Produce Safety Risks and an Update on Two Ongoing Research Projects – Risks imposed by wild birds and risks associated with EHEC during post harvest leafy greens

Organized by: IAFP's Fruit and Vegetable Quality and Safety PDG

Moderator: Kristin Esch, FDA and Chair of the Fruit & Vegetable Quality & Safety PDG

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- This webinar is being recorded and will be available for access by IAFP members at www.foodprotection.org within one week.

Kristin Esch

Kristin graduated from Michigan State University with a bachelors in Animal Science and a masters degree in Agricultural Extension Education.

For almost 20 years she worked for the Michigan Department of Agriculture (MDA) conducting on-site farm inspections mostly with livestock but also conducted cropping and produce farm inspections. She eventually transitioned to the Produce Safety Program at MDA for four years before going to FDA.

Currently Kristin works in the FDA Center for Food Safety and Applied Nutrition Produce Safety Network in the North Central Region. Kristin is also the current chair of the IAFP Fruit and Vegetable Safety and Quality PDG.

Daniel Karp

Daniel Karp is an associate professor in the Department of Wildlife, Fish, and Conservation Biology at UC Davis. Daniel completed his Ph.D. in 2013 and undergraduate studies in 2009 at Stanford University's Department of Biology. Following his graduate studies, Daniel became an inaugural NatureNet postdoctoral fellow at the University of California, Berkeley and the Nature Conservancy.

He then received a Killam Postdoctoral Fellowship to conduct research at The University of British Colombia, before beginning his position at UC Davis in 2017. A critical challenge for this century is transitioning towards sustainable farming systems that simultaneously produce safe and sufficient food while conserving wildlife.

Daniel uses ecological research to develop strategies for co-managing agriculture for bird conservation, crop production, and food-safety outcomes.

Teresa Bergholz

Teresa earned her Ph.D. in Food Science from Michigan State University in 2007 and was a postdoctoral researcher in Food Science at Cornell University from 2007 to 2012.

She was a faculty member in Microbiological Sciences at North Dakota State University from 2012 to 2020 and joined the faculty at Michigan State University in the fall of 2020 as an associate professor.

Her research focuses on understanding stress resistance capabilities of foodborne pathogens in the food supply and assessing inactivation methods. Current research in her lab is funded by USDA NIFA, the Center for Produce Safety, and the Michigan Department of Health



Towards a holistic assessment of the food-safety risks imposed by wild birds

Daniel Karp

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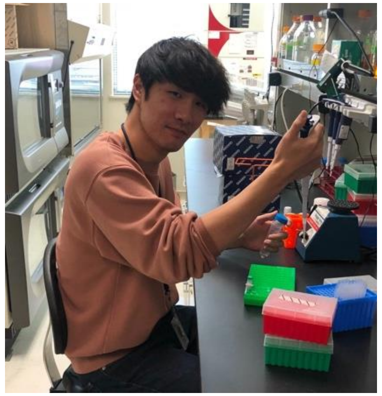
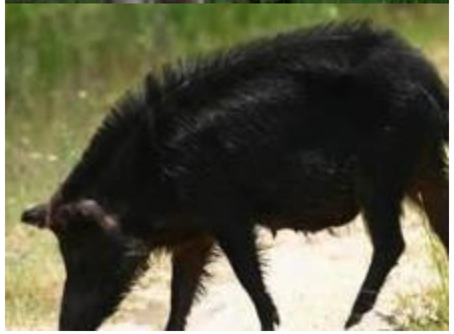


Sangin Lee
(Technician)

