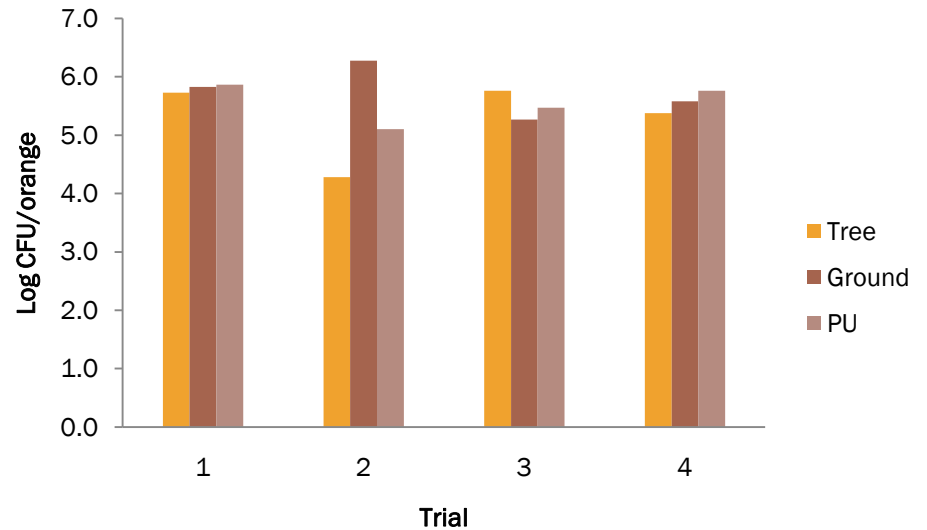
    - × Total counts, Acidophilic count, *Salmonella*, generic *E. coli*

# 2005 - 2006 - FRUIT



Total Plate Count  
Significant differences only in  
Trial 1 and Trial 2

Acidophilic Count  
Significant differences only in  
Trial 2





# 2005 - 2006 - FRUIT

*E. coli* and *Salmonella* enrichment

	<b>Tree</b>	<b>Ground</b>	<b>PU</b>
<i>E. coli</i>	0/20 <sup>z</sup>	0/20	0/20
<i>Salmonella</i>	0/20	0/20	0/20

<sup>z</sup> (number of positive tests)/ (number of total enrichments)

## Take Away message:

1. No consistent significant differences in fruit surface microflora
2. No pathogens or fecal indicators isolated.

# STUDY OBJECTIVES

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- × Four year study:

