

Sanitization of Fresh Produce Using Fit Produce Rinse

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Fresh Produce Studies

- Alfalfa Seeds
- Alfalfa Sprouts
- Radish
- Lettuce
- Tomatoes

General Produce Methods

- Pathogen cocktail - 5 to 6 strains
 - *E. coli* O157:H7 (Gram -)
 - *Salmonella* (Gram -)
 - *Listeria* (Gram +)
- Fit applications
 - Designed as neat spray
 - Spray & rub - tomato
 - Spray - Radish & lettuce
 - Optimize efficacy on difficult fresh produce
 - Soak - Alfalfa seed/sprouts

Gram (-) Pathogen Cocktails

- *E. coli* O157:H7

- E0018 (calf feces)
- 932 (human feces)
- 994 (salami)
- H1730 (human feces)
- F4546 (human feces)

- *Salmonella*

- *agona* (alfalfa sprouts)
- *enteritidis* (human feces)
- *gaminarum* (orange juice)
- *michigan* (cantaloupe)
- *montevideo* (tomato)
- *typhimurium* (human feces)

Gram (+) Pathogen Cocktail

- *Listeria monocytogenes*
- Scott (human)
- LCDC-81-861 (cabbage)
- V7 (milk)
- 101M (beef)
- 108 (beef)

Alfalfa Seeds Methods

- *E. coli* O157:H7 & *Salmonella* cocktails
 - 6 ml - 24 h culture/strain in 1 L DI water
 - No soil load
- 1 kg alfalfa seeds in L, mix 1 min
- Seed Prep
 - Decant, seed/cheese cloth; hood dry 48 h
 - Store (age) in baggie 5°C \geq 1 week
- Soak Treatments

dry to
6-7% moisture
content of seeds
(similar to
what industry
uses)

Alfalfa Seeds Methods

- 10 g aged, inoculated seed
- 40 ml desired soak treatments
 - Water control
 - D/E neutralizer control
 - 200 & 20,000 ppm Cl⁻
 - Fit
- Mix - rotary shaker (100 rpm)
- Exposure - 15 or 30 mins.
- Duplicate 10 g samples/rep; 3 reps

pH = 6.8 - (Cl⁻) used approximate ratio recommended for industry - probably industry using

Microbial Analysis

- Soak Solution

- Serial dilute in 0.1% peptone
- Plate
 - *E. coli* - SMAN
 - *Salmonella* - BSAN

- Seeds (decanted)

- 20 ml D/E
- Stomach 30 s
- Plate
 - *E. coli* - SMAN
 - *Salmonella* - BSAN
- Enrichment
 - Plate on SMAN or BSAN
 - Biochemical confirmation

Alfalfa Seed Data
***E. coli* O157:H7 cocktail**

Treatment	Time	Wash (peptone)	log Reduction vs water	D/E Stomach	log Reduction vs water
<i>E. coli</i> O157:H7		log ₁₀ cfu/gm seed ¹		log ₁₀ cfu/gm seed ¹	
Water Control	15 min	5.12 ab	NA	5.31 a	NA
	30 min	5.34 a	NA	5.25 a	NA
D/E Control	15 min	4.95 b	0.39	4.65 b	0.66
	30 min	4.78 b	0.56	5.01 ab	0.24
200 ppm Cl-	15 min	4.32 c	0.80	3.96 c	1.35
	30 min	4.21 c	1.13	3.95 c	1.30
20,000 ppm Cl-	15 min	BDL*	~5.12	2.04 e	3.27
	30 min	BDL*	~5.34	3.06 d	2.19
Fit	15 min	BDL*	~5.12	BDL**	5.31
	30 min	BDL*	~5.34	BDL**	5.25

¹ Mean values in the same column that are not followed by the same letter are significantly different (P ≤ 0.05)

* < 1 cfu/2.5 g seeds - Below detectable level

** < 1 cfu/5 g seeds

(Plating Media - SMAN; Sorbitol MacConkey Agar plus 50 ppm nalidixic acid)

Alfalfa Seed Data
***Salmonella* cocktail**

Treatment	Time	Wash (peptone)	log Reduction vs water	D/E Stomach	log Reduction vs water
<i>Salmonella</i>		log ₁₀ cfu/gm seed ¹		log ₁₀ cfu/gm seed ¹	
Water Control	15 min	6.54 a	NA	6.20 b	NA
	30 min	6.56 a	NA	6.26 a	NA
D/E Control	15 min	6.46 a	0.08	5.94 ab	0.26
	30 min	6.50 a	0.06	6.10 ab	0.16
200 ppm Cl-	15 min	4.81 b	1.73	4.37 c	1.83
	30 min	4.84 b	1.72	4.29 c	1.97
20,000 ppm C	15 min	BDL*	~6.54	3.76 d	2.44
	30 min	BDL*	~6.56	3.94 d	2.32
Fit	15 min	BDL*	~6.54	3.32 e	2.88
	30 min	BDL*	~6.56	3.64 d	2.62

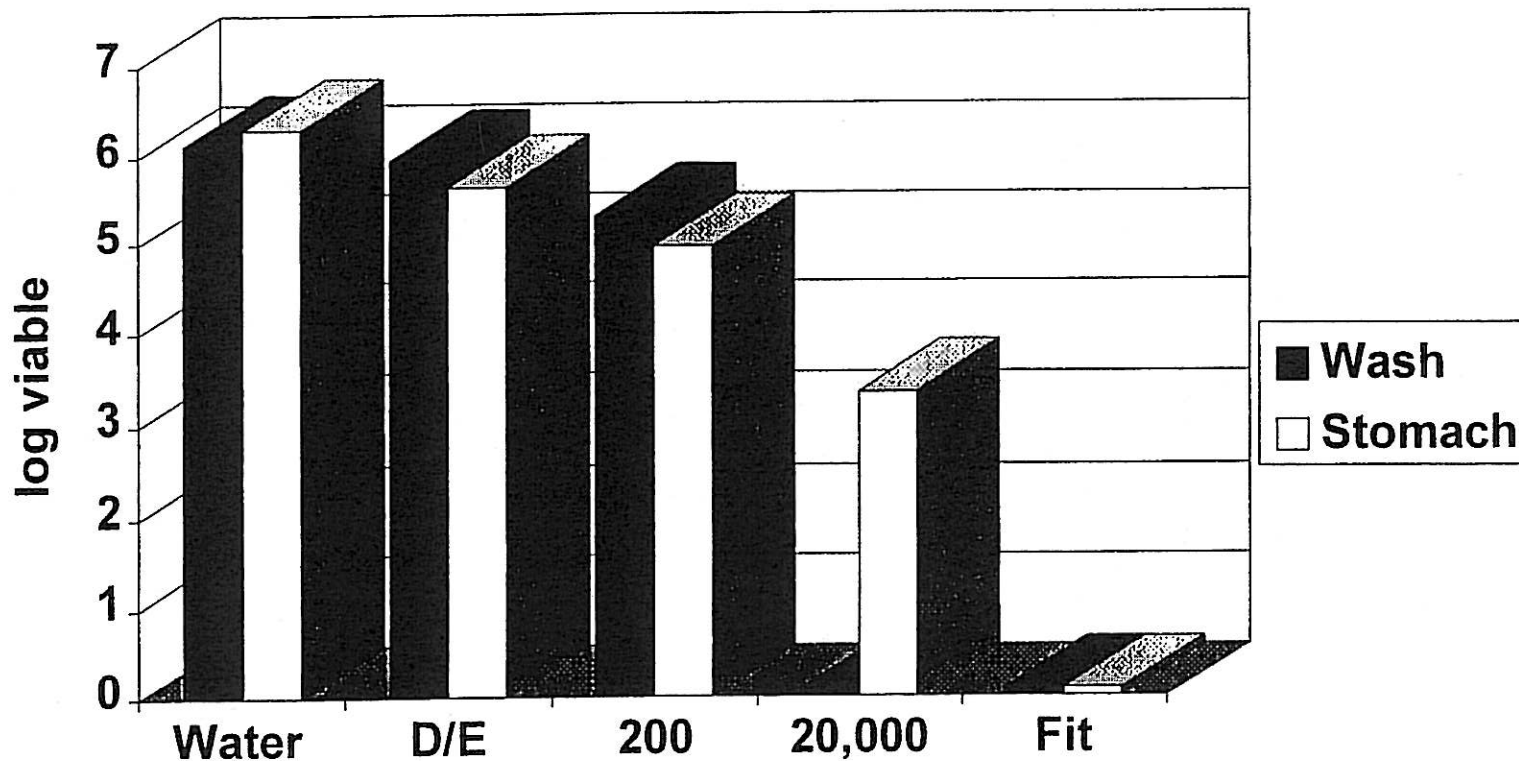
¹ Mean values in the same column that are not followed by the same letter are significantly different ($P \leq 0.05$)