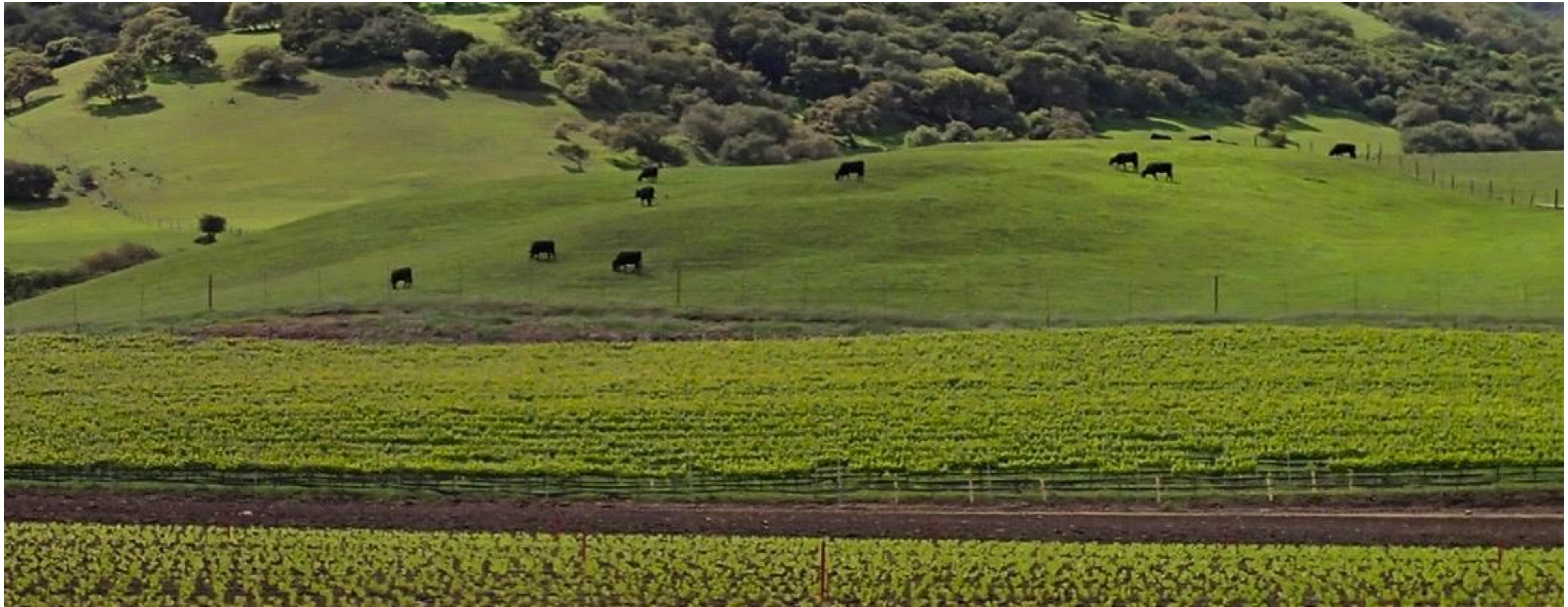

EASY OPEN
All Natural
Dole
SPINACH
CHERRY ALMOND BLEU
ALL NATURAL
Kit
SPINACH CHERRY ALMOND BLEU


California's
Central Coast




NO TRESPASSING
NO
ANIMALS!
IT'S A FOOD SAFETY
VIOLATION!
5
MPH
DUST

P. Baur

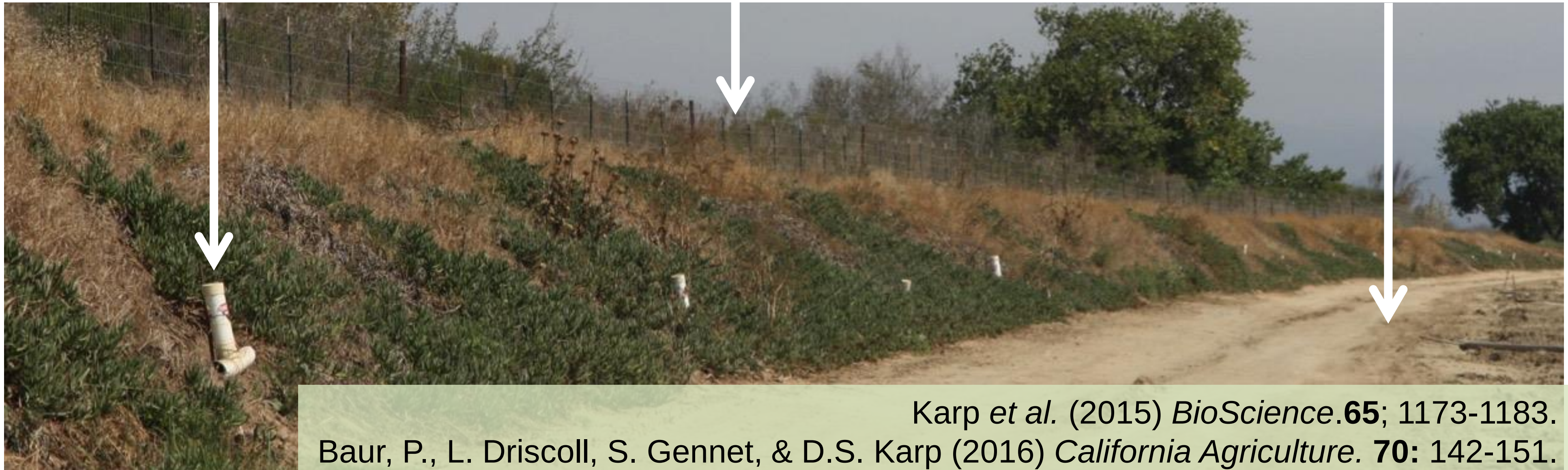


- **~13% of the remaining riparian habitat along the Salinas River was cleared between 2005 and 2009**
- **A survey from 2015 indicated that ~40% of California produce growers are still clearing vegetation**

Rodent traps

Wildlife fences

Vegetation removal



Karp *et al.* (2015) *BioScience*.**65**; 1173-1183.

Baur, P., L. Driscoll, S. Gennet, & D.S. Karp (2016) *California Agriculture*. **70:** 142-151.

Birds and Food Safety

- Known to carry foodborne pathogens (STEC, *Salmonella,* and *Campylobacter*)
- Difficult to exclude
- Move large distances (from livestock operations to produce fields)
- Defecate throughout fresh produce fields

"The thing that worries me more actually are birds. . . you can't control birds and they constantly like flying over your field. They come and sit in the field. They carry Salmonella"

Holistic Risk Assessment

Does the species carry the pathogen?

No → No extra risk

Yes → Does contact occur?

No → No extra risk

Yes → Do bacteria survive for long periods?

No → No extra risk

Yes → Higher risk for spillover

Smith et al. (2020) *Biol. Rev.*

Guiding Questions

1. Which species carry the greatest food-safety risks?
    - Pathogen prevalence
    - Crop contact
    - Pathogen survival

2. How do farm management and surrounding landscapes affect the food-safety risks from birds?

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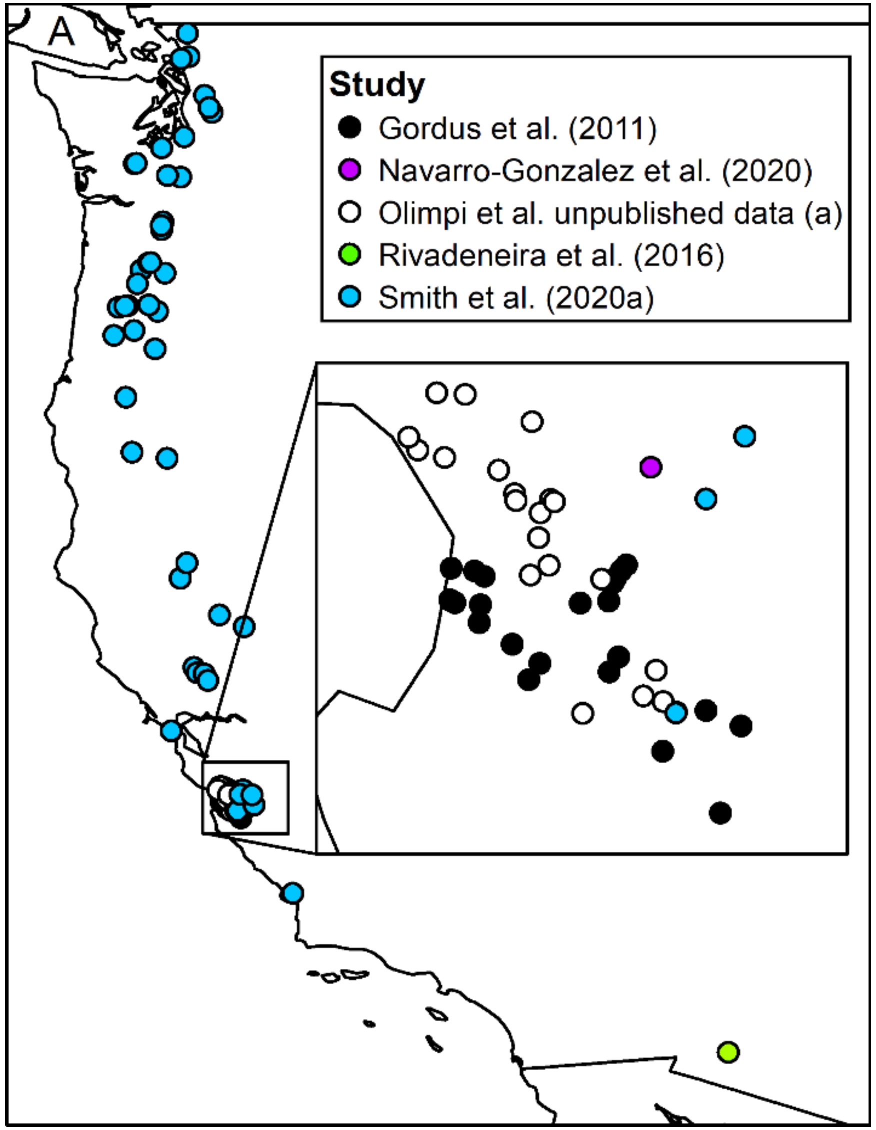
Which species carry the greatest risks?

