How did that get there? Case studies in produce packing and production

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Green Skin Avocado

 Looks very different from the 'Hass' avocado grown in California (86% of domestic production)





Green Skin Avocado

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Green Skin Avocado

- Falls into one of three main types
 - West Indian
 - Guatemalan
 - Mexican



http://edis.ifas.ufl.edu/st435 http://edis.ifas.ufl.edu/fe956



Second most valuable tree crop in Florida

- 7,000 acres in Florida
- 60% of the tropical fruit industry
- 95% of commercial production is in Miami-Dade
- Orchard size between
 0.1 500 acres
 - 93% of farms are less than 15 acres

http://edis.ifas.ufl.edu/st435 http://edis.ifas.ufl.edu/fe956





Avocados and Food Safety Recalls

2014-2016 FDA sampling assignment – Whole fresh avocados

- 1,615 avocado samples tested for *Salmonella* and *Listeria monocytogenes*
- 70% imported, 30% domestic
- After 3 months (out of 18 total), sampling approach changed to only test the flesh for *L. monocytogenes*. Not the whole fruit.
- Salmonella prevalence at 0.74%
- *L. monocytogenes* prevalence at 17.73 for whole fruit and 0.24 of pulp



Avocados and Food Safety Recalls

2014 FDA initiated a sampling project to evaluate presence of foodborne pathogens on avocados in the US

- Included avocados that were grown and packed in Florida
- At least 4 recalls initiated for Florida Packers in 2014
- Two recalls for one firm



Avocados and Food Safety – What do we know?



Salmonella, Escherichia coli O157:H7, *Staphylococcus aureus*, and *Listeria monocytogenes* can grow in avocado pulp or juice at a range of temperature conditions (Arvizu-Medrano et al. 2001; Yigeremu et al., 2001; Iturriaga et al., 2002; Mutaku et al., 2005).

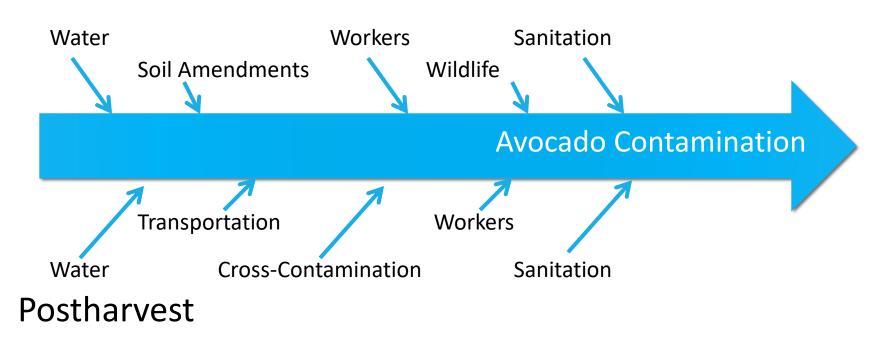
No work published on survival on whole fruit, potential for pathogen internalization during handling, or mitigation strategies to remove contamination once present.

No work done on green skin varieties grown in Florida.

Where was the *Salmonella* coming from?

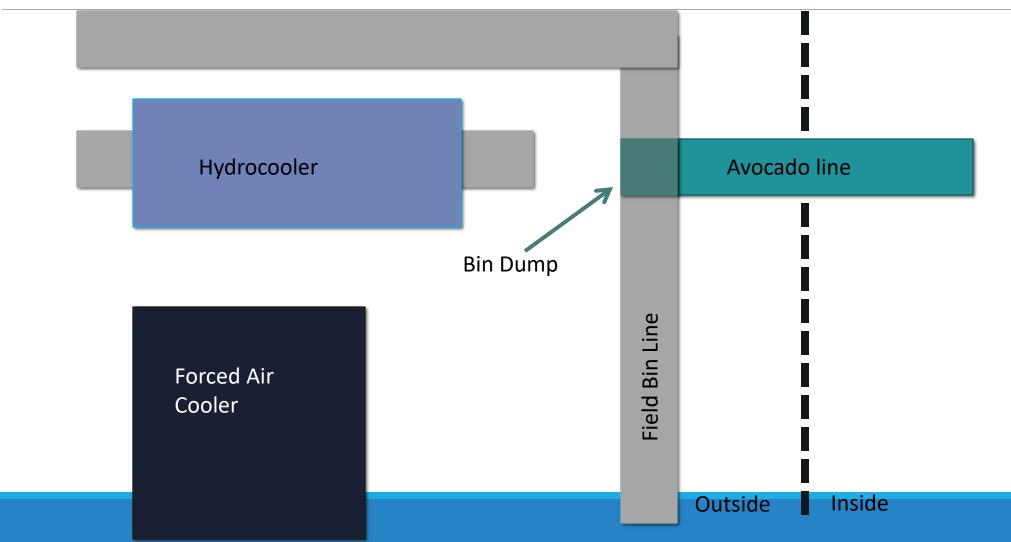


Following the second recall in the 2014 season from the same facility, we were asked to help identify the source of the *Salmonella*