* < 1 cfu/2.5 g seeds

(Plating Media - BSAN; Bismuth Sulfite Agar plus 50 ppm nalidixic acid)

Viability Counts (log) on Seeds *E. coli* O157:H7 Cocktail

2 samples/rep; 3 reps; n = 6
30 min data; 15 min similar

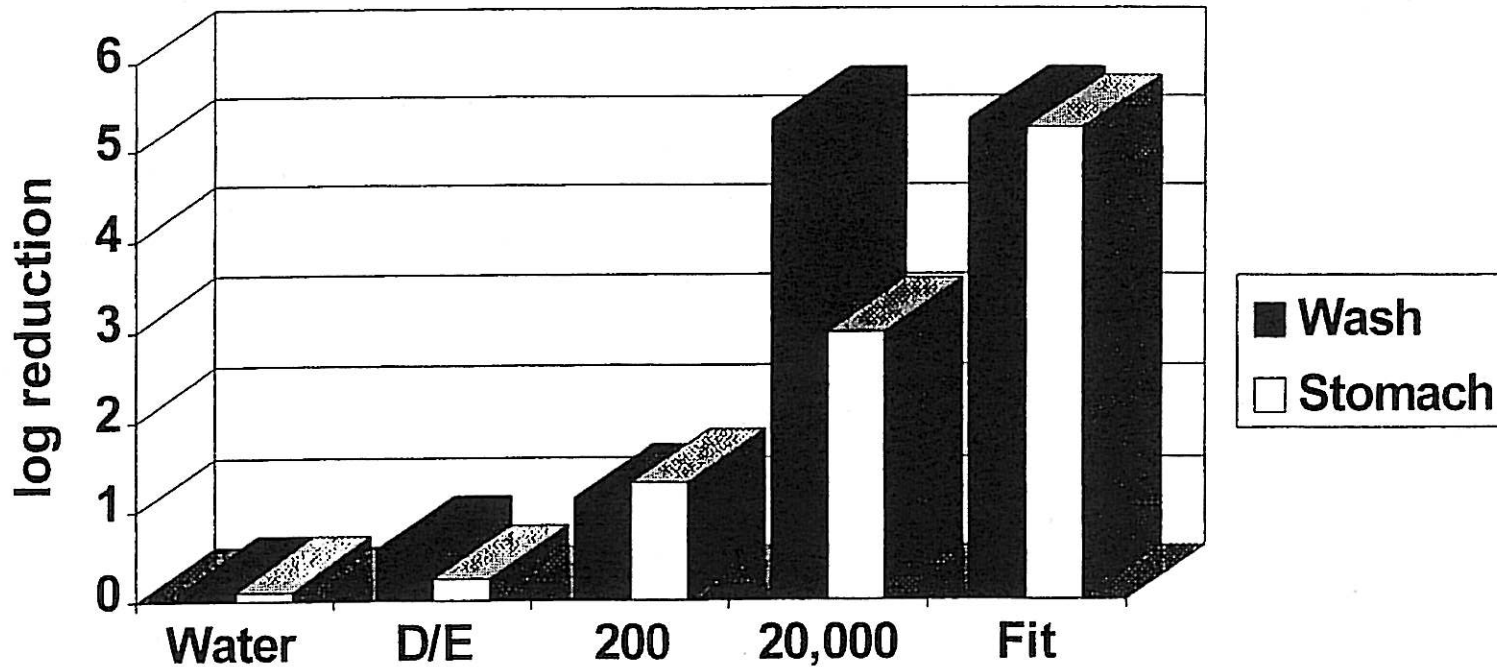


always get more off after stomaching vs original wash
- what's left

log Reduction *E. coli* O157:H7

(Reduction cfu/gm seed; 30 min exposure)

Alfalfa Seeds - samples n = 6

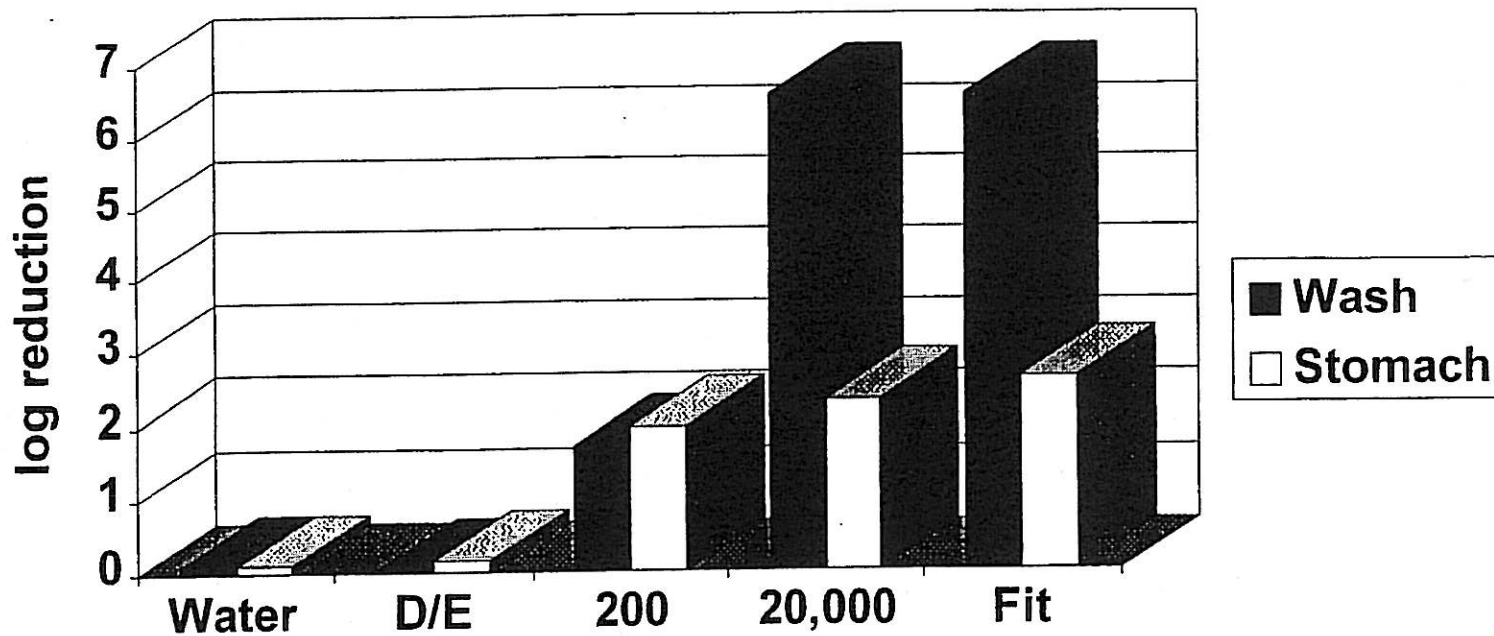


log reduction

log Reduction *Salmonella*

(Reduction cfu/gm seed; 30 min exposure)

