RECENT OBSERVATIONS ENABLING BETTER MANAGEMENT OF SAFETY RISKS ASSOCIATED WITH PRODUCE AND PECANS

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Joint Meeting of Governor's Food Safety and Defense Task Force and North Carolina Fresh Produce Task Force
September 8-9, 2010, Raleigh, N.C.

Research Leaders in Produce and Pecan Safety (UGA Center for Food Safety)

- Dr. Larry Beuchat
- Dr. Jennifer Cannon
- Dr. Faith Critzer
- Dr. Mike Doyle
- Dr. Marilyn Erickson
- Dr. Ynes Ortega
- Dr. Tong Zhao

Overview

- Internalization of *E. coli* O157:H7 in leafy greens
- Behavior of foodborne pathogens in compost and soil
- Improved methods for detecting foodborne pathogens on fresh produce
- Behavior of *Salmonella* on pecans

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Surface and Internalized *E. coli* O157:H7 on Spinach and Lettuce Sprayed with Contaminated Irrigation Water

- *E. coli* O157:H7 was applied by spraying spinach 48 and 69 days after transplanting seedlings
  - Initially detected on the surface of leaves dosed at 10^4 CFU/ml and internally in leaves dosed at 10^6 CFU/ml
  - Seven days post spraying, leaves tested negative for *E. coli* O157:H7

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Surface and Internalized *E. coli* O157:H7 on Spinach and Lettuce Sprayed with Contaminated Irrigation Water

- *E. coli* O157:H7 (10^8 CFU/ml) was spray inoculated onto the abaxial (under side) and adaxial (top side) sides of lettuce leaves of plants grown under sunny or shaded conditions
  - Detectable 27 days post-spraying
  - Survived better on the under side of leaves than on the top side
  - Internalization was also greater on the under side (up to 14 days) than on the top side (2 days)

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Internalization of *E. coli* O157:H7 in Leafy Greens as Affected by Insect and Physical Damage

- Internalization of *E. coli* O157:H7 in lettuce and spinach infested with cabbage loopers, instars, aphids, thips, and whiteflies or physically damaged was studied
  - Internalization of lettuce leaves inoculated at a population of 10^4 CFU/leaf did not occur but did occur when leaves were inoculated at 10^6 CFU/leaf
  - Internalization of *E. coli* O157:H7 in leaves exposed to insects or physically damaged was reduced compared to control leaves
  - Phytoalexins may be involved

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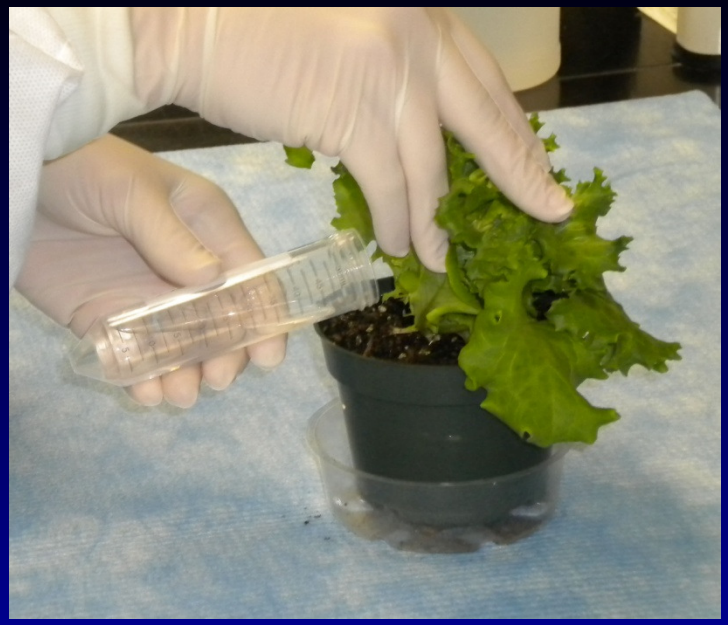
Threshold Populations of *E. coli* O157:H7 in Soil Required for Internalization into Leafy Greens