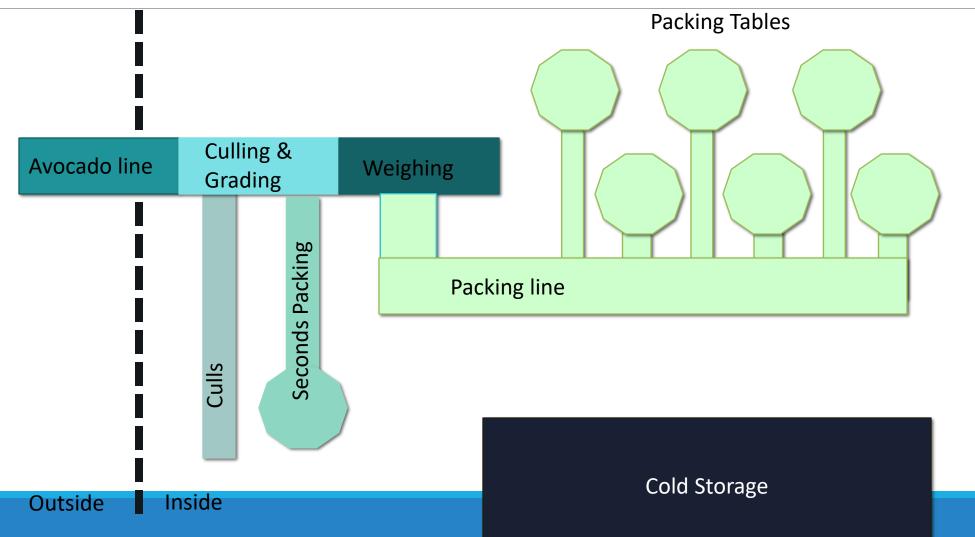


Preharvest











Visited the facility following a full day of operation to collect swab samples

- 198 samples, from zones 1-4
- Included water samples from hydrocooler

Facility Sanitation

- Food contact surfaces cleaned either daily (packing tables), or weekly (weighing line)
- Hydrocooler water maintained at 14-17 ppm free Chlorine, 6.5-7.5 pH, concentration measured hourly by titration, water changed weekly