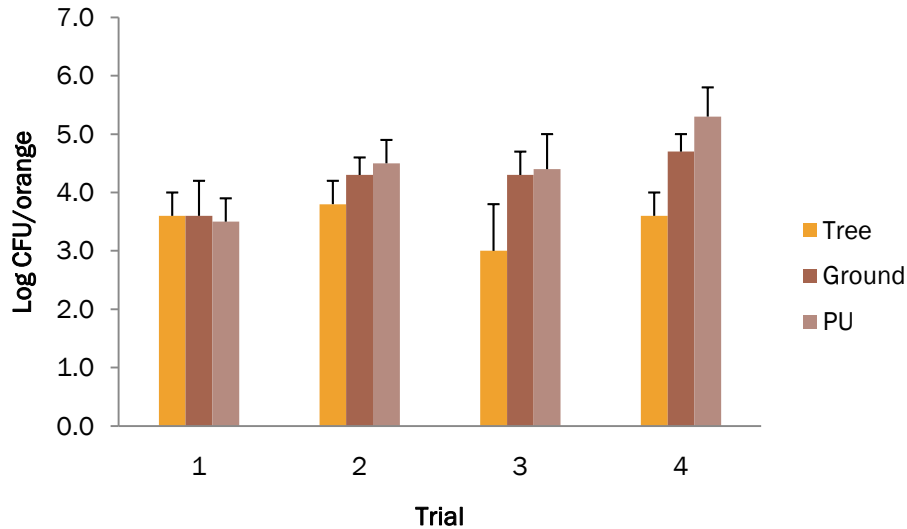
  - + 2006-2007

    - × Hand, ground, pick up machine

    - × Fruit and **juice**

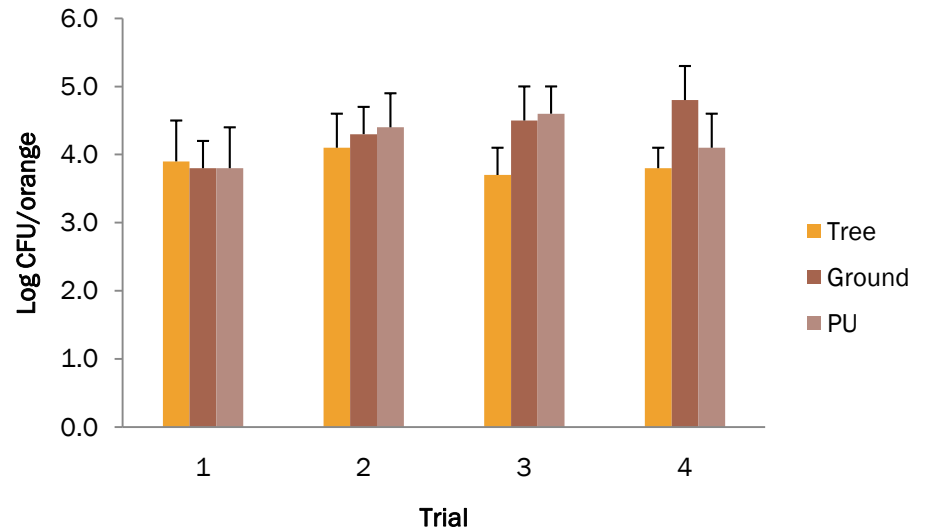
    - × Total counts, Acidophilic count, *Salmonella*,  
generic *E. coli*

# 2006 - 2007 - FRUIT



Total Plate Count  
Significant differences only in  
Trial 4

Acidophilic Count  
Significant differences only in  
Trial 3





# 2006 - 2007 - FRUIT

## *E. coli* and *Salmonella* enrichment

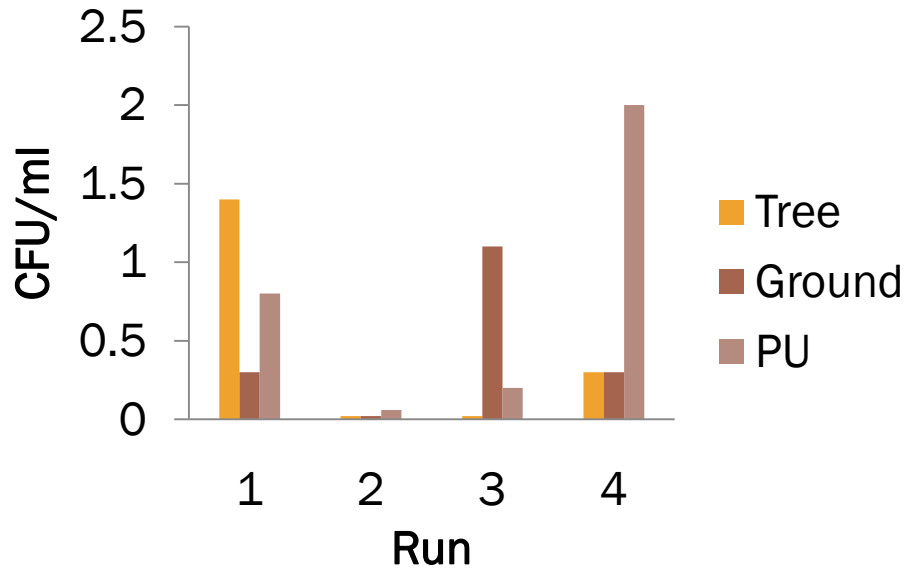
Trial	Tree	Ground		PU
		<i>E. coli</i> enrichments		
1	0 <sup>z</sup>	<b>3</b>	<b>3</b>	
2	0	0	0	
3	0	<b>1</b>	0	
4	0	0	0	
<i>Salmonella</i> enrichments				
1	0	0	0	
2	0	<b>1</b>	0	
3	0	0	0	
4	0	0	0	