- Survival and population in irrigation water needed to internalize roots, and mobilization to leaf tissue were studied
  - Lettuce, spinach, and parsley plants at different stages of development were irrigated with water containing *E. coli* O157
    - Moisture saturation of soil enhanced survival
    - Application of irrigation water to give 10^6 CFU/g of soil led to uptake via roots
    - Internalized *E. coli* O157:H7 was infrequently found in leaves 3 days post-application of contaminated water and did not persist after 6 days

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Inactivation of *Salmonella* and *Listeria monocytogenes* in Compost as Affected by Different Carbon Amendments

- Survival of *Salmonella* and *L. monocytogenes* in cow manure mixed with wheat straw, peanut hulls or pine needles, and chicken manure mixed with wheat straw, peanut hulls, pine needles, or rice hulls during aerobic composting was determined
- Systems simulating internal (bioreactors) and surface (trays) sites in compost piles were studied
  - Type of carbon amendment did not significantly affect inactivation of *L. monocytogenes* in bioreactors
  - *Salmonella* was inactivated faster in compost containing wheat straw
  - In tray compost, *Salmonella* died more rapidly in mixtures containing wheat straw or peanut hulls than in mixtures containing pine needles or rice hulls

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Survival of *E. coli* O157:H7 and *Salmonella* in Soil During Cultivation of Lettuce

- Effects of soil moisture and fertility on survival of *E. coli* O157:H7 and *Salmonella* were studied
  - Manure compost:top soil ratios of 0:5, 1:5, and 2:5 (w/w) were used
  - Pathogens (10^3 and 10^6 CFU/ml) in irrigation water were applied to water-stressed (watering rate reduced by 50% for 2 - 3 weeks preceding inoculation) and non-stressed plants
  - *E. coli* O157:H7 populations on root surfaces were lower on water-stressed plants than on non-stressed plants
  - *E. coli* O157:H7 and *Salmonella* did not internalize roots or leaves
  - Behavior of pathogens was unaffected by fertility of soil

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Risk Associated with Field Core (Core and Cut) Practices for Harvesting Lettuce

- Knife blades contaminated with soil containing *E. coli* O157:H7 (10^4 and 10^6 CFU/blade) were not decontaminated by immersing blades in chlorinated water (200 µg/ml)
  - Reduction was 1.6 log CFU/blade
- Contaminated blades (10^3 CFU/blade) were used to sequentially cut and core 10 heads of iceberg lettuce
  - *E. coli* O157:H7 was recovered from all 10 heads
  - Cores remained contaminated after spraying with chlorinated water (100 µg/ml) for 2 min

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Improved Methodologies to Detect Foodborne Pathogens in Romaine Lettuce

- Non-selective short-term enrichment and real-time PCR methodologies to detect low numbers of *Salmonella* and Shiga-toxigenic *E. coli* were studied
  - *Salmonella* and *E. coli* serotypes 0145, 0157, 026, 0111, and 0103 were inoculated onto Romaine lettuce leaves at population of 1 to 9 CFU/g
  - Pathogens could be detected by DNA extraction of 10-h enrichment cultures (BPW and UPB) followed by molecular assay
  - Results can be obtained within 12 h of beginning the enrichment procedures

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Recovery of Norovirus from Produce and Produce-Contact Surfaces

- Alternative buffers for virus elution were evaluated for recovering norovirus and a norovirus surrogate (murine norovirus) from carrots, lettuce, berries, stainless steel knives, brushes, and latex nitrile gloves
  - Among test buffers, phosphate-buffered saline (1M NaCl) containing 0.05% Tween 20 gave the lowest limit of detection
- A concentration method using positively-charged magnetic beads was also evaluated
  - Positively charged magnetic beads can be used to improve recovery from some produce matrices by concentrating norovirus, but is not an effective method for concentrating virus from all produce types.