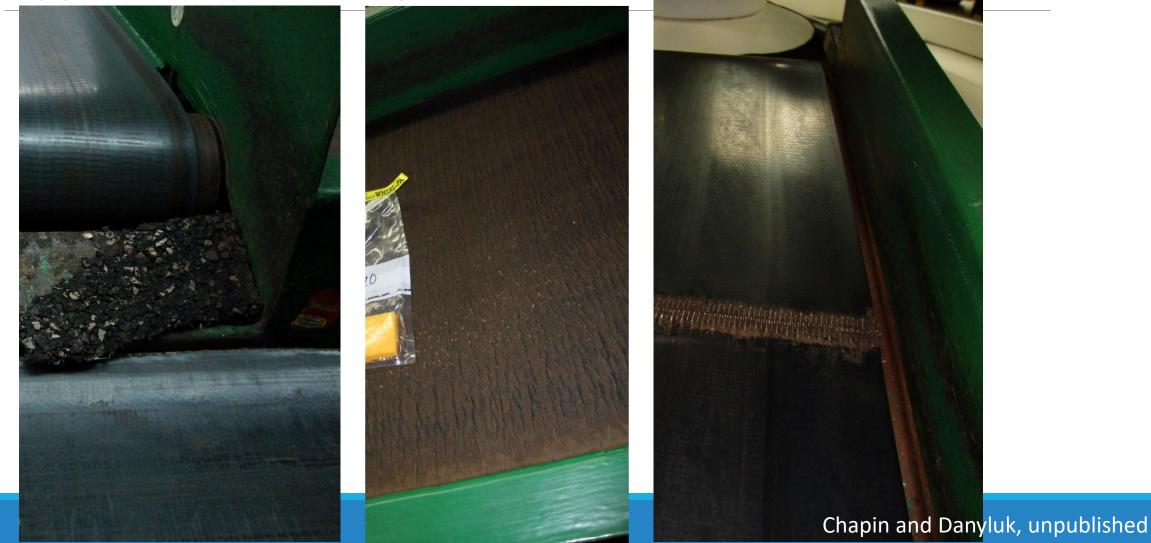
Methodology

All samples enriched following FDA BAM for Salmonella

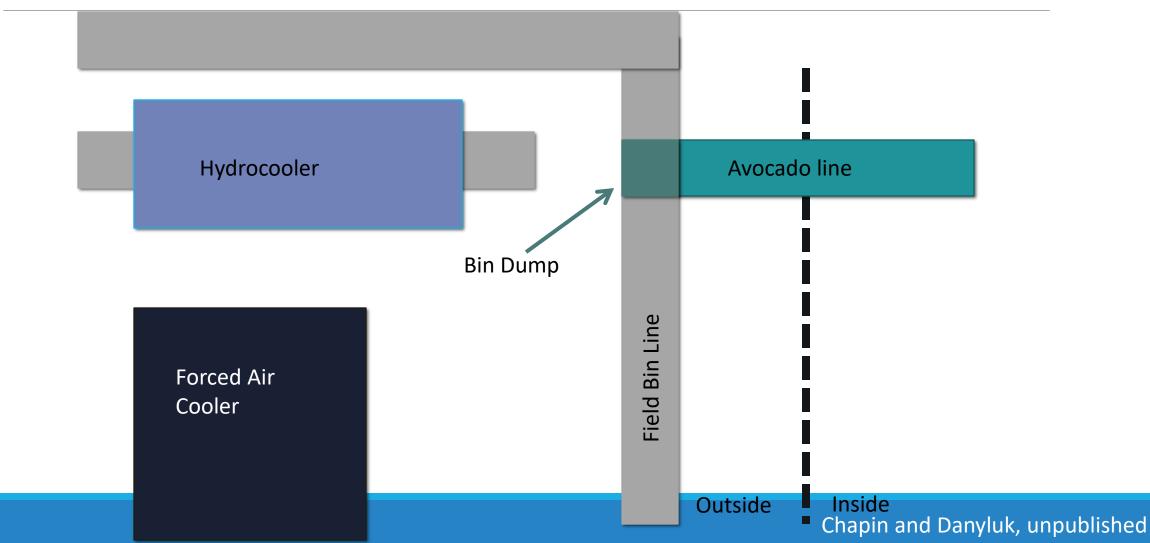
- Preenrichment (Lactose broth)
- Selective enrichment (Rv-R10 and TT broths)
- Selective & differential plating media (XLD, XLT-4, HE, and Chrome)
- Colony isolation and confirmation (TSI and LIA)
- PCR confirmation (invA)
- Serotyping
- Genetic Fingerprinting

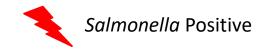


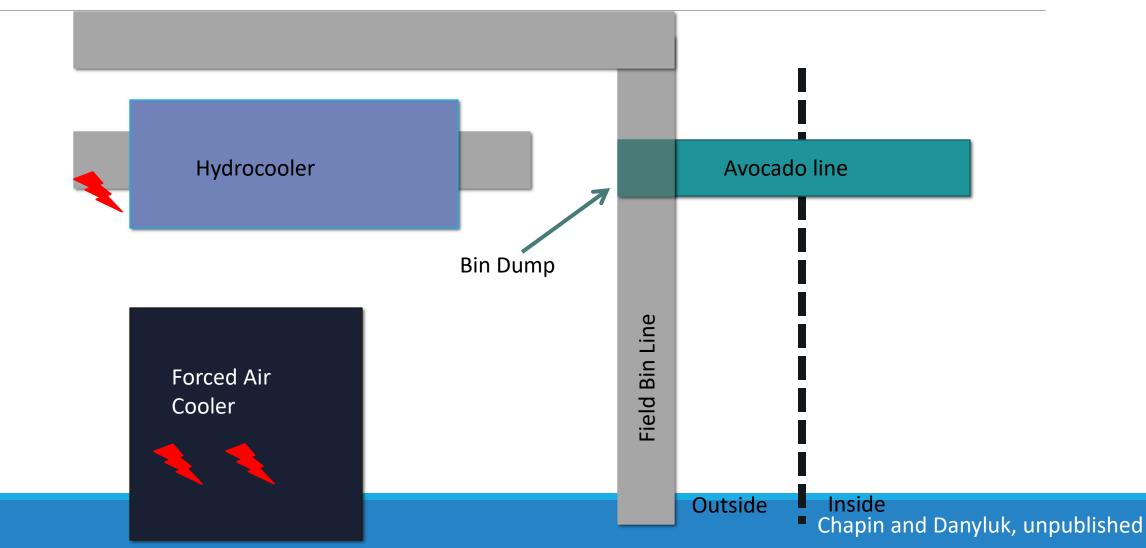












Postharvest Contamination





Preharvest Contamination

Visited three groves, two associated with the recalls, one currently storing soil amendments