Alfalfa Seeds n = 6



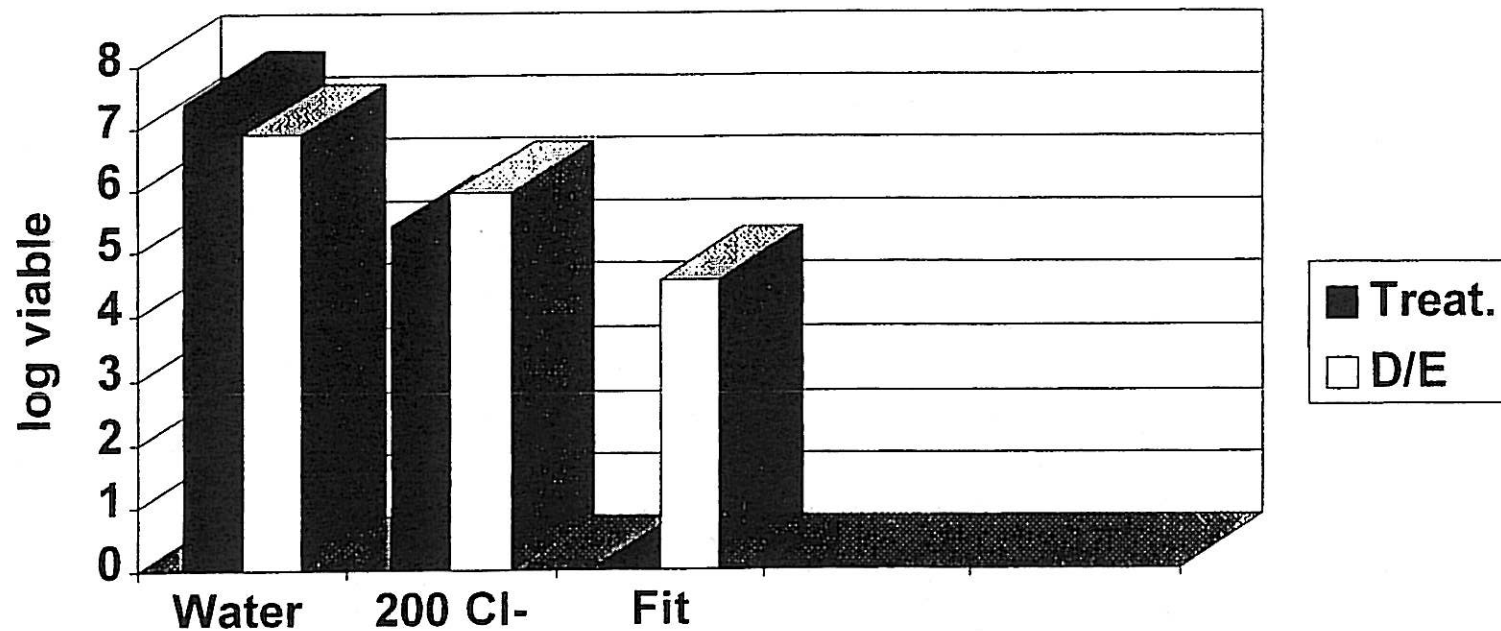
Alfalfa Sprouts

- Mature sprouts purchased
- 20 g inoculated sprouts
- 80 ml desired soak treatment
 - Water; 200 ppm Cl; Fit
 - 3, 5, 10 mins exposure
- Mix - rotary shaker
- Decant, add 80 ml D/E
- Wash 30 sec. - Enumerate

Viabile *E. coli* O157:H7 on Sprouts

(Total cfu/20 gm inoculated sprouts; 10 min exposure)

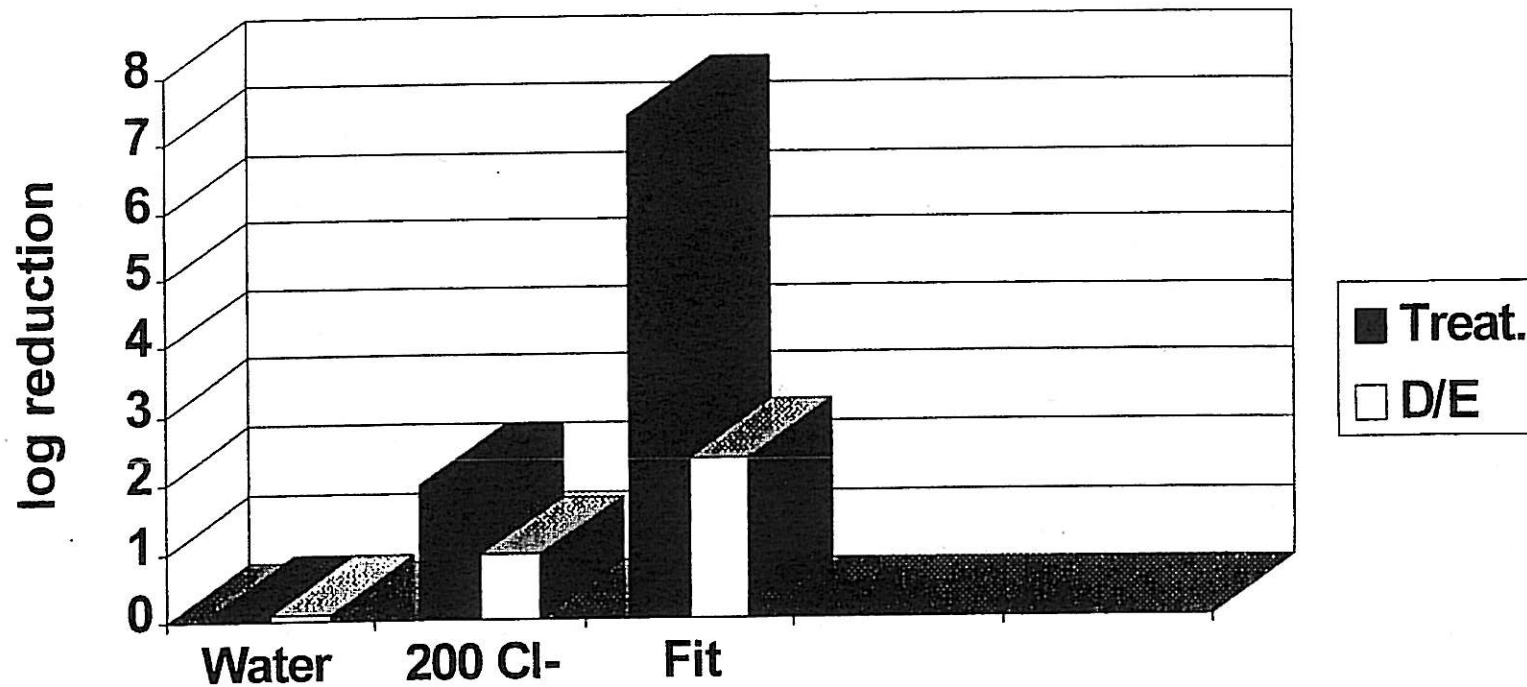
2 samples/rep; 1 rep; . n = 2



log Reduction on Sprouts

(Total cfu/20 gm inoculated sprouts; 10 min exposure)