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Lower detection limits of infectious MNV from (a) food and (b) food contact surfaces using 0.1M PBS, 1M NaCl, 0.05% Tween-20 elution buffer using a standard plaque assay

(a) MNV	Strawberries	Carrots	Cantaloupe
Input (pfu)	Ratio of positive replicates	Ratio of positive replicates	Ratio of positive replicates
10^3	9/9	12/12	9/9
10^2	9/9	7/9	9/9
10^1	8/9	7/9	7/9
10^0	8/9	2/9	5/9
10^{-1}	3/9	0/9	3/9
Detection Limit (pfu)	0.1	6.8	0.1

(b) MNV	Knives	Nylon Brushes	Nitrile Gloves*
Input (pfu)	Ratio of positive replicates	Ratio of positive replicates	Ratio of positive replicates
10^3	6/6	6/6	9/9
10^2	6/6	6/6	5/6
10^1	3/6	5/6	1/6
10^0	0/6	2/6	0/6
Detection Limit (pfu)	11	7.0	50

*0.1M NaCl, 1M NaCl *without* Tween-20

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Lower detection limits of (a) MNV and (b) huNoV GI.1 on produce using a 0.1M PBS, 1M NaCl, 0.05% Tween-20 elution buffer elution buffer and detection by real time RT-qPCR

(a) MNV	Strawberries	Carrots	Cantaloupe	Romaine Lettuce
Input (copy #)	Ratio of positive replicates	Ratio of positive replicates	Ratio of positive replicates	Ratio of positive replicates
7×10^7	15/15	18/18	15/15	9/9
7×10^6	15/15	15/15	15/15	9/9
7×10^5	15/15	15/15	15/15	9/9
7×10^4	10/15	12/15	15/15	6/9
7×10^3	1/6	5/15	10/15	4/9
7×10^2	0/6	0/6	3/6	2/9
Lower Detection Limit (copy #)	7,000	7,000	700	700

(b) GI.1	Strawberries	Carrots	Cantaloupe	Romaine Lettuce
Input (copy #)	Ratio of positive replicates	Ratio of positive replicates	Ratio of positive replicates	Ratio of positive replicates
6×10^5	6/6	6/6	6/6	6/6
6×10^4	5/6	6/6	5/6	6/6
6×10^3	3/6	3/6	2/6	5/6
6×10^2	0/6	1/6	1/6	1/6
6×10^1	0/6	0/6	0/6	0/6
Lower Detection Limit (copy #)	6,000	600	600	600