 57 samples, including soil, water, drag swabs, standing water, bird feces, snails, biosolids, fence bordering horse pasture

Visited field bin storage

15 additional swabs



Methodology

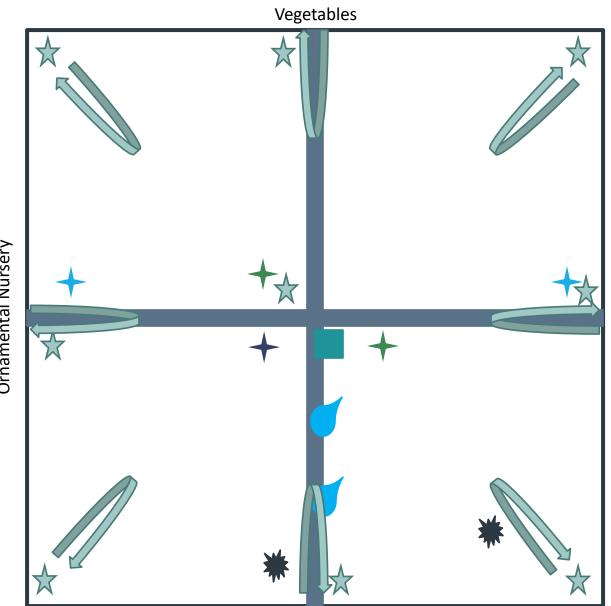
All samples enriched following FDA BAM for *Salmonella*

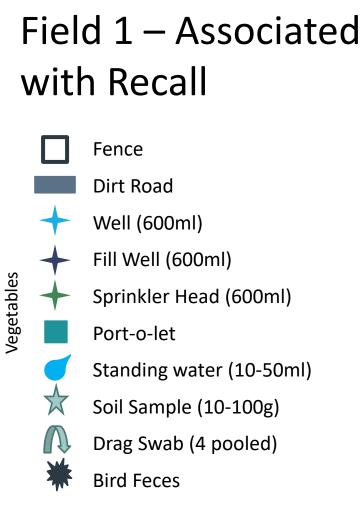
When water (14), soil (13) or biosolids (9) samples were big enough, samples were split, and ½ went through pre-enrichment in lactose, the other ½ went through pre-enrichment in buffered peptone water.

• Why?

 We keep seeing FDA reports using buffered peptone instead of lactose for pre-enrichment of environmental samples







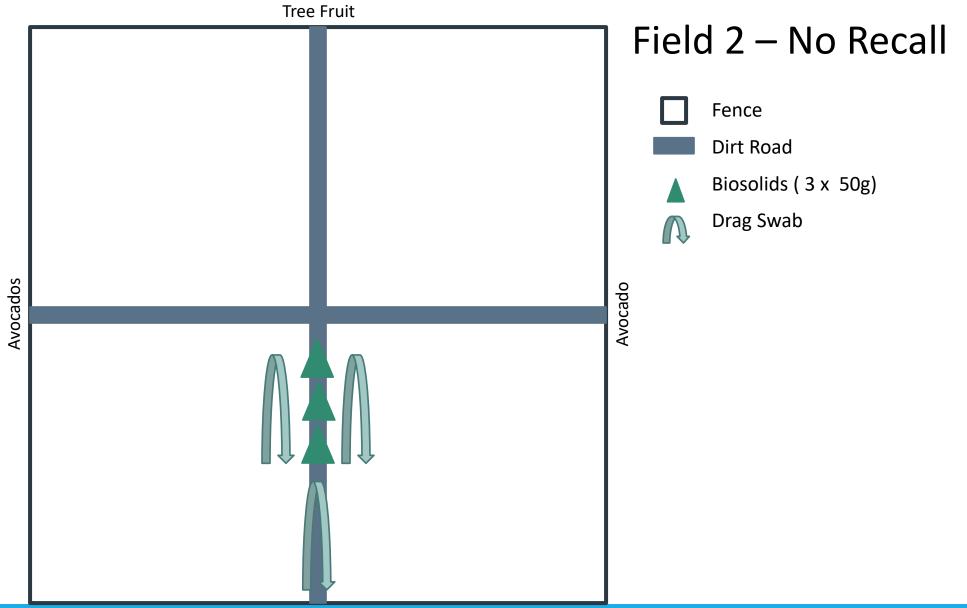
Vegetables

Ornamental Nursery

Horse Pasture









Biosolid Use

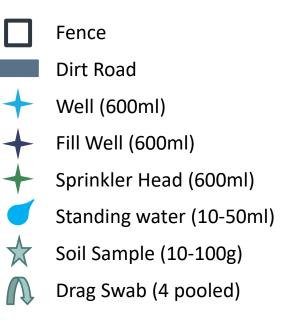
How were biosolids used in the orchard?