- We compiled three datasets…

- Pathogen dataset
  - ~11,000 tests of STEC, *Salmonella*, & *Campylobacter*
  - ~90 produce farms
  - ~95 bird species

- Bird survey database
  - ~1500 point counts
  - ~350 sites

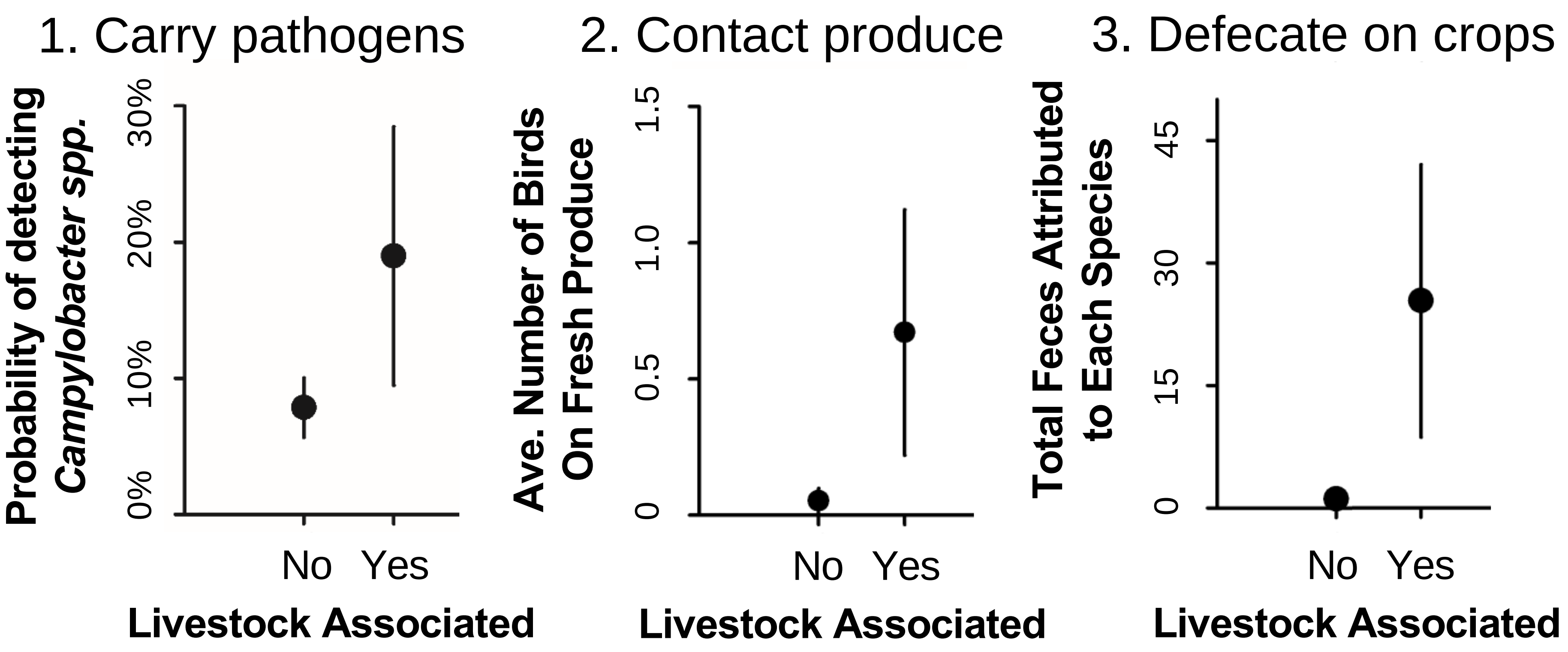
- Fecal database
  - ~460 feces
  - ~35 farms



Smith *et al.* (2021) *Ecological Applications.*

Which species carry the greatest risks?

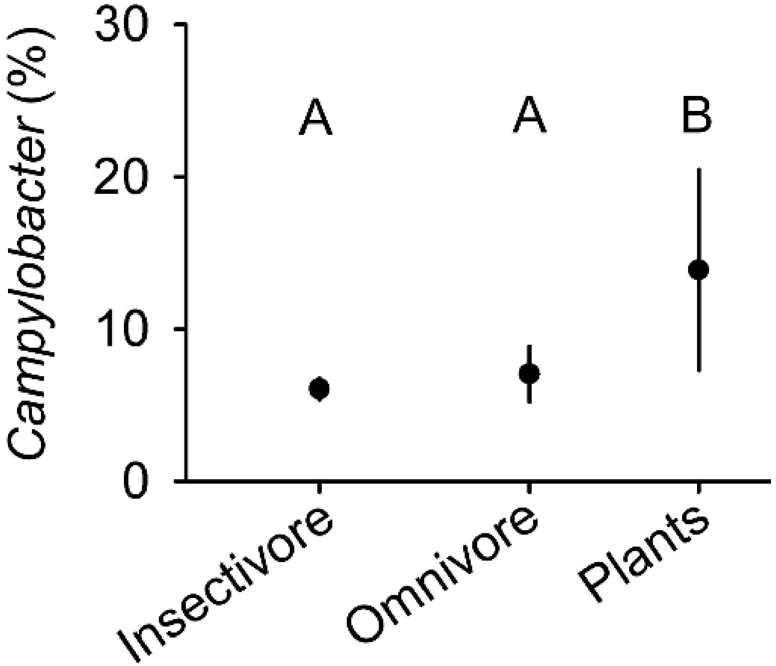
- Positivity rates
  - STEC: 0.2%; *Salmonella*: 0.5%; *Campylobacter*: 8.0%
- Livestock-associated species are more likely to…



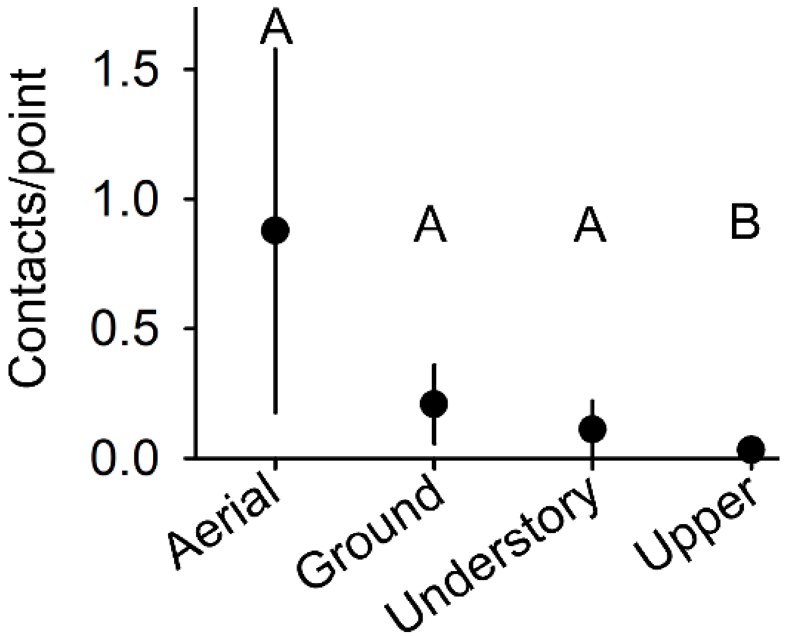
Smith *et al.* (2022) *Ecological Applications.*

Which species carry the greatest risks?