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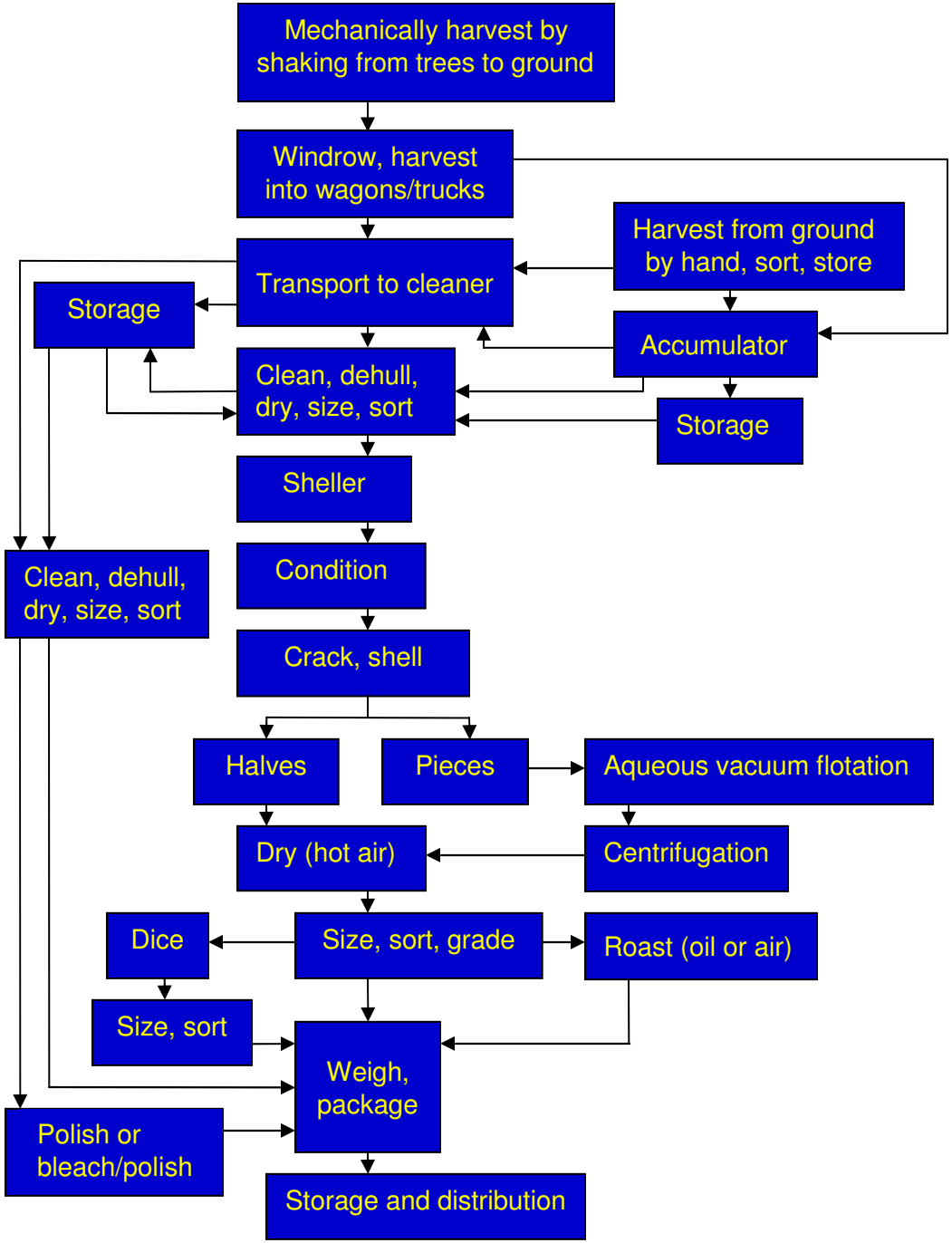