- Trees are side dressed with in throughout the year
- Can be close to harvest and can generate dust

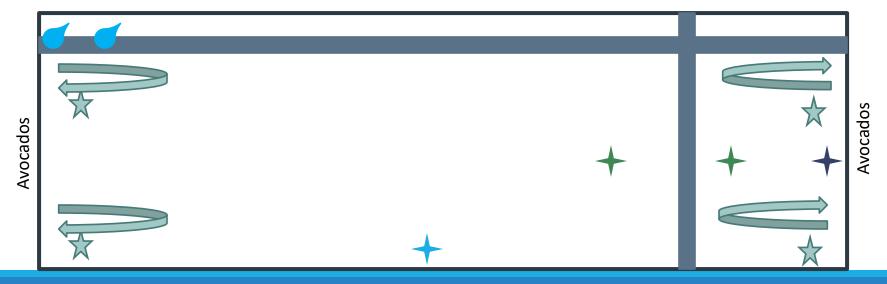




Field 3 – Associated with Recall



Avocados



Fallow Field



Field 3 – Grove was approximately 70 years old Chapin and Danyluk, unpublished



Field 3 – And home to a Burmese Python

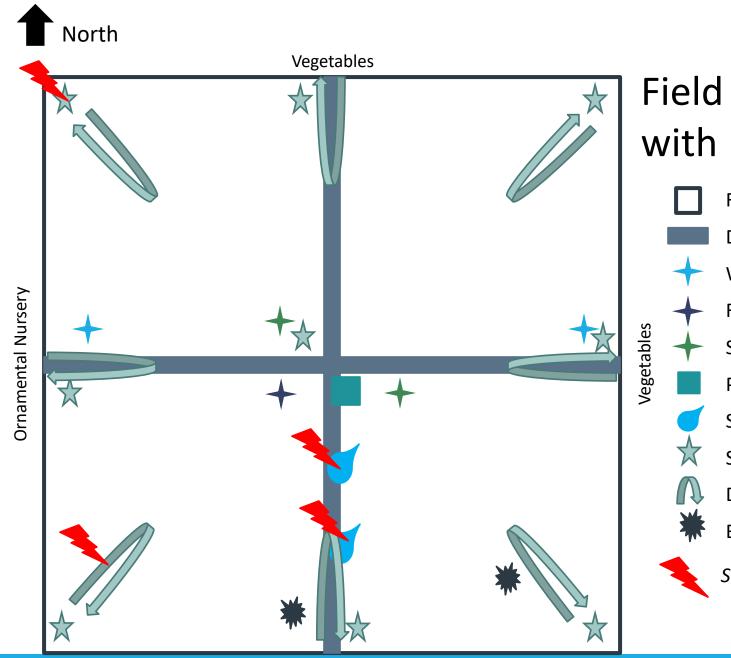
(that we found out about after we had sampled and wondered what was hissing at us)



Vegetables Field 1 – Associated 57 with Recall Fence Dirt Road Well (600ml) **Ornamental Nursery** Fill Well (600ml) Vegetables Sprinkler Head (600ml) Port-o-let \overline{X} Standing water (10-50ml) Soil Sample (10-100g) Drag Swab (4 pooled) **Bird Feces**