<sup>z</sup>Number positive out of 5 enrichments

## Take Away message:

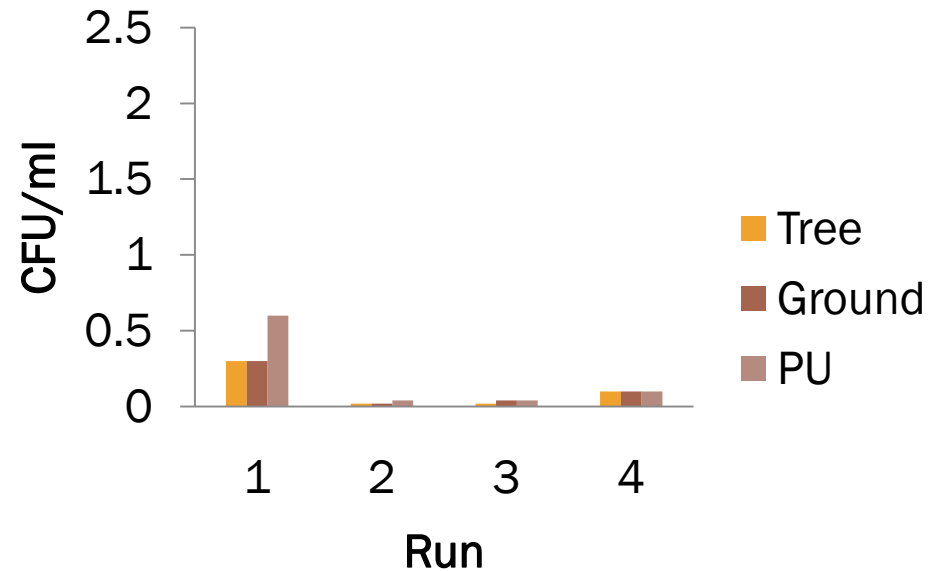
1. No consistent significant differences in fruit surface microflora
2. Pathogens and fecal indicators isolated, but not in the same trial, all from fruit that has touched the ground

# 2006 - 2007 - JUICE



Total Plate Count  
No significant differences

Acidophilic Count  
No significant differences



# 2006 - 2007 - JUICE

## *E. coli* and *Salmonella* enrichment

Trial	Tree	Ground	PU
	<i>E. coli</i> enrichments		
1	0 <sup>z</sup>	0	0
2	0	0	0
3	0	0	0
4	0	0	0
<i>Salmonella</i> enrichments			
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0

<sup>z</sup>Number positive out of 5 enrichments

## Take Away message:

1. No consistent significant differences in juice microflora
2. Pathogens and fecal indicators not isolated



# STUDY OBJECTIVES

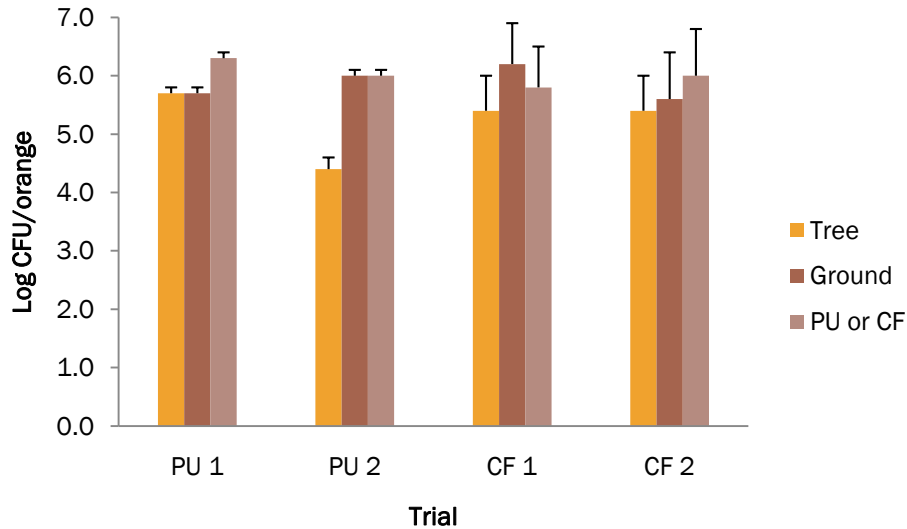
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- × Four year study:

  - + 2007-2008

    - × Hand, ground, pick-up machine, **catch frame**
    - × Fruit and juice
    - × Total counts, Acidophilic count, *Salmonella*, generic *E. coli*