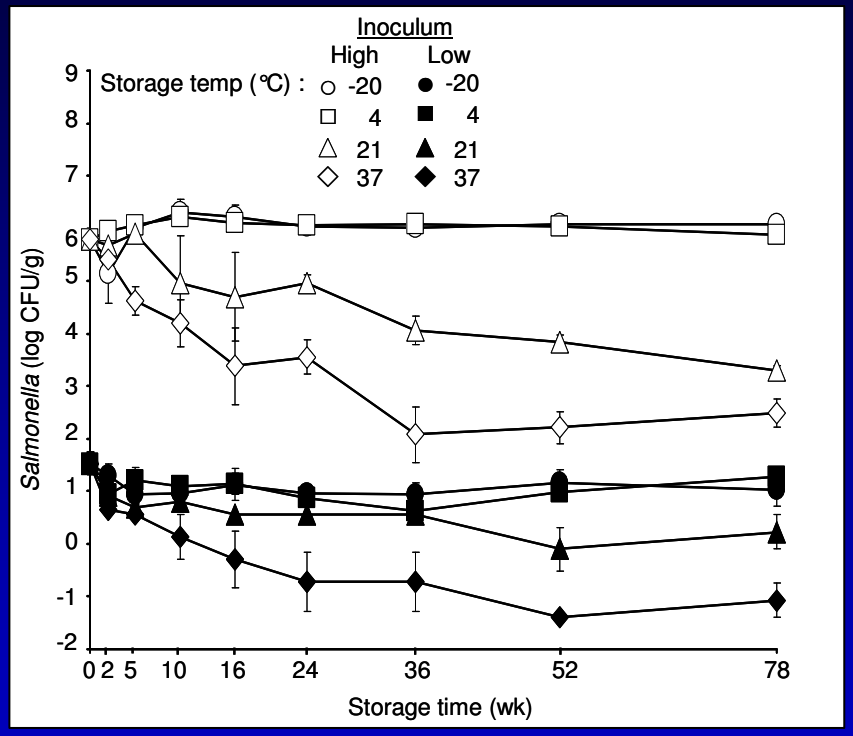
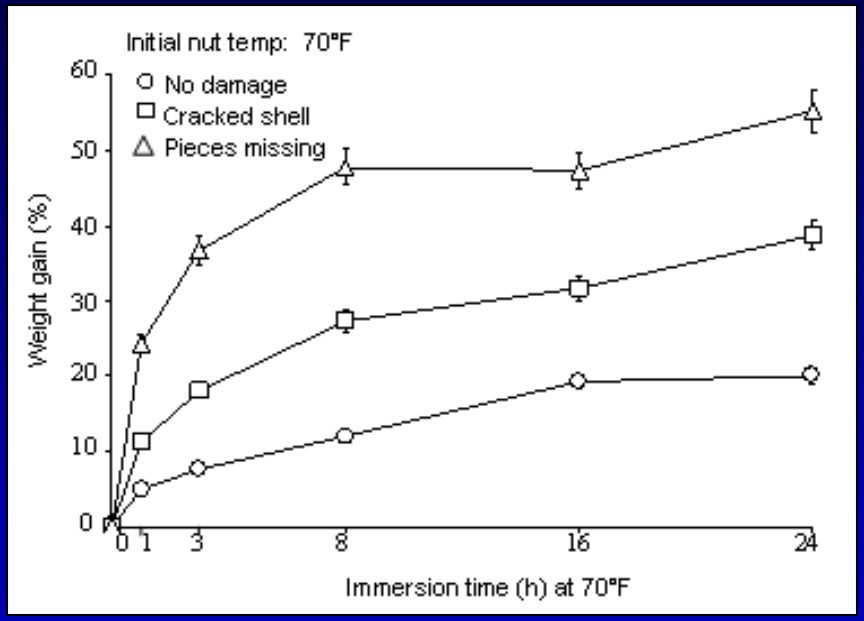