Insectivores and omnivores are less likely to carry pathogens



Canopy-foraging species are less likely to contact fresh produce



Ongoing Work: Pathogen Prevalence

- 100 of 130 species in our database have <20 birds tested for *Campylobacter, Salmonella*, and STEC
- **Goal:** Expand pathogen prevalence database for understudied species
- 8 more species now adequately sampled (>20 birds)
- 13 species with larger sample sizes (but still <20)
- 215 total fecal samples for undersampled species

Nest box birds	High-risk birds	Winter birds	Migrant birds
			

Ongoing Work: Bird Intrusion

- Across 20 leafy-greens farms in the Central Coast
  1. Survey birds in summer, fall, and winter, noting which individuals contact produce
  2. Quantify fecal densities along 20m transects
  3. Collect ~1000 feces from transects to quantify pathogen prevalence AND identify which species are defecating on crops

Ongoing Work: Pathogen Survival

- Experiment 1: Field conditions
- Experiment 2: Variation among 10 bird species
- Experiment 3: Pathogenic vs. non-pathogenic *E. coli*

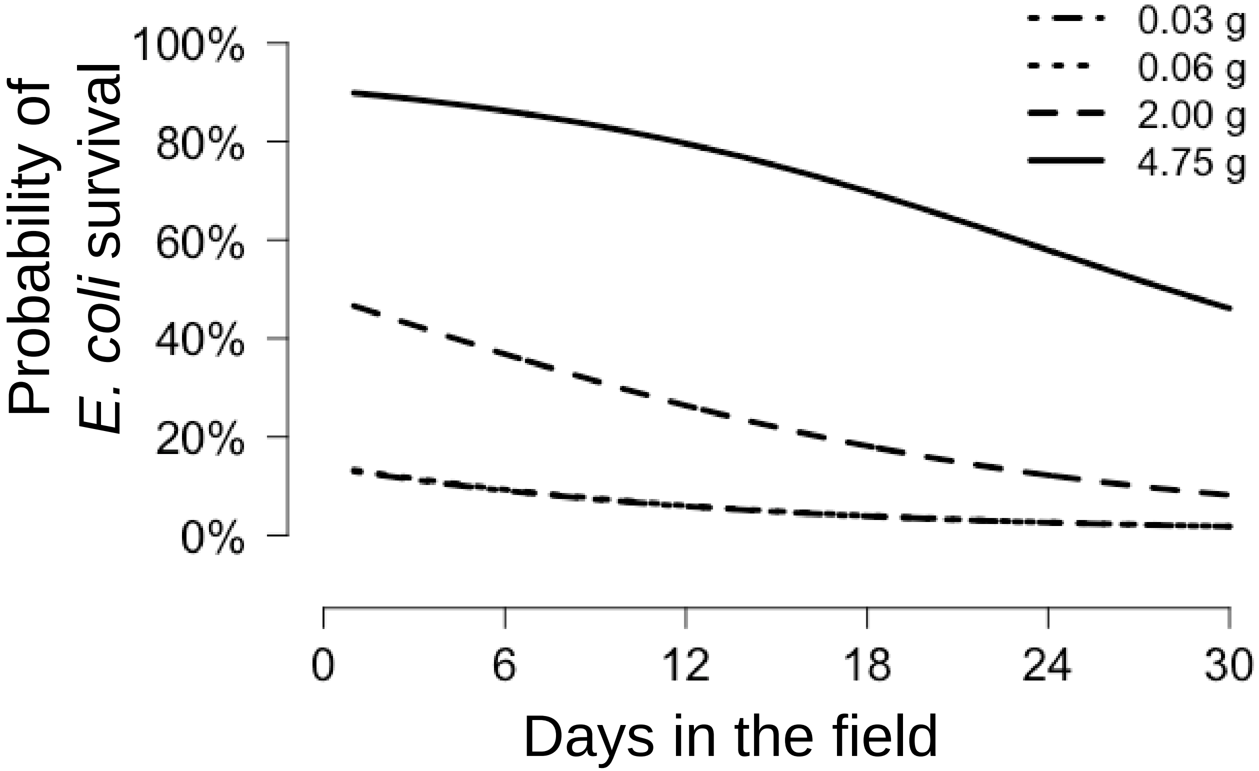
Grow *E. coli* in the lab

Collect bird feces & inoculate with *E. coli*

Deploy in field, collect at set times, and quantify fraction remaining

Ongoing Work: Pathogen Survival

- Field experiment: Wild Turkey and Western Bluebird
- Standard and natural fecal sizes
- Size, not bird species identity, determined pathogen survival



Implications

- Farmers are told to apply no-harvest buffers (often 1 m) around wildlife feces
- We ran >170 bird fecal transects on 43 farms…
  - 50% of 1 m^2 quadrats on strawberry farms had feces
  - 35% on lettuce farms had feces
- Can we judge food-safety risks by species according to fecal size?
- Can farmers ignore small songbird feces?

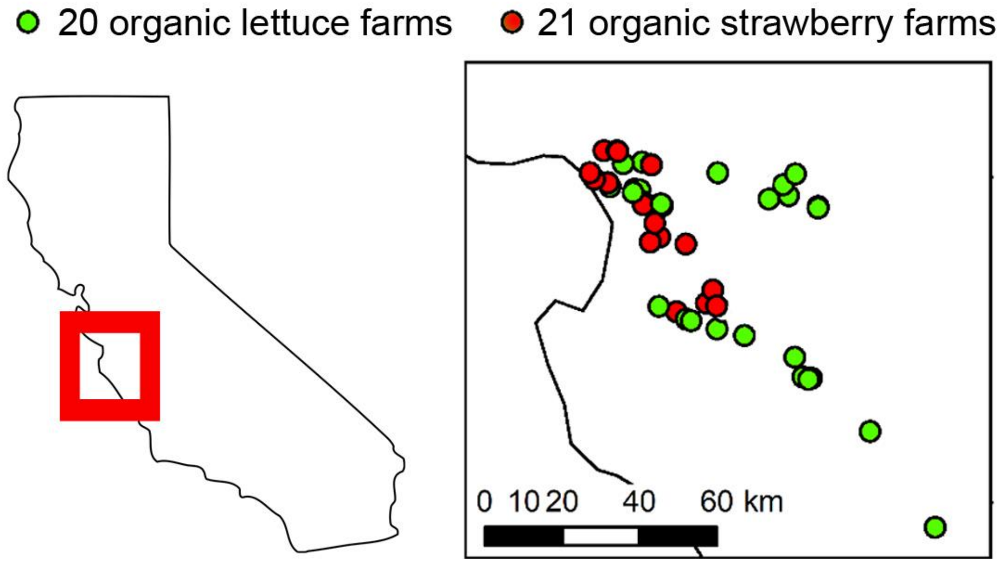
Guiding Questions

1. Which species carry the greatest food-safety risks?
    - Pathogen prevalence
    - Crop contact
    - Pathogen survival