Vegetables

Horse Pasture

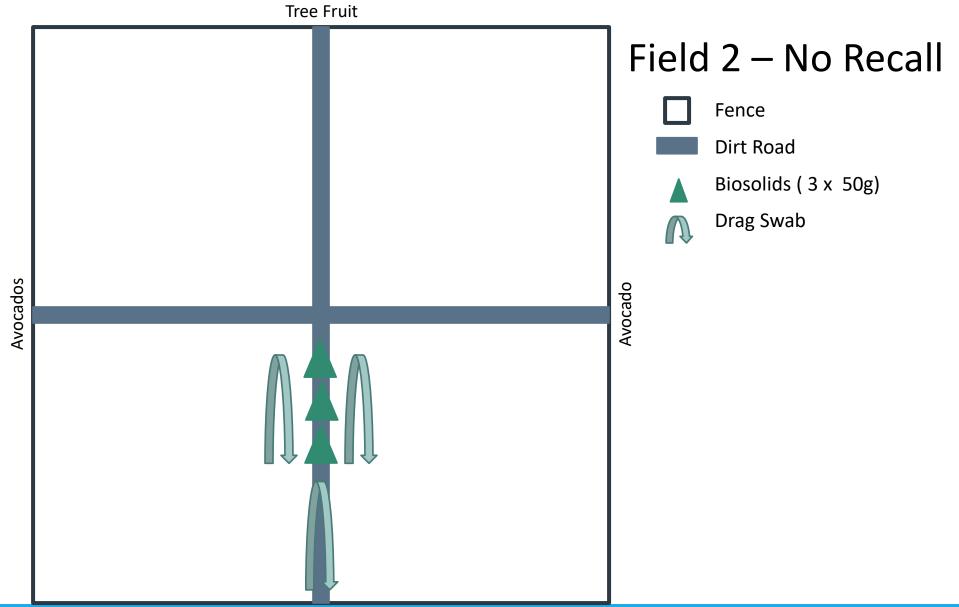




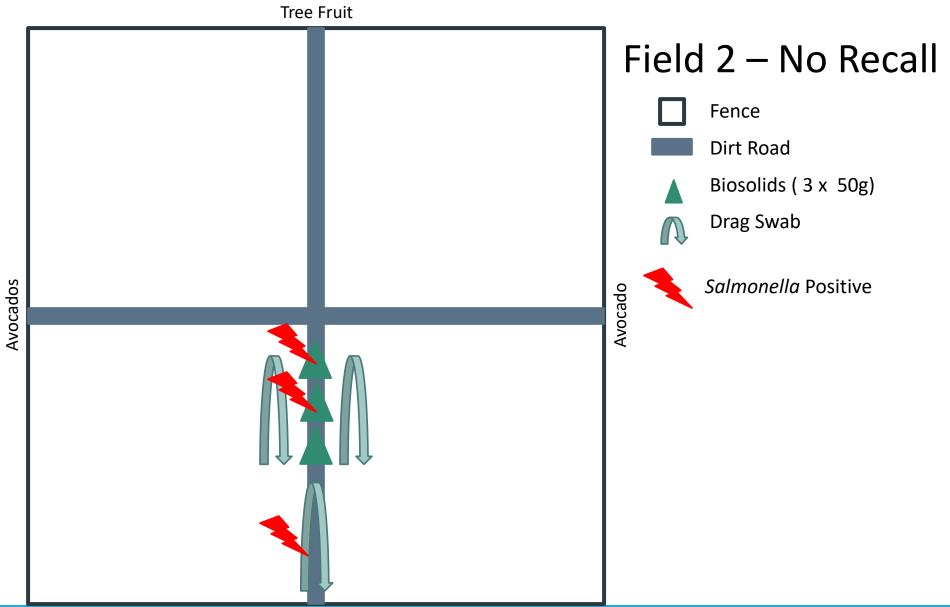
Horse Pasture

Vegetables









Vegetables



Methodology

With the remaining biosolids samples, we performed a 3 tube MPN for *Salmonella*

 Report results in MPN/4 g, based on the proposed standards from the initial proposed Produce Safety Rule for treated compost

L. monocytogenes	0 CFU / 5 g		
Salmonella	< 3 MPN / 4 g	< 3 MPN / 4 g	
<i>E. coli</i> O157	<0.3 MPN / 1 g		
Fecal coliforms	OF	<1000 MPN / 1 g	

Amount of *Salmonella* in the three biosolids piles

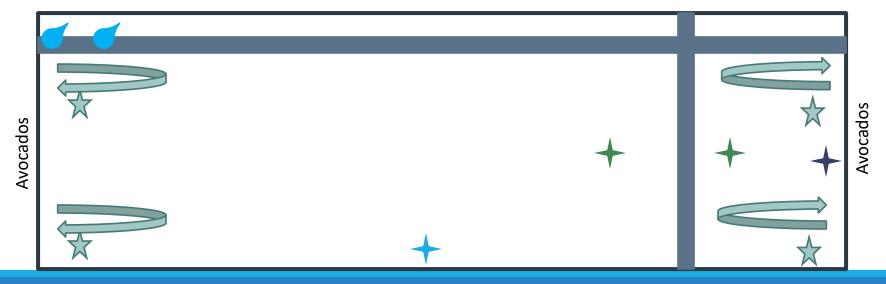
Biosolid Pile	Salmonella MPN/4 g Dry Weight		
1	0.64		
2		2.96	
3		3.38	