Mechanically harvest by shaking from trees to ground
Windrow, harvest into wagons/trucks
Harvest from ground by hand, sort, store
Transport to cleaner
Storage
Accumulator
Clean, dehull, dry, size, sort
Storage
Sheller
Clean, dehull, dry, size, sort
Condition
Crack, shell
Halves
Pieces
Aqueous vacuum flotation
Dry (hot air)
Centrifugation
Dice
Size, sort, grade
Roast (oil or air)
Size, sort
Weigh, package
Polish or bleach/polish
Storage and distribution

Behavior of *Salmonella* on Post-harvest Pecans



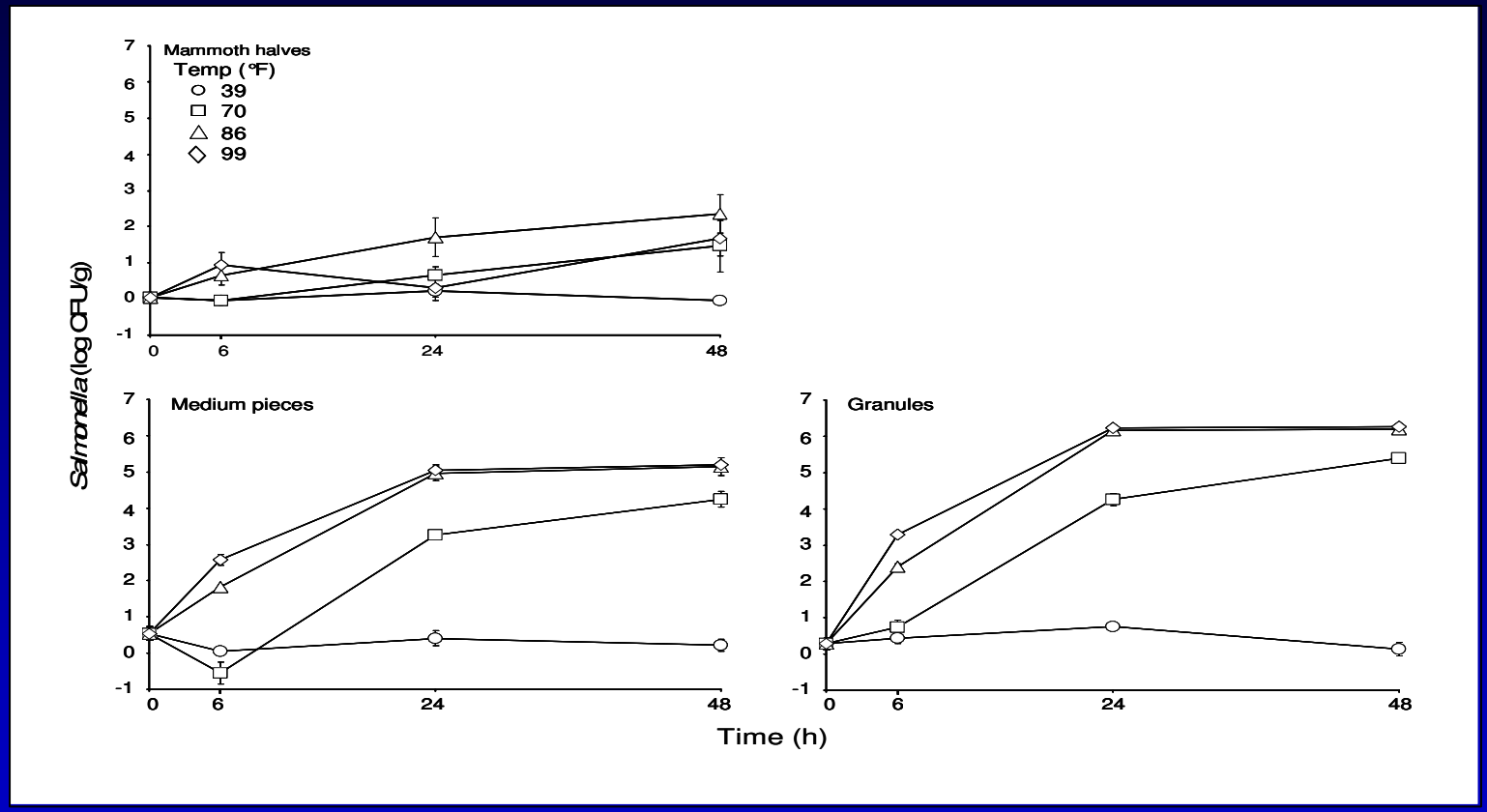
- *Salmonella* can infiltrate in-shell pecans, reach the kernel, and remain viable for at least 18 months at -20 to 37°C
  - Can also survive on shelled nuts (halves and pieces)