2. How do farm management and surrounding landscapes affect the food-safety risks from birds?

Farm Management/Landscape Context Effects

- ~20 organic strawberry farms and ~20 lettuce farms surveyed per year from 2018-2020
  - Local diversity (*e.g.*, # of crops, non-crop vegetation, etc).
  - Surrounding ungrazed seminatural habitat (within 1 km)
  - Surrounding grazed seminatural habitat (within 1 km)
- Strawberry study: fecal samples from captured birds
- Lettuce study: bird counts and feces collected from lettuce



Local Diversity

Ungrazed Habitat

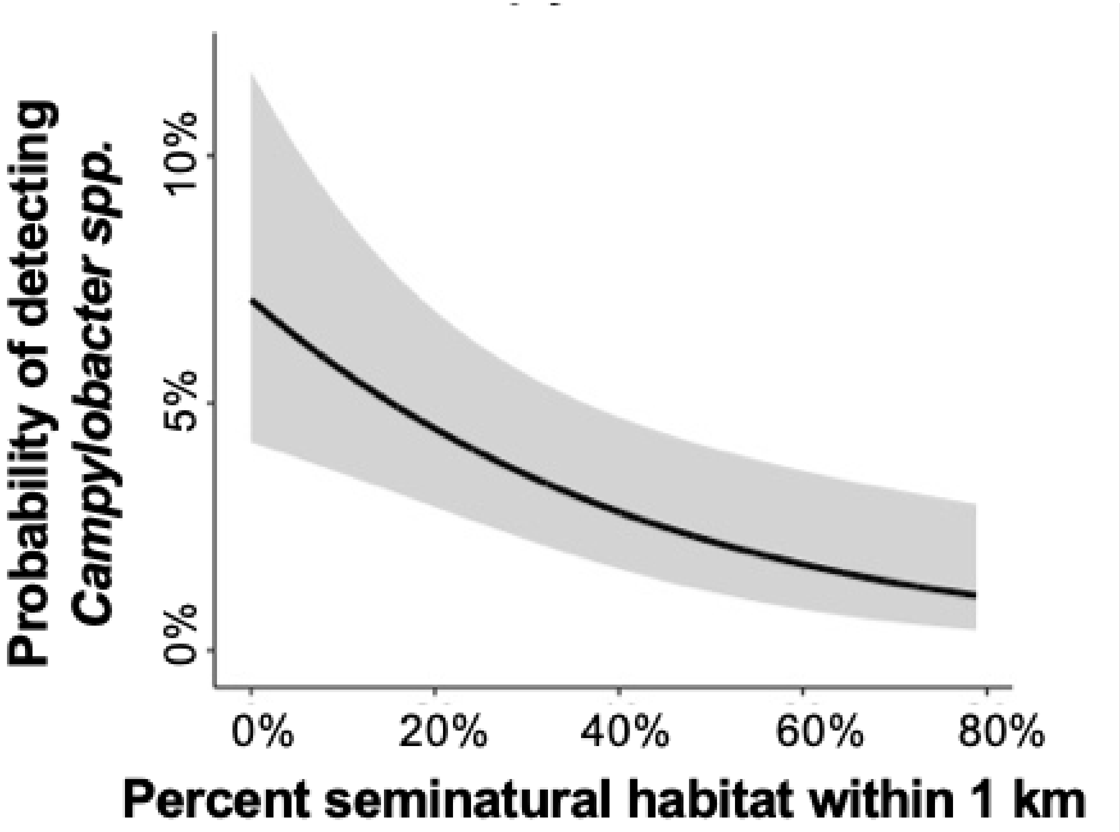
Grazed Habitat

Farm Management/Landscape Context Effects

Strawberry Study:

- Feces contaminated 2 of >10,000 strawberries
- Positivity (out of 980 feces from captured birds)
  - STEC: 0.1%; *Salmonella*: 0%; *Campylobacter*: 3.6%

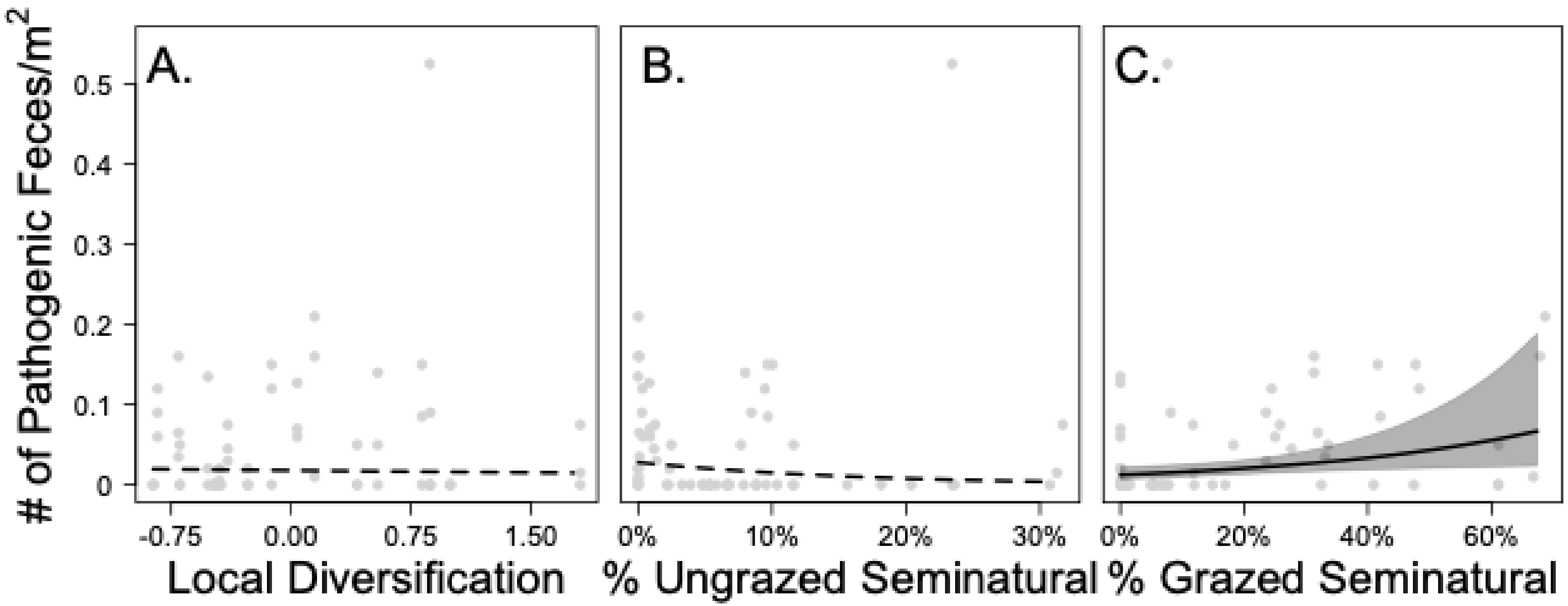
Olimpi *et al.* (2021)
Journal of Applied Ecology.