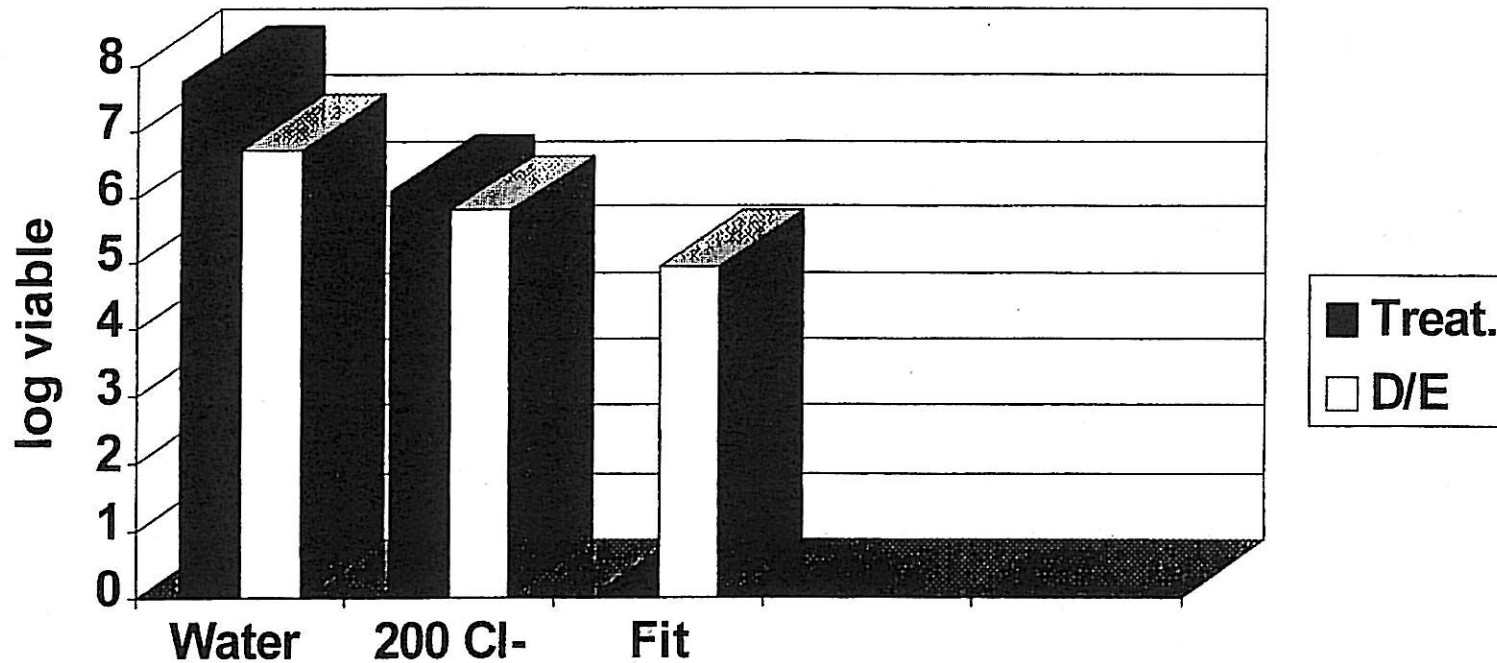
E. coli n = 2



Viabile *Salmonella* on Sprouts

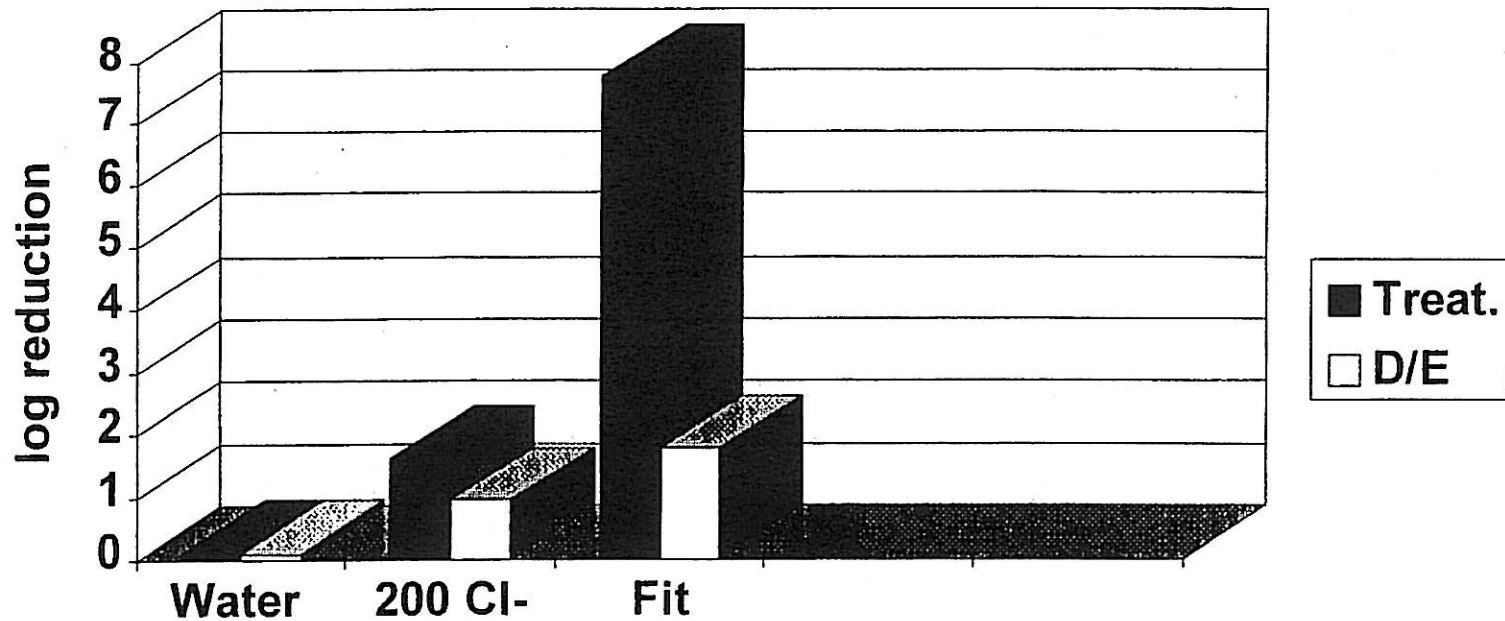
(Total cfu/20 gm inoculated sprouts; 10 min exposure)

Salmonella n = 2



log Reduction *Salmonella* on Sprouts (10 min exposure)

Salmonella n = 2



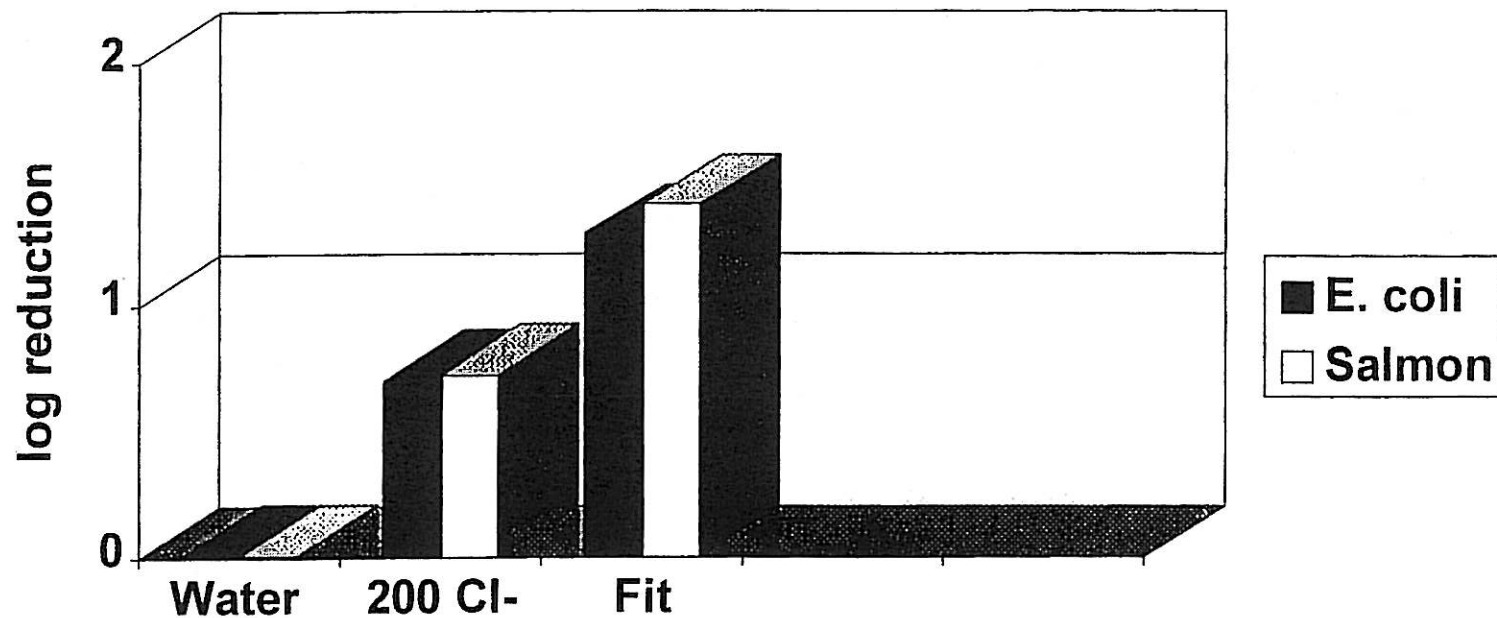
reductions
compared to H₂O

Radish Methods

- *E. coli* O157:H7 & *Salmonella* cocktails
 - Spot inoculation - 50 ul in 5% serum
- 4 radishes - *inoculate on side*
 - (~50 g sample)/rep; 3 reps
 - Dry in hood 20-25 min; 37°C
- Spray (4 sprays) Treatments
 - Water, 200 ppm Cl⁻, Fit
 - 1 min static exposure
 - Wash - 40 ml D/E 20 sec. shake & rub
- Analyze for pathogens

log Reduction *E. coli* & *Salmonella* on Radish (1 min exposure)