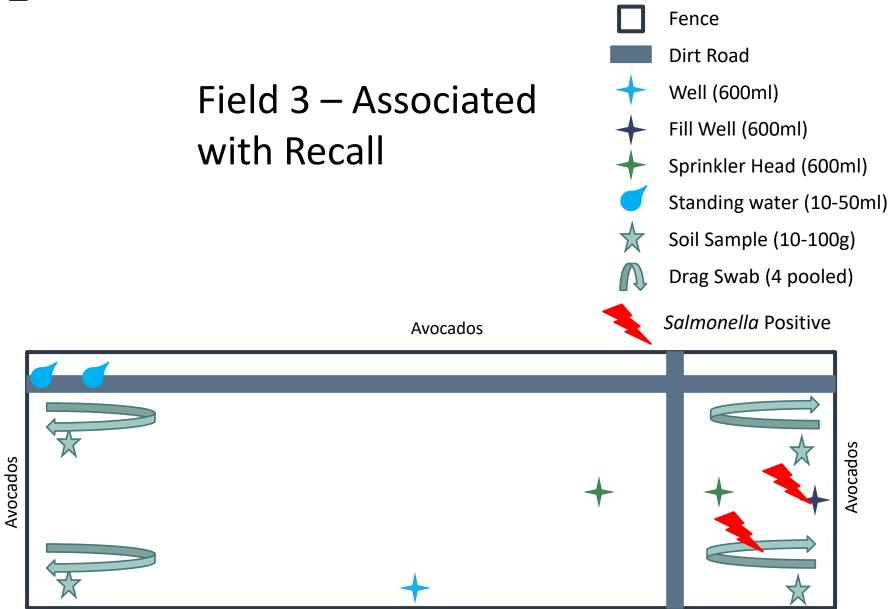
Field 3 – Associated with Recall

Fence
 Dirt Road
 Well (600ml)
 Fill Well (600ml)
 Sprinkler Head (600ml)
 Standing water (10-50ml)
 Soil Sample (10-100g)
 Drag Swab (4 pooled)

Avocados







Fallow Field



What Types of Salmonella?

Field	Location	Serotype	
Packinghouse	Field Bin in cold storage	Tennessee	
	Field Bin in cold storage	Tennessee	
	Ground by hydrocooler	Tennessee	
Field 1	Standing water 1	Madjorio	
	Standing water2	Infantis	
	NW Soil	Tennessee	
	SW Drag	Muenchen	
Field 2	Biosolids Pile 2	Cerro	
	Biosolids Pile 3	Branderup	
	South Biosolids Drag	Cerro	
Field 3	Fill Well	Kedougou	
	SE Soil	Tennessee	



What Types of Salmonella?

Key

MDD 437/#1

MDD 437/#2

MDD 441

MDD 443

MDD 435

MDD 443/#1

MDD 435/#1

MDD 440/#1

MDD 440/#2

MDD 440

MDD 436

MDD 438

MDD 436/#1

kb 0.00 500

PFGE-Xba1

PFGE-Xba1

Sample NuSample Location		Serotype
237	Pile #2 Biosolids	Cerro
237	Pile #2 Biosolids	Cerro
223	Standing Water with Birds (Entrance)	Infantis
245	Grove Fill Well	Kedougou
245	Grove Fill Well	Kedougou
175	Packinghouse Floor	Tennessee
175	Packinghouse Floor	Tennessee
211	Grove SW drag swab	Muenchen
211	Grove SW drag swab	Muenchen
211	Grove SW drag swab	Muenchen
240	Pile #3 Biosolids	Braenderup
240	Pile #3 Biosolids	Braenderup
205	Grove Standing Water	Madjorio

Salmonella Pre-Enrichment Lactose broth vs BPW



No difference seen between two preenrichment media

We used the full spread of enrichment broths and plating media, following each preenrichment

• Not all samples positive, or typical colonies on all media types

When working with environmental samples, use all enrichment media, if possible

Sample Type	Number Tested	Total Positive	Positive Lactose Broth	Positive from BPW
Water	14	4	4	4
Soil	13	2	2	2
Biosolids	9	2	2	2

Summary of Sampling Results & Recommendations



Biosolids contained Salmonella, and could be introducing it into the grove

 Subsequent testing of biosolids, as they arrived at the fields confirmed they were Salmonella positive

If field bins contacted the ground, or if dust was generated while field bins were in the grove, field bins could be contaminated.

Stacking of field bins could lead to contaminated product

An SSOP for field bin cleaning should be established



Changes Implemented

Discontinued use of Biosolids

• Shared results with other growers who were also using these biosolids

Implemented an SSOP for field bin cleaning, each bin following each use.