# 2007 - 2008 - FRUIT

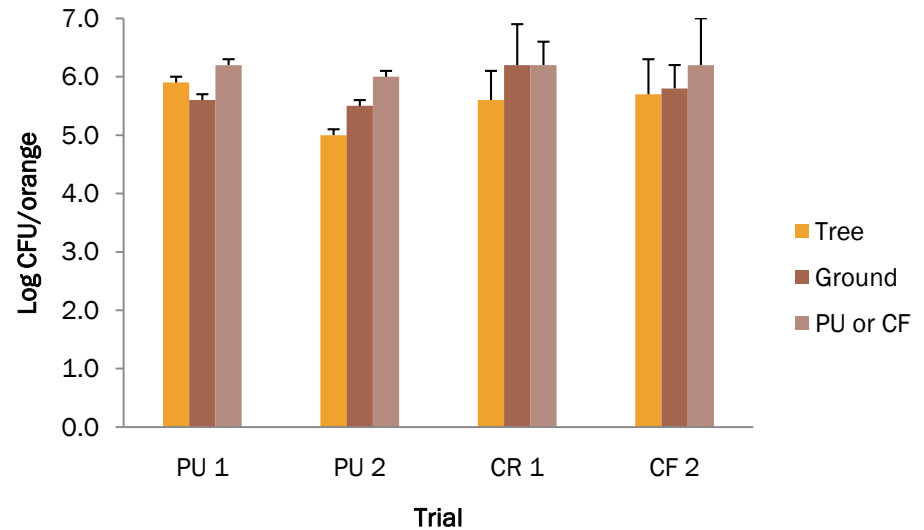


## Total Plate Count

Significant differences both Pick Up trials, but not in Catch Frame trials

## Acidophilic Count

Significant differences both Pick Up trials, but not in Catch Frame trials



# 2007 - 2008 - FRUIT

## *E. coli* and *Salmonella* enrichment

Trial	Tree	Ground	PU/CF
	<i>E. coli</i> enrichments		
PU 1	0 <sup>z</sup>	0	0
PU 2	0	0	<b>5</b>
CF 1	0	<b>1</b>	<b>4</b>
CF 2	0	0	0
<i>Salmonella</i> enrichments			
PU 1	0	0	0
PU 2	0	0	0
CF 1	0	0	0
CF 2	0	0	0

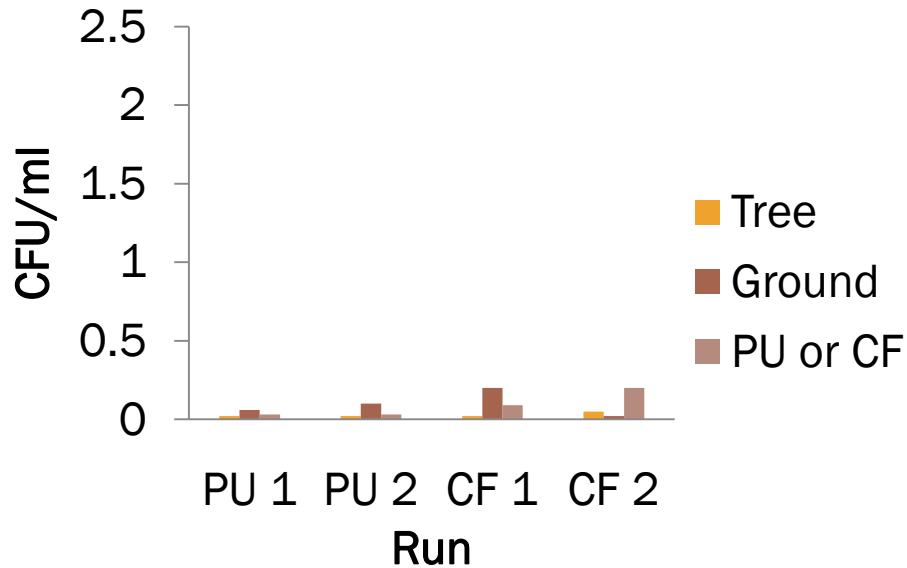
<sup>z</sup>Number positive out of 5 enrichments

## Take Away message:

1. No consistent significant differences in fruit surface microflora
2. Fecal indicators isolated in all enrichments from PU 2, and in four of five enrichments from CF 1. **These data indicate a potential for cross contamination during MH.**