3 samples/rep; 2 plates/sample; 3 reps; n = 18



Lettuce Methods

(Single Sanitizer Application)

- *E. coli* O157:H7 & *Listeria* cocktails
 - Spot inoculation - 50 ul in 5% serum
 - 3 discs ~ 7.5 cm dia; ~20 g sample/rep; 3 reps
- Dry in hood
 - 30-40 min; 37°C; 35% RH
- Spray (5 sprays) Treatments
 - Water, D/E, 200 ppm Cl⁻, Fit
 - 30 s, 1, 5 & 10 min static exposure

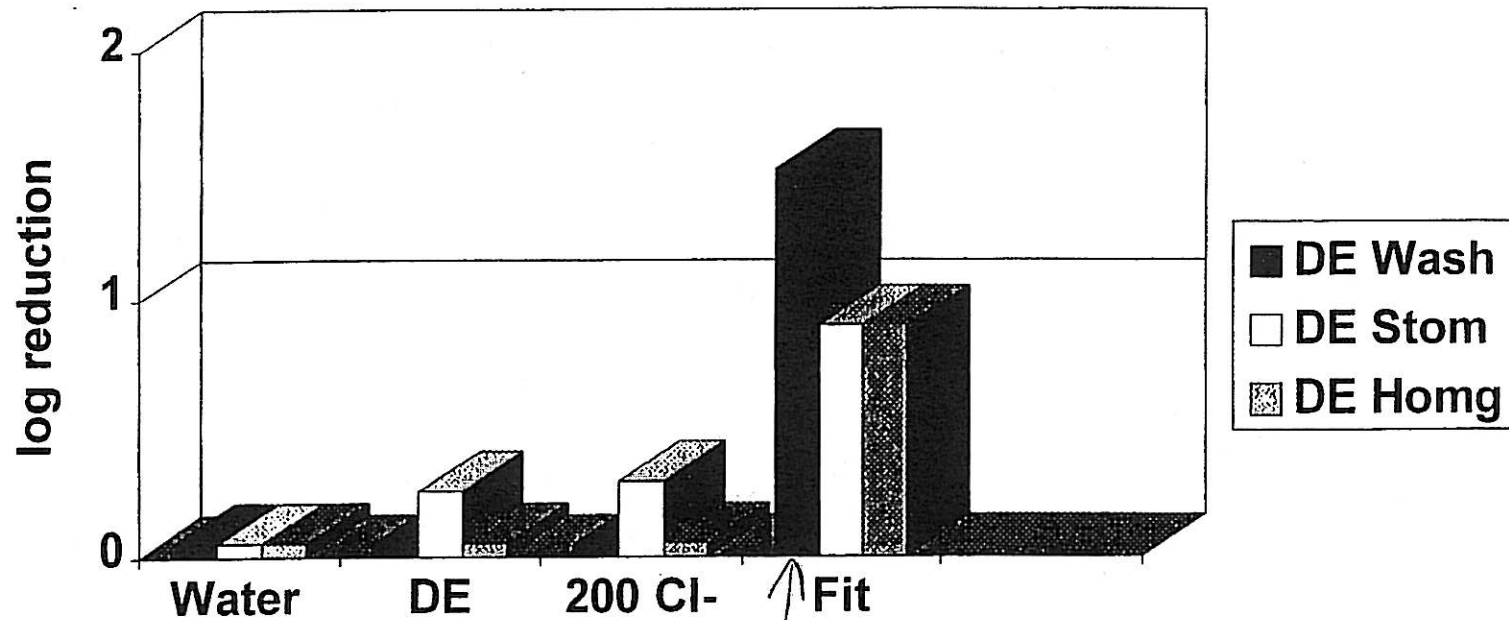
Lettuce Methods

(Single Sanitizer Application)

- Treated Leaf & 50 ml D/E
 - Wash/shake 20 sec - analyze D/E wash
 - ~ Loosely held cells on surface
- Leaf & 20 ml D/E - stomach 30 sec.
 - Analyze D/E
 - ~More permanently attached cells on surface
- Homogenize stomached leaf
 - Analyze
 - ~ Cells trapped inside leaf (stomata, cracks, etc)

log Reduction *E. coli* on Lettuce (30 sec exposure; single sanitizer application)

n = 18; Trends similar for 30 s, 1, 5 & 10 min

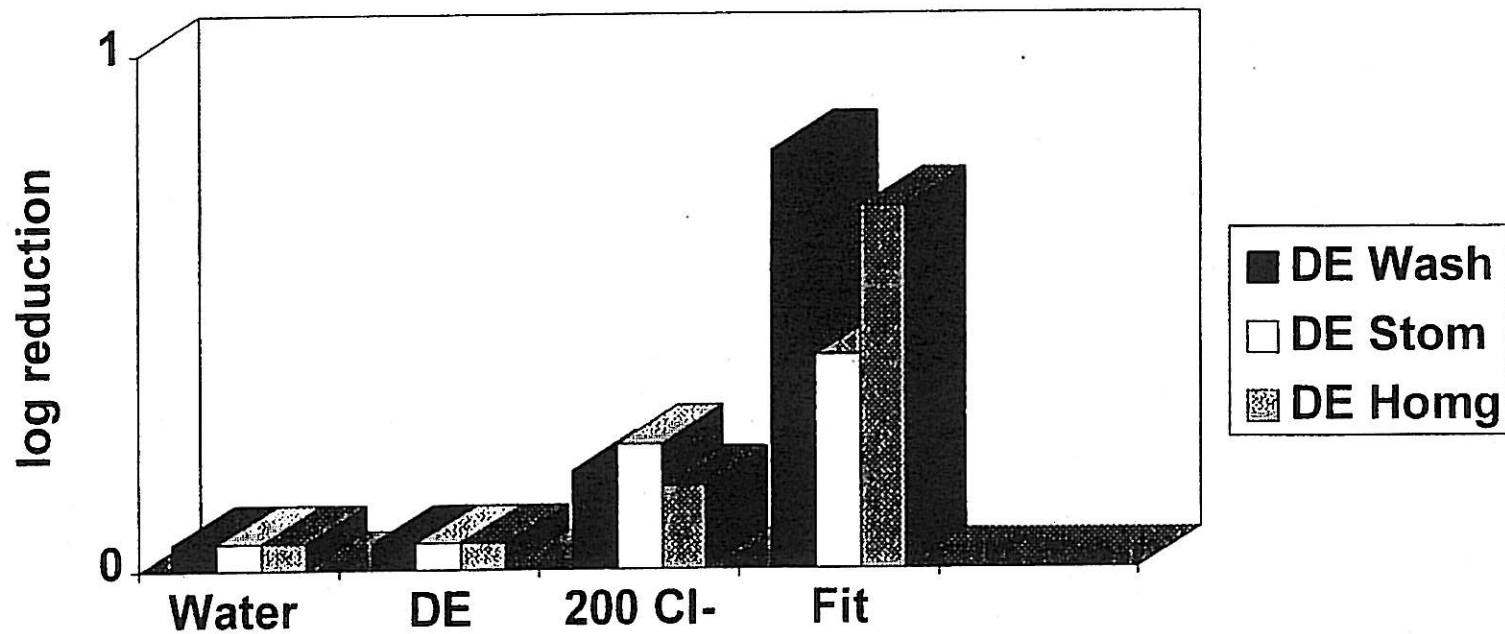


relative to
H₂O

↓ viable counts much higher here

log Reduction *Listeria* on Lettuce (30 sec exposure; single sanitizer application)

3 samples/rep; 2 plates; 3 reps; n = 18



Lettuce Methods

(Sanitizer 3X Repetitive Applications)