Farm Management/Landscape Context Effects

Lettuce Study:

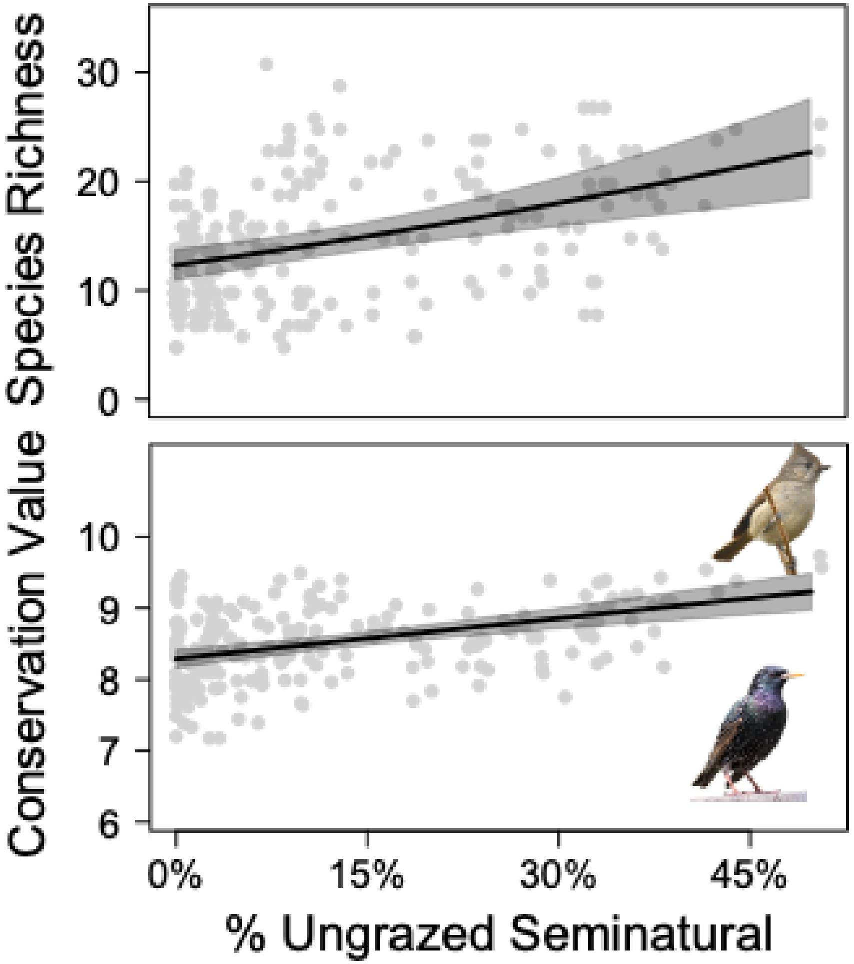
- Positivity (out of 601 feces from lettuce plants)
  - STEC: 0%; *Salmonella*: 0%; *Campylobacter*: 5.7%
- Potentially pathogenic feces: Positive for *Campylobacter* or possible *E. coli* virulence genes

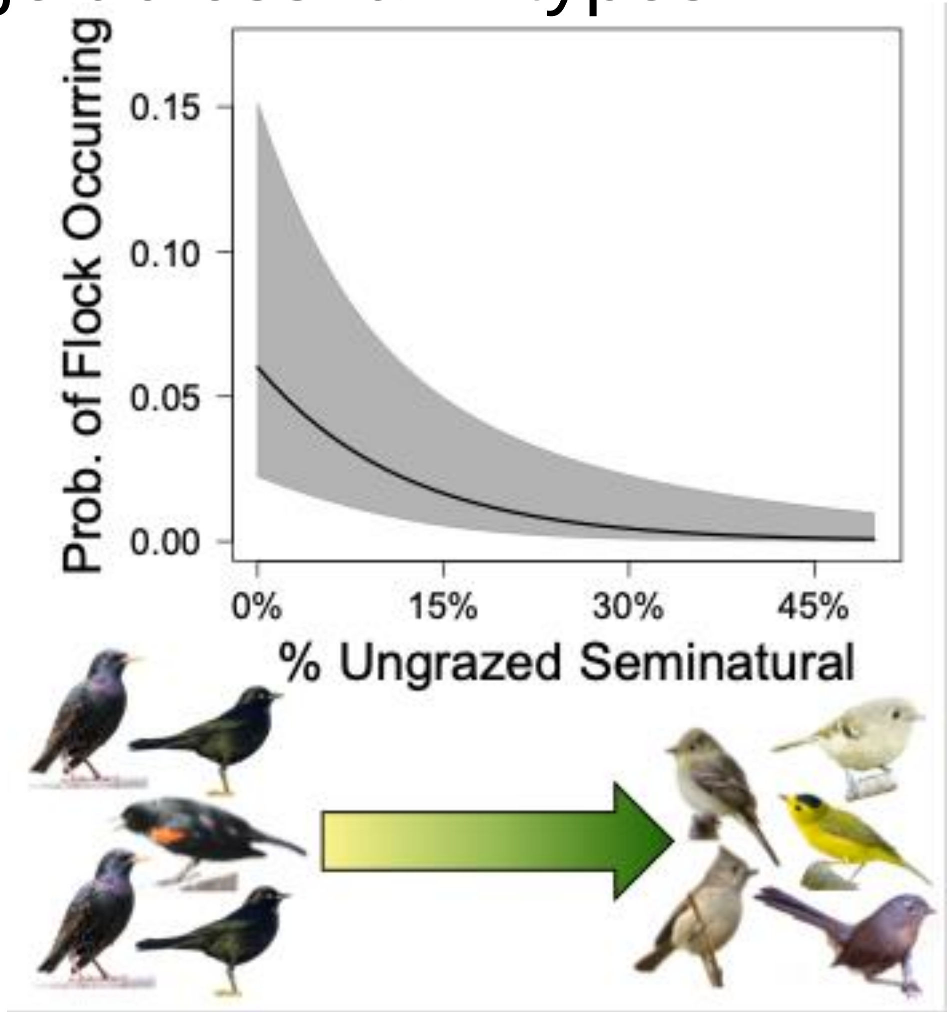


Farm Management/Landscape Context Effects

Lettuce Study:

- What is driving these trends? Specifically, how do bird communities change across farm types?