Populations (\log_{10} CFU/avocado; mean <u>+</u> std dev) of *Salmonella* on avocado surfaces following treatment on a spray roller system with polyvinyl chloride rollers (n=15).

Time (s)	NaOCl (100 mg/L)	ClO ₂ (5 mg/L)	PAA (80 mg/L)	Water
0	6.16 <u>+</u> 0.39 Aa ^a	6.35 <u>+</u> 0.25 Aa	5.94 <u>+</u> 0.42 Aa	6.37 <u>+</u> 0.18 Aa
5	< 2.13 <u>+</u> 0.24 BCa	4.31 <u>+</u> 0.55 Bb	< 2.39 <u>+</u> 0.61 BCDa	4.55 <u>+</u> 0.66 Bb
15	< 2.14 <u>+</u> 0.31 BCa	3.69 <u>+</u> 0.47 Cb	< 2.05 <u>+</u> 0.14 BCDa	3.42 <u>+</u> 0.63 Cb
30	< 2.11 <u>+</u> 0.23 BCa	2.86 <u>+</u> 0.45 Db	< 2.00 <u>+</u> 0.00 CDa	< 2.66 <u>+</u> 0.68 Db
60	< 2.00 <u>+</u> 0.00 Cab	2.47 <u>+</u> 0.34 Dbc	< 1.87 <u>+</u> 0.52 Da	2.57 <u>+</u> 0.45 Dc

^a Populations with different uppercase letters within a column or lowercase letters within a row are statistically different (P \leq 0.05).

Valdés Garrido and Danyluk, unpublished



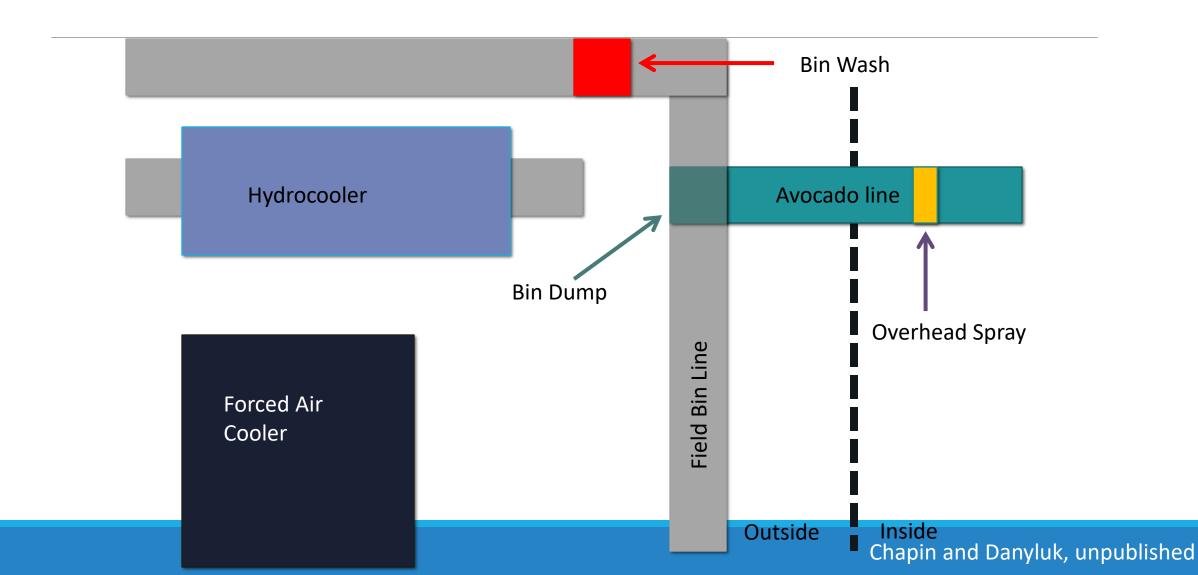
Changes Implemented

Added an overhead spray bar with sanitizer and single pass water to wash avocados



Upon retesting, all locations previously positive, no Salmonella was detected

Changes Implemented



Final thoughts...

Produce outbreaks continue to happen

- Fruits and vegetables contribute to the burden of foodborne disease
- We can learn a lot from outbreaks and recalls

Produce Safety is critical during production

- Growing produce outside means there are many risks
- Good management can help mitigate, manage, and minimize risks
- Each situation is different, tailored solutions are key

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Scientific Challenges and Cost-Effective Management of Risks Associated with Implementation of Produce Safety Regulations

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Questions?



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