- *E. coli* O157:H7 cocktails
 - Spot inoculation - 50 ul in 5% serum
 - High ($\sim 10^7$ cfu/50 ul) level
 - Low ($\sim 10^5$ cfu/50 ul) level
 - 3 discs ~ 7.5 cm dia; ~ 20 g sample/rep
- Dry in hood
 - 30-40 min; 37°C; 35% RH
- Spray (5 sprays) Treatments
 - Water, D/E, 200 ppm Cl⁻, Fit
 - 1 min static exposure

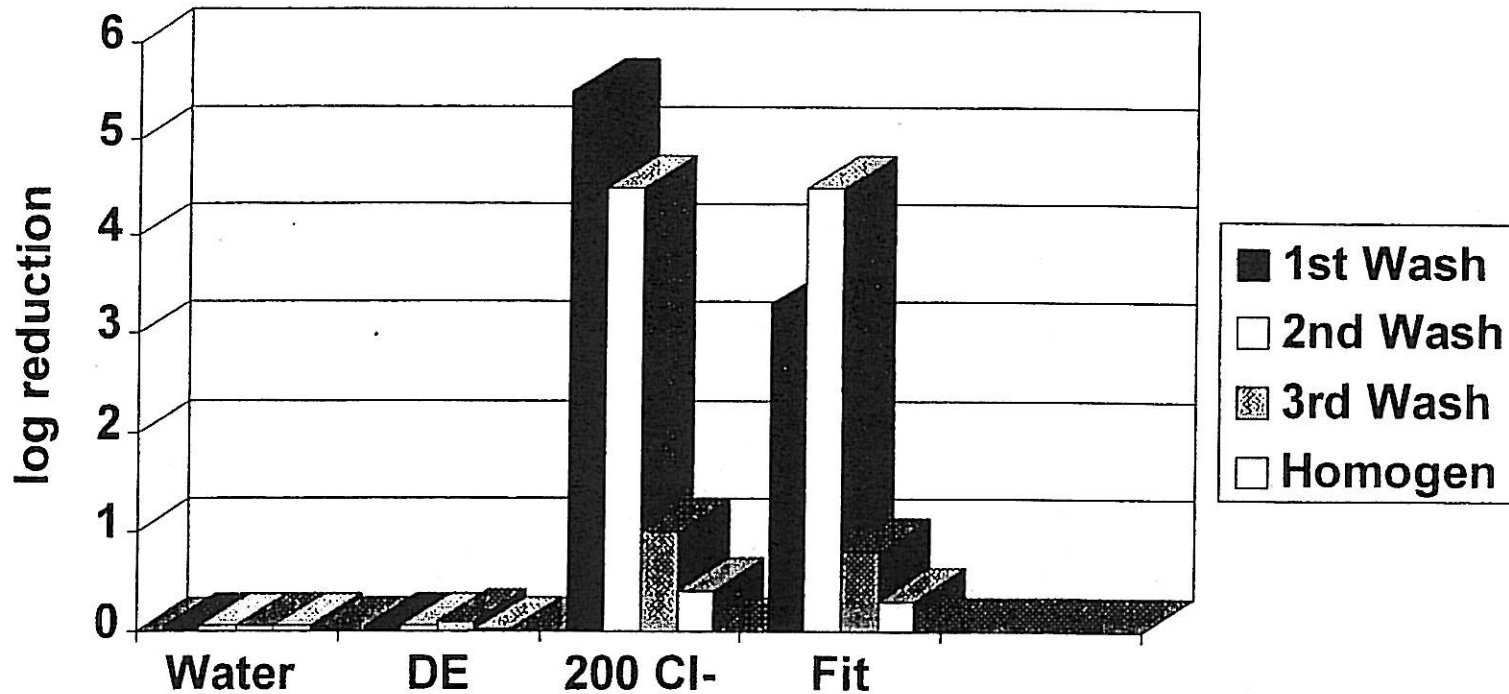
Lettuce Methods

(Sanitizer 3X Repetitive Applications)

- Add 40 ml DI water rinse; shake 20 sec
 - Analyze 1st sanitizer & rinse steps
- Repeat sanitizer application 2X; Rinse
 - Analyze 2nd & 3rd sanitizer & rinse steps
- Homogenize residual leaf 60 - 70 sec
 - Analyze homogenate for pathogen

log Reduction *E. coli* on Lettuce (1 min exposure; 3X sanitizer application)

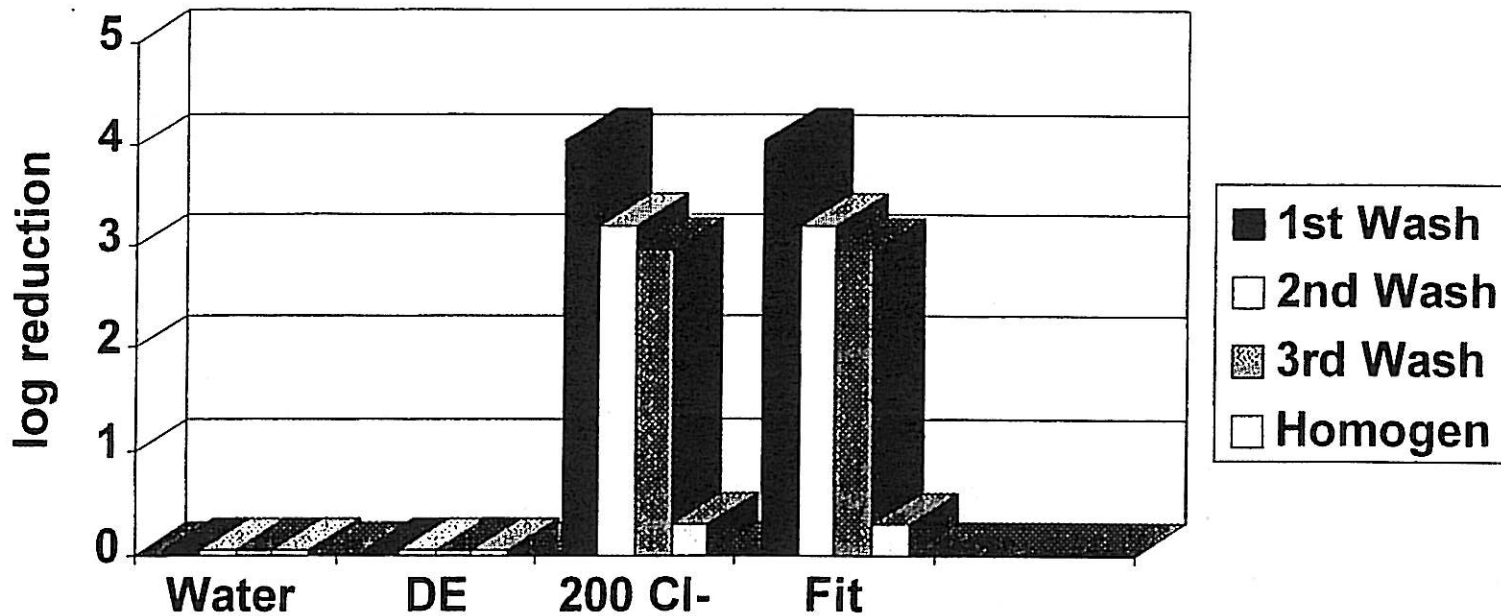
2 samples; 2 plates; 1 rep; n = 4
High level inoculum



Visible counts
on H₂O

log Reduction *E. coli* on Lettuce (vs Water; 1 min exposure; 3X sanitizer application)

2 samples; 2 plates; 1 rep; n = 4
Low Level Inoculum



Fresh Produce
(Tomato)

(1) Inoculate 50 μ l Pathogen(s)
in 5% Horse Serum
 $\sim 10^9$ cfu/ml in inoculum
 $\sim 10^{6-7}$ cfu/produce
(Nalidixic acid acclimated)

(3) Spray Sanitizer (To saturate)
• Water
• 200 ppm HOCl
• Sanitizer

(4) Exposure time
30 sec.