Ongoing Work: Bird Intrusion

- Objectives:
  1. Identify which species are contaminating crops
  2. Pinpoint effects of surrounding rangeland on food-safety risks from birds
  3. Unpack seasonal variation in habitat effects on bird communities & associated food-safety risks
- Early results: Birds are more abundant in fall
  - 3,081 vs. 6,140 birds
  - 59 vs. 201 flocking events

Conclusions/Implications

1. Pathogen prevalence is low, but species vary in risk
    - Higher risk: livestock-associated, flocking birds that defecate large feces
2. No-harvest buffers near small feces *may* be unnecessary
3. Nest boxes for pest-eating birds seem to be low risk
4. Removing non-crop vegetation likely harms species of conservation concern without improving food safety

Acknowledgements

- Our amazing team!
- Growers and landowners
- The Center for Produce Safety (and NSF/USDA)
- All of you for listening!

Quantifying risk associated with changes in EHEC physiology during post harvest pre-processing stages of leafy green production

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Project Team

- Dr. Jade Mitchell, co-PI

- Dr. Joshua Owade, postdoc

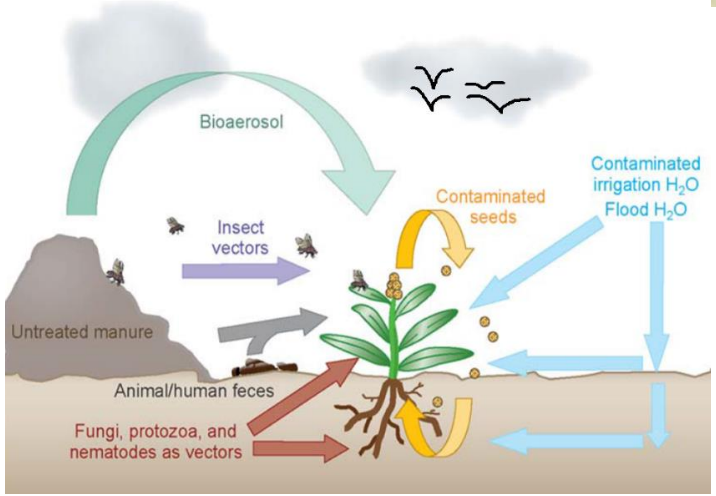
- Dimple Sharma, graduate student

- Cleary Catur, undergraduate student

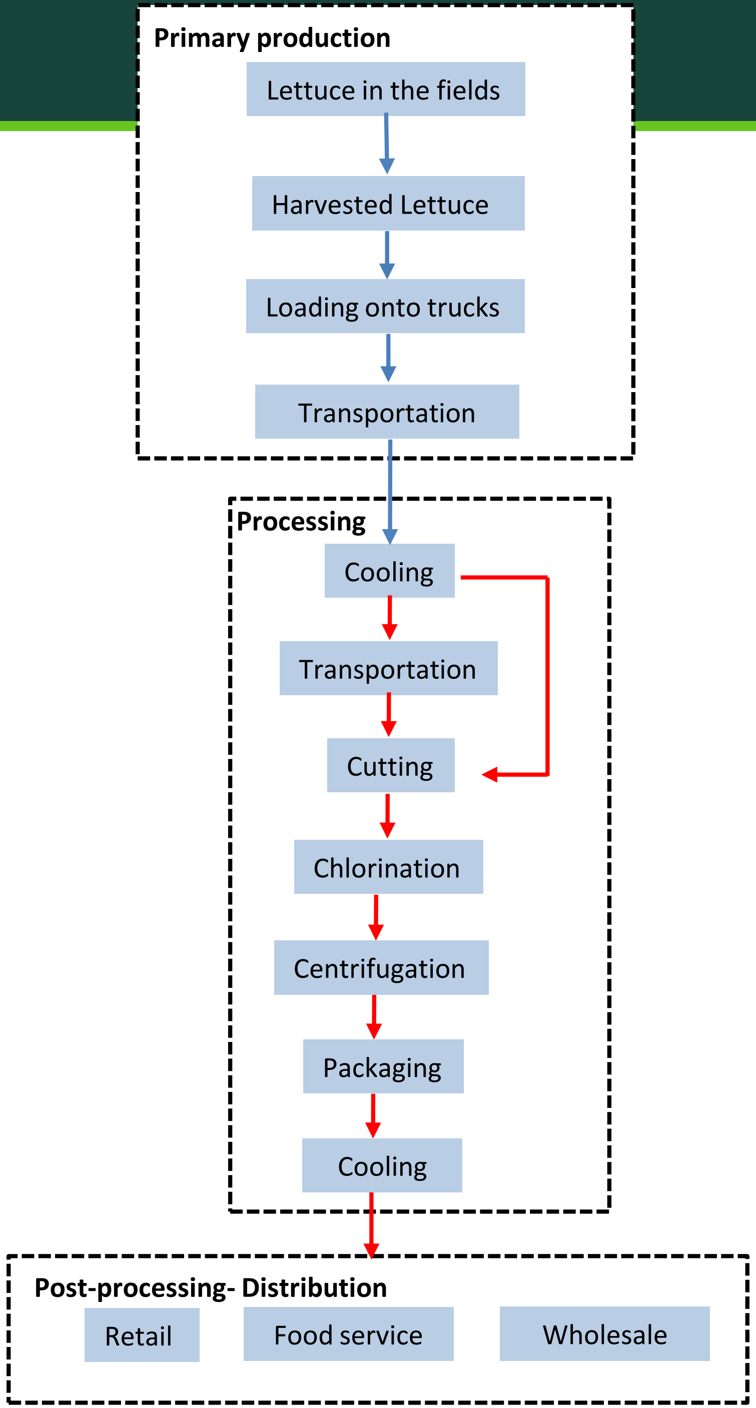
Recurring U.S. outbreaks of STEC O157 linked to leafy greens

Year	Food
2021	Packaged salads
2021	Baby spinach
2020	Leafy greens
2019	Romaine lettuce
2019	Salad mix
2018	Romaine lettuce
2018	Romaine lettuce

- Likely sources of contamination
  - Irrigation water
  - Runoff from cattle operations
  - Improperly treated manure