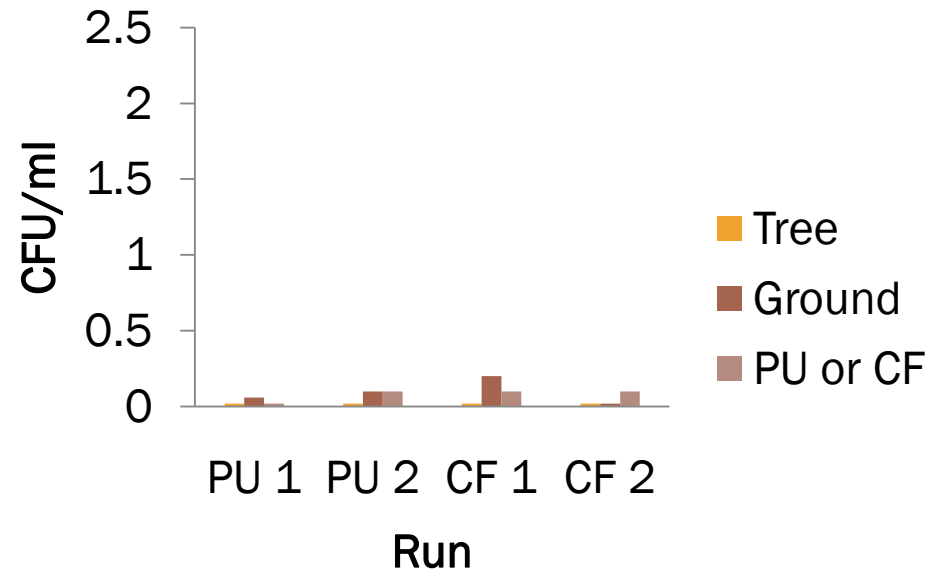


# 2007 - 2008 - JUICE



Total Plate Count  
No significant differences

Acidophilic Count  
No significant differences



# 2007 - 2008 - JUICE

## *E. coli* and *Salmonella* enrichment

Trial	Tree	Ground	PU
	<i>E. coli</i> enrichments		
1	0 <sup>z</sup>	0	0
2	0	0	0
3	0	0	0
4	0	0	0
<i>Salmonella</i> enrichments			
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0

<sup>z</sup>Number positive out of 5 enrichments

## Take Away message:

1. No consistent significant differences in juice microflora
2. Pathogens and fecal indicators not isolated

# STUDY OBJECTIVES

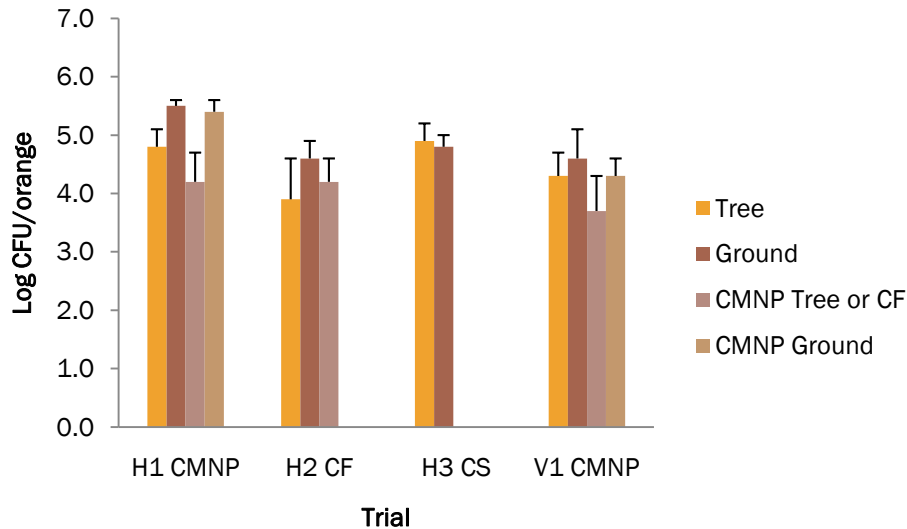
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- × Four year study:

  - + 2008-2009

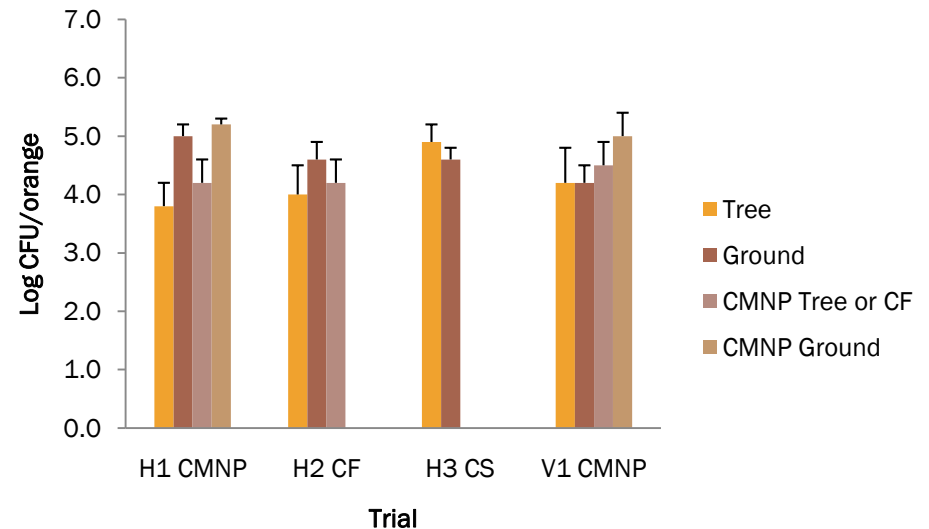
    - × Hand, ground, catch frame, **CMNP application**
    - × Fruit and juice
    - × Total counts, Acidophilic count, *Salmonella*, generic *E. coli*, ***Alicyclobacillus* count** (Fruit only)

# 2008 - 2009 - FRUIT



**Total Plate Count**  
Significant differences in Hamlin CMNP trial between tree and ground in both cases, no other significant problems

**Acidophilic Count**  
Significant differences only Hamlin CMNP trial between tree and ground in both cases





# 2008 - 2009 - FRUIT

## *E. coli* and *Salmonella* enrichment

Trial	Tree	Ground	CMNP Tree or CF	CMNP Ground
<i>E. coli</i> enrichments				
H1 CMNP	0 <sup>z</sup>	5	<b>1</b>	<b>1</b>
H2 CF	0	0	<b>4</b>	
H3 CS	0	0		
V1 CMNP	0	0	0	0
<i>Salmonella</i> enrichments				
H1 CMNP	0	0	0	0
H2 CF	0	0	0	
H3 CS	0	0		
V1 CMNP	0	0	0	0
<i>Alicyclobacillus</i> counts (CFU/orange)				
H1 CMNP	>0.04	<b>32 ± 22</b>	> 0.04	<b>32 ± 39</b>
H2 CF	> 0.04	> 0.04	> 0.04	
H3 CS	> 0.04	> 0.04		
V1 CMNP	> 0.04	> 0.04	> 0.04	> 0.04

<sup>z</sup>Number positive out of 5 enrichments

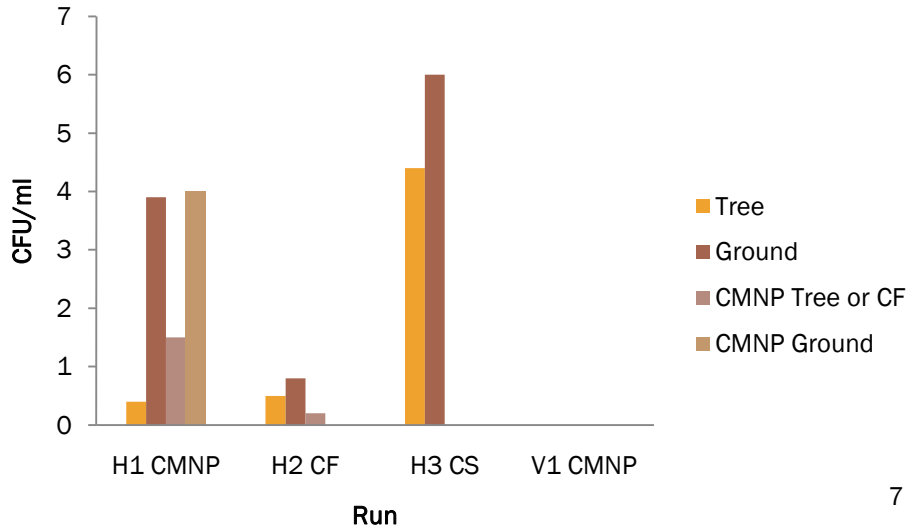
# 2008 - 2009 - FRUIT

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## Take Away message:

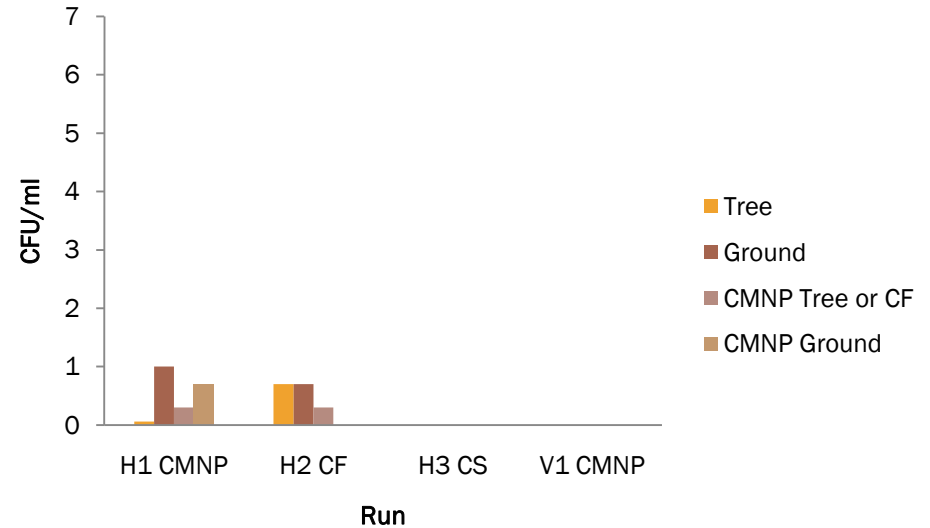
1. No consistent significant differences in fruit surface microflora, **CMNP application does not appear to impact total microflora levels.**
2. *E.coli* isolated in all but one CF enrichment again highlight **potential for cross contamination.**
3. Presence of *E. coli* on CMNP tree sample indicate potential contamination from spray. This highlights need **for good quality water use.**
4. Hamlin 1 trial occurred during rain and following irrigation.
5. *Alicyclobacillus* only isolated during one trial, and only from ground samples. **Weather may play a role** in contamination levels.

# 2008 - 2009 - JUICE



Total Plate Count  
Significantly higher counts in  
Hamlin 1, ground samples

Acidophilic Count  
No significant differences



# 2008 - 2009 - JUICE

## *E. coli* and *Salmonella* enrichment

Trial	Tree	Ground	CMNP Tree or CF	CMNP Ground
<i>E. coli</i> enrichments				
H1 CMNP	0 <sup>2</sup>	0	0	0
H2 CF	0	0	0	
H3 CS	0	0		
V1 CMNP	0	0	0	0
<i>Salmonella</i> enrichments				
H1 CMNP	0	0	0	0
H2 CF	0	0	0	
H3 CS	0	0		
V1 CMNP	0	0	0	0

<sup>2</sup>Number positive out of 5 enrichments

## Take Away message:

1. No consistent significant differences in juice microflora
2. Pathogens and fecal indicators not isolated



# SUMMARY 2005 - 2009

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- ✘ No indication that fruit in contact with ground is consistently and significantly higher in microbial surface contamination
- ✘ During PU and CF trials multiple samples positive for *E. coli* indicate the potential for cross contamination during MH
  - + Highlights the need for cleaning and sanitizing harvesting equipment
- ✘ Salmonella was only isolated in one sample in 2006 – 2007
  - + Further classified as *Salmonella* Munchen
- ✘ *E. coli* isolation from one tree orange following CMNP application indicate a need for good quality water use.
- ✘ Weather conditions and grove floor maintenance during the season may contribute to these results

# NEXT STEPS

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- ✘ Continue to sample 2 additional Valencia trials in 2009
- ✘ Repeat 2009 experiments in 2009-2010
- ✘ Continue to sample from fruit juice following CMNP adding:
  - + Brix, acid, and color

# ACKNOWLEDGEMENTS

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## ✘ Collaborators

- + Renee Goodrich-Schneider
- + Reza Eshani
- + Tim Spann
- + Bob Ebel

## ✘ Technical assistance

- + Lorrie Friedrich
- + Gwen Lundy

## ✘ OXBO International Corp.

## ✘ Grower cooperators

## ✘ State of Florida Citrus Industry Initiative funding



**QUESTIONS?**

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