(2) Dry in hood
1 hour

(5A) 30 second rub
with sanitizer

(5D) Water Rinse
(200 ml D. H₂O)

(6) Serial Dilutions in Neutralizer (D/E)
Duplicate Plate Counts

- *L. monocytogenes* - Oxford Media & Nalidixic acid (OXN)
- *Salmonella* - Bismuth Sulfite Agar & Nalidixic acid (BSAN)

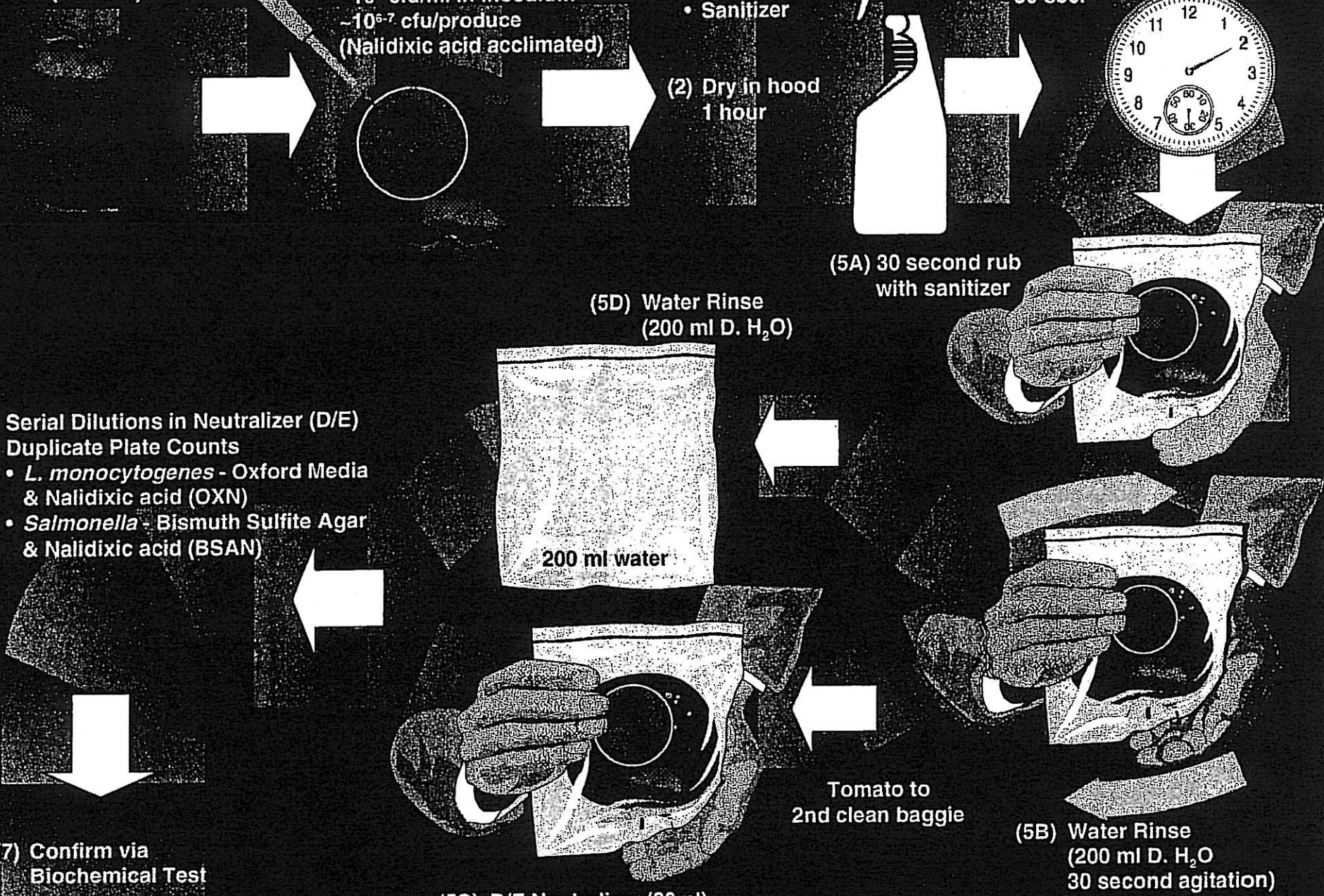
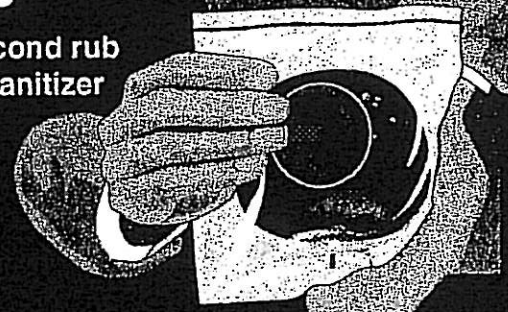
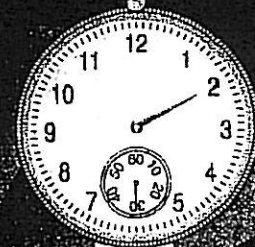
(7) Confirm via
Biochemical Test

(5C) D/E Neutralizer (20ml)
Rub 30 sec.

Tomato to
2nd clean baggie

(5B) Water Rinse
(200 ml D. H₂O
30 second agitation)

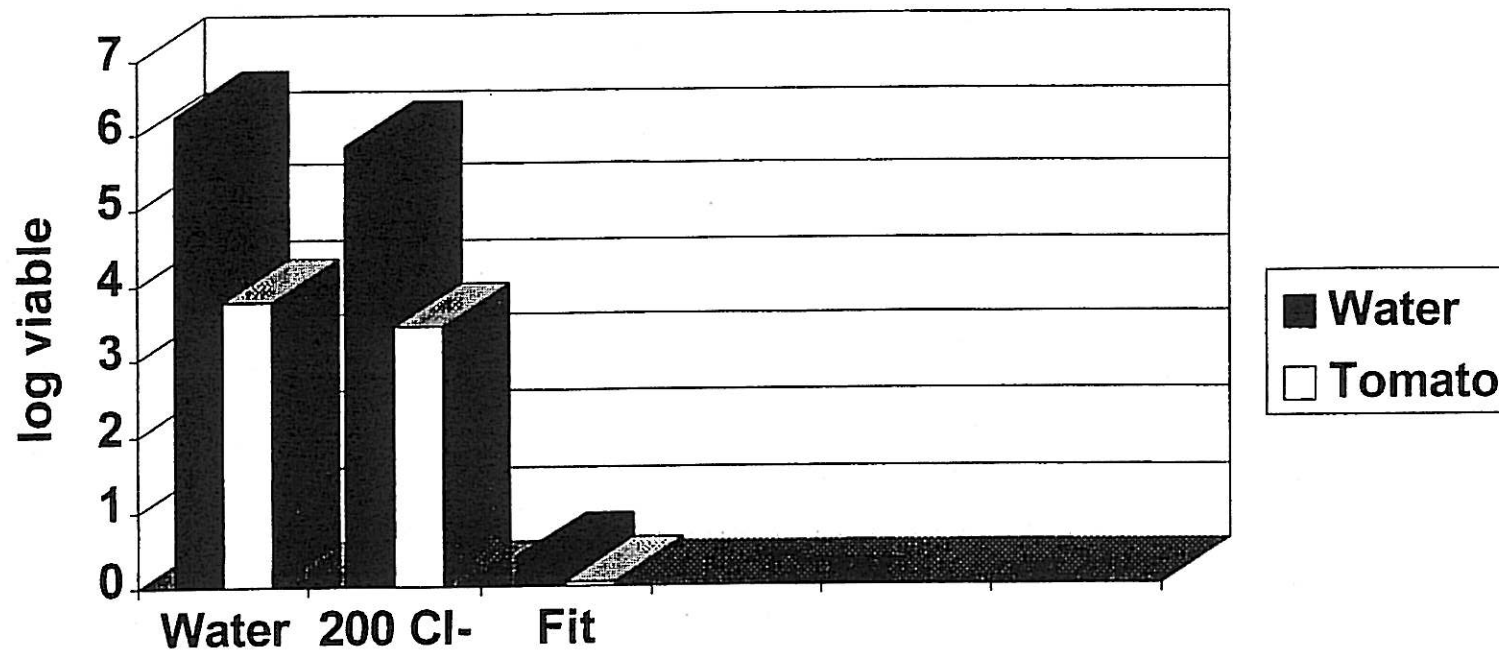
200 ml water



log Viable *Salmonella* on Tomato

(Representative 1 of 2 studies; 30 s exposure & 30 s rub)