Behavior of *Salmonella* on Post-harvest Pecans



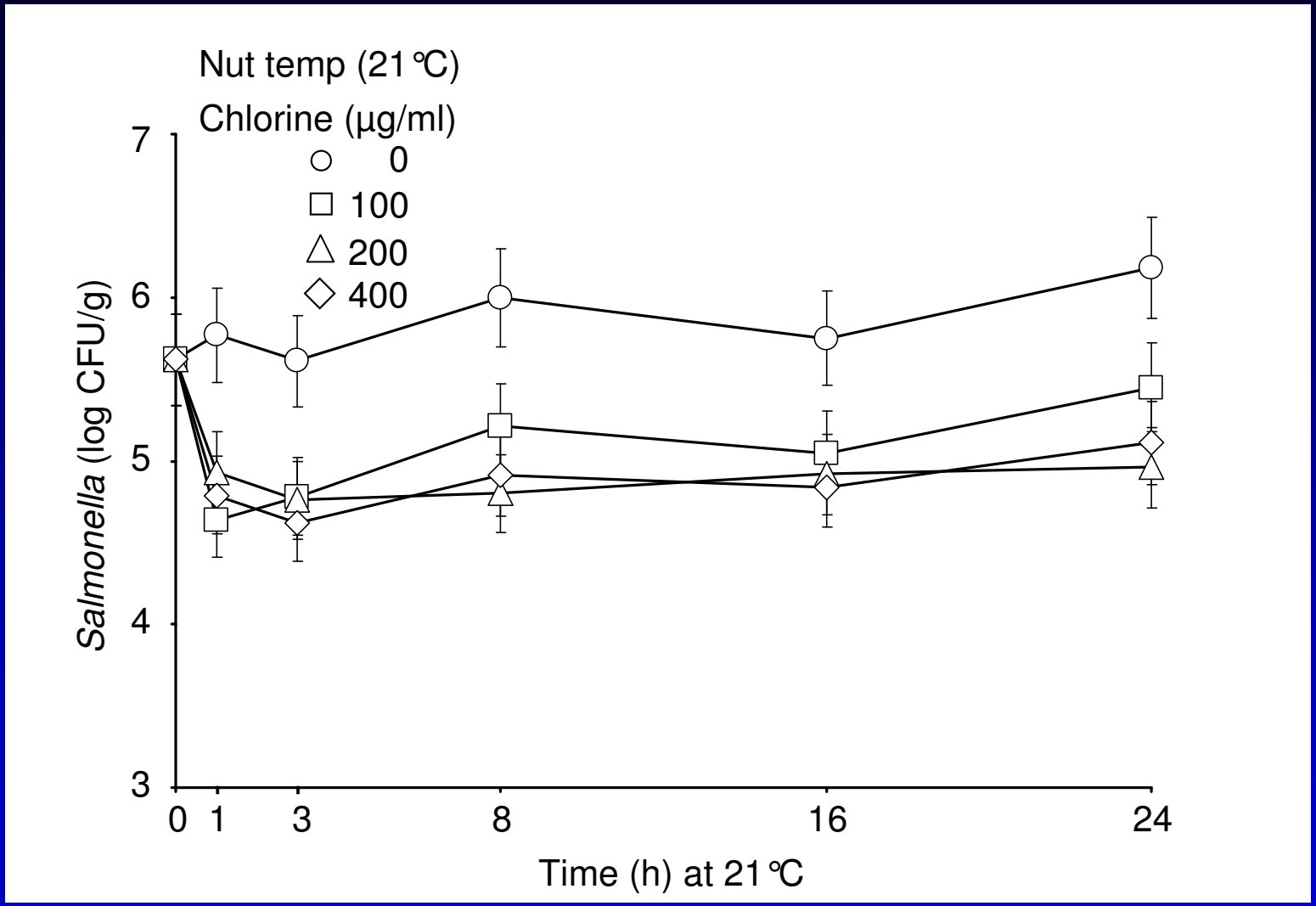
- Rate of infiltration is affected by temperature differential between in-shell nuts and water containing *Salmonella*

Behavior of *Salmonella* on Post-harvest Pecans



- *Salmonella* can grow on high-moisture (a_w > 0.94) pecan nutmeats and on the surface of pecans shells and hulls, but is inactivated on middle septum tissue

Behavior of *Salmonella* on Post-harvest Pecans



- Treatment of in-shell pecans in chlorinated water (up to 400 µg/ml) cannot be relied on/in in-shell pecans

Behavior of *Salmonella* on Post-harvest Pecans

- Five-log reduction in *Salmonella* can be achieved by conditioning treatments, but heat resistance varies, depending on the method used to prepare inoculated pecans
  - Most resistant: Immersion inoculated, dried, stored
  - Less resistant: Surface inoculated, dried, stored
  - Least resistant: Surface inoculated, not thoroughly dried, not stored
- Hot air drying of pecan nutmeats does not kill large numbers of *Salmonella* without negatively affecting raw quality characteristics

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THANK YOU