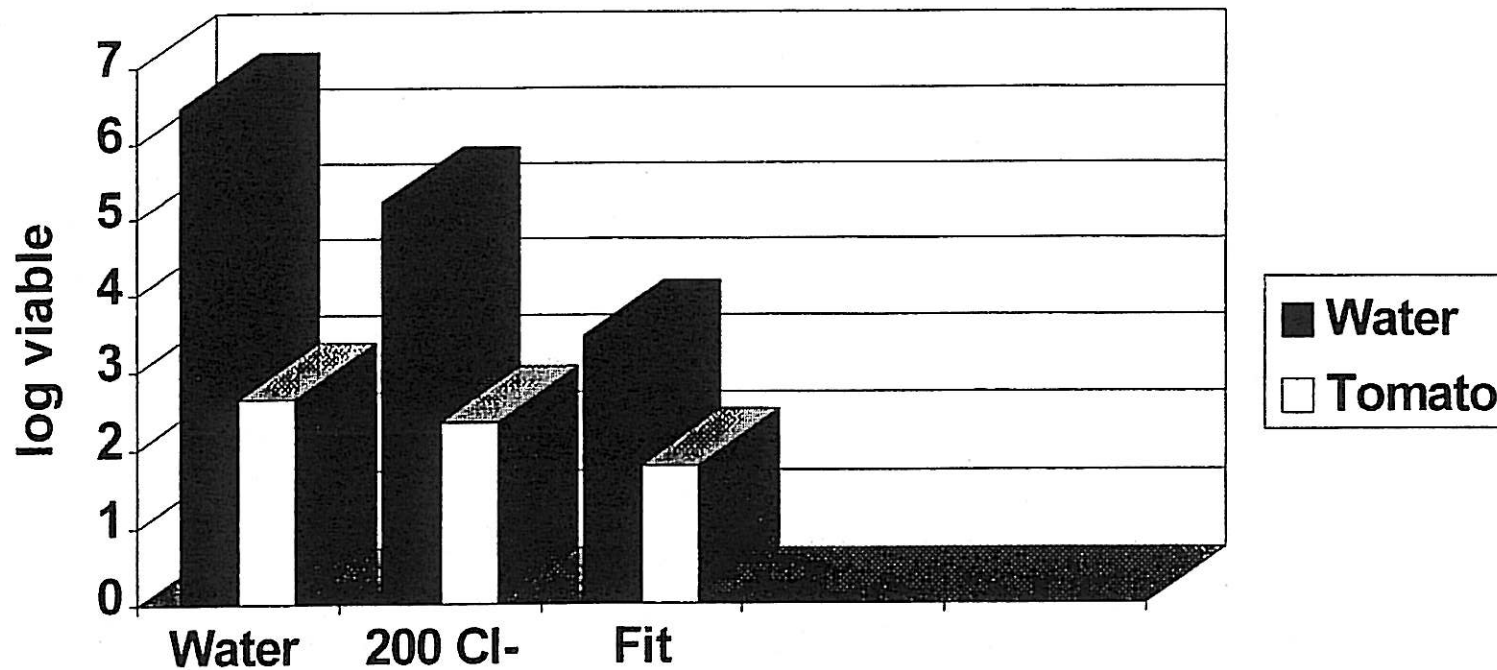
3 tomatoes/rep; 2 plates; 3 reps; n = 18



log Viable *Listeria* on Tomato

(Representative 1 of 2 studies; 30 s exposure & 30 s rub)

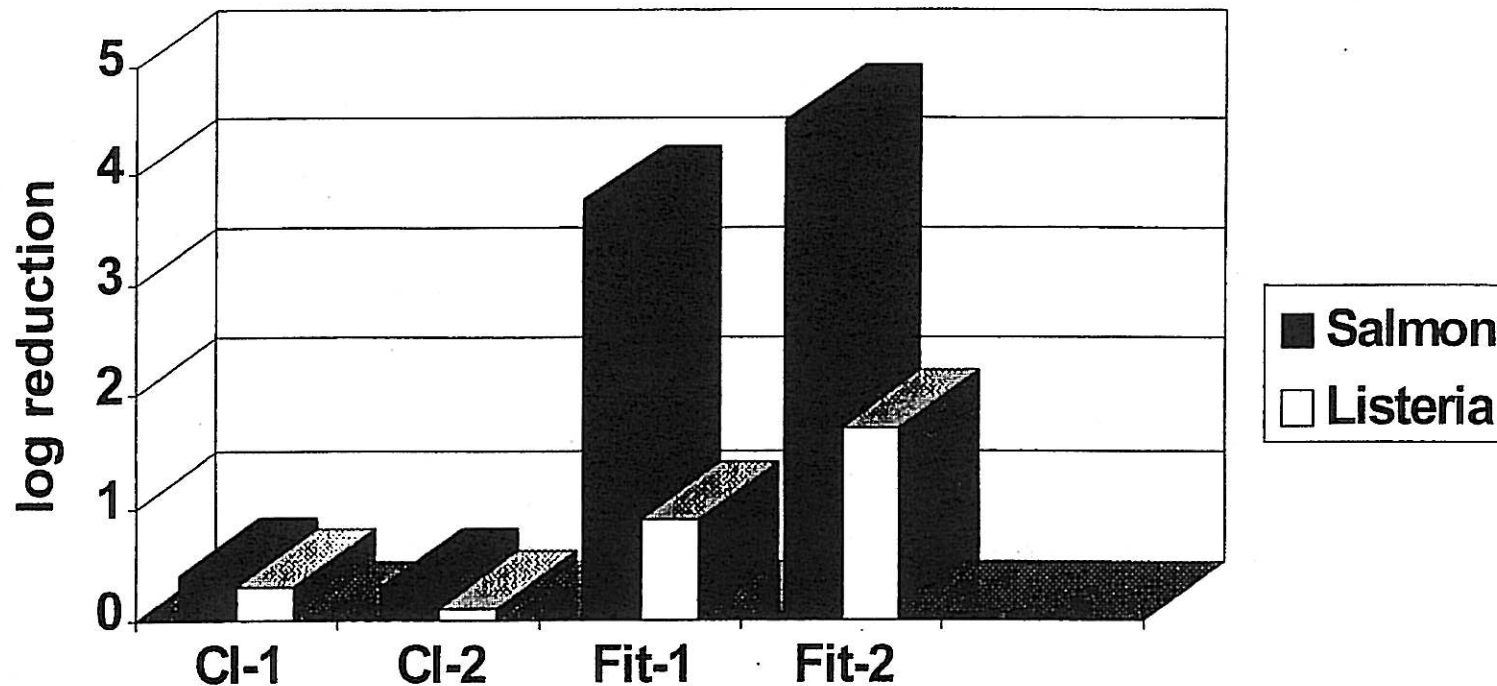
3 tomatoes/rep; 2 plates; 3 reps; n = 18



log Reduction *Salmonella* & *Listeria* on Tomato

(Results of 2 studies; 30 s exposure; 30 s rub)

Salmonella & *Listeria*



50% horse serum by mistake

Summary - Fit Produce Sanitization

- Spray applications
 - Tomatoes
 - Efficacious for spray/rub strategies
 - e.g. *Salmonella*
 - Optimize efficacy/methods vs *Listeria*
 - Radish
 - Efficacy \geq 200 ppm Cl⁻
 - Optimize efficacy/methods
 - *Listeria* testing needed
 - Lettuce
 - Efficacy \geq 200 ppm Cl⁻
 - Optimize efficacy/methods

Summary - Fit Produce Sanitization

- Soak applications

- Alfalfa - Seeds & Sprouts

- Efficacious for seeds
 - Efficacy $\geq 20,000$ ppm Cl⁻
 - Efficacy on sprouts ≥ 200 ppm Cl⁻
 - Optimize efficacy/methods on sprouts

- Next Steps

- Integrate consumer produce washing habits & practices
 - Consider soaking strategies for difficult produce