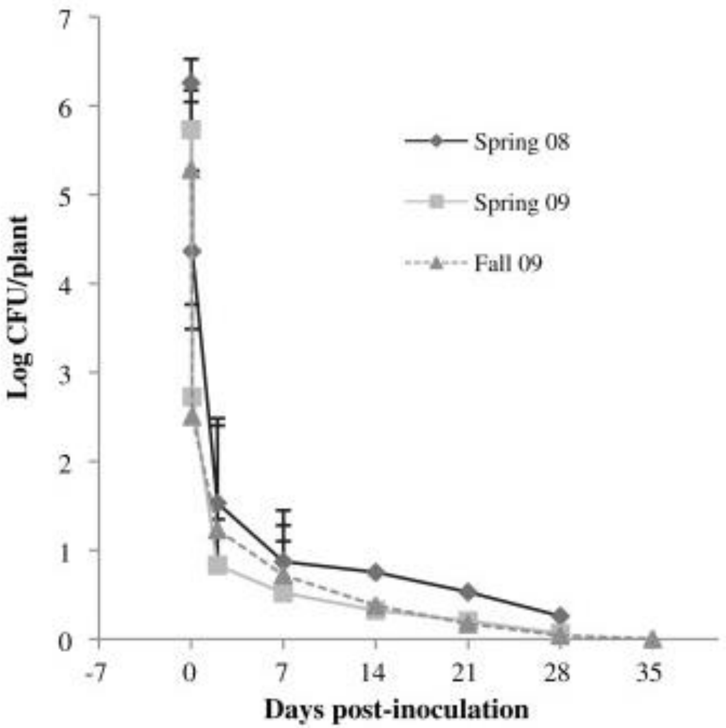


Brandl 2006
Ann. Rev. Phytopathology



Microbes on lettuce experience numerous environmental changes during production

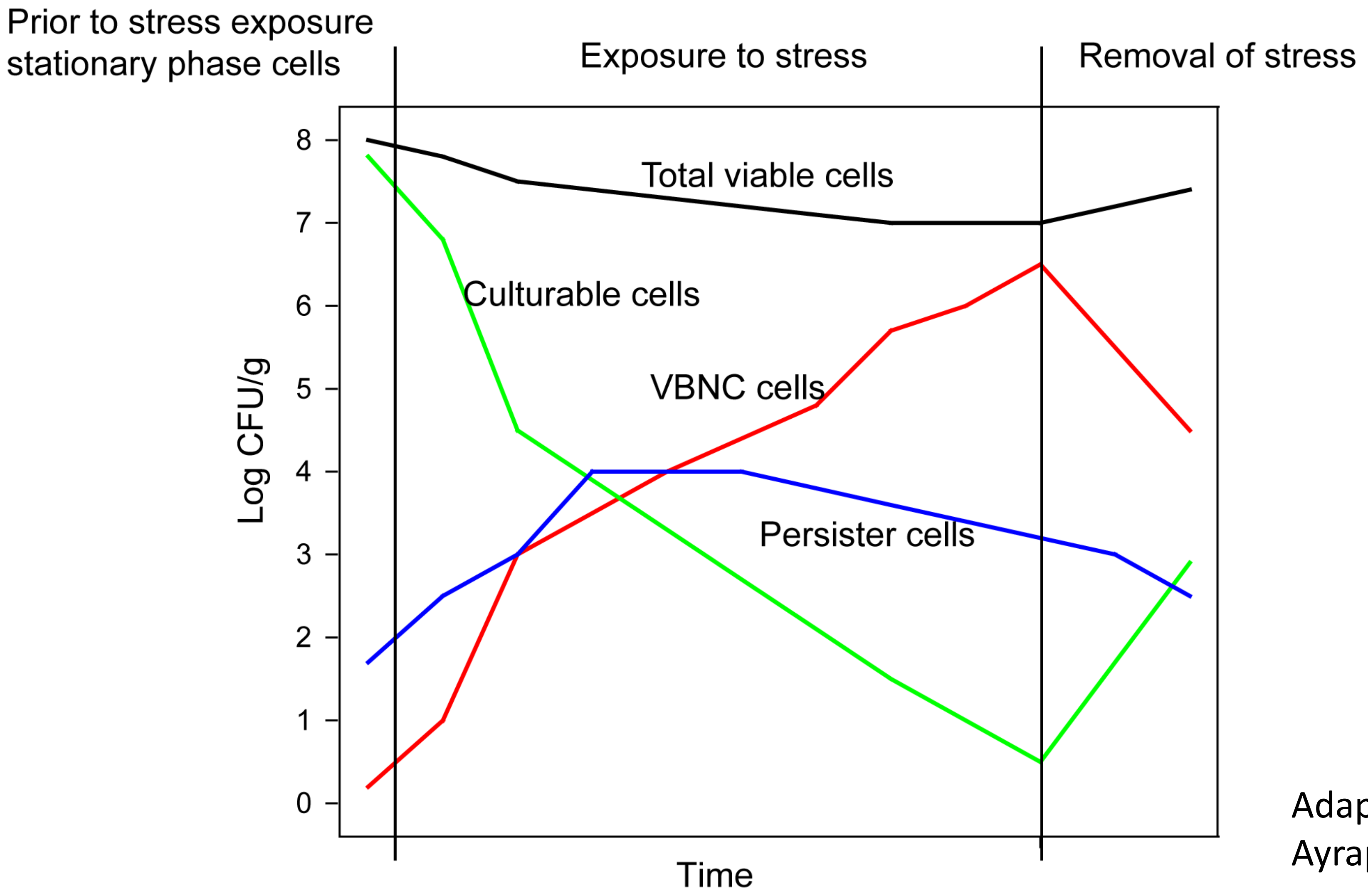
- Primary production
  - Bi-phasic decay on plants (Moyne et al. 2011)
- Processing
  - Chlorine, other sanitizers
    - ~ 1 log reduction in pathogen numbers
- Post-processing
  - Temperature most influential



Physiological state of pathogens in the pre- and post-harvest environment

- Typically monitor changes in culturable cells
- Physiological adaptations -> better stress tolerance and survival
- Persister state
  - sub-population of cells resistant to antibiotics when no antibiotic resistance mechanism present
  - Induced by environmental stress
- Viable but non-culturable (VBNC)
  - Sub-population of cells that are viable but unable to grow on culture media

Potential dormancy dynamics for a bacterial population exposed to stress



Adapted from Fig. 1 from
Ayrapetyan et al. J. Bac. 2018

Increase in STEC persister cells under pre-harvest conditions

E. coli O157 inoculated on lettuce plants (Munther et al. AEM 2020)

- 50 fold increase in persister cells when plants exposed to low relative humidity

Multiple strains of STEC inoculated into samples of irrigation water (Thao et al. Food Microbiology 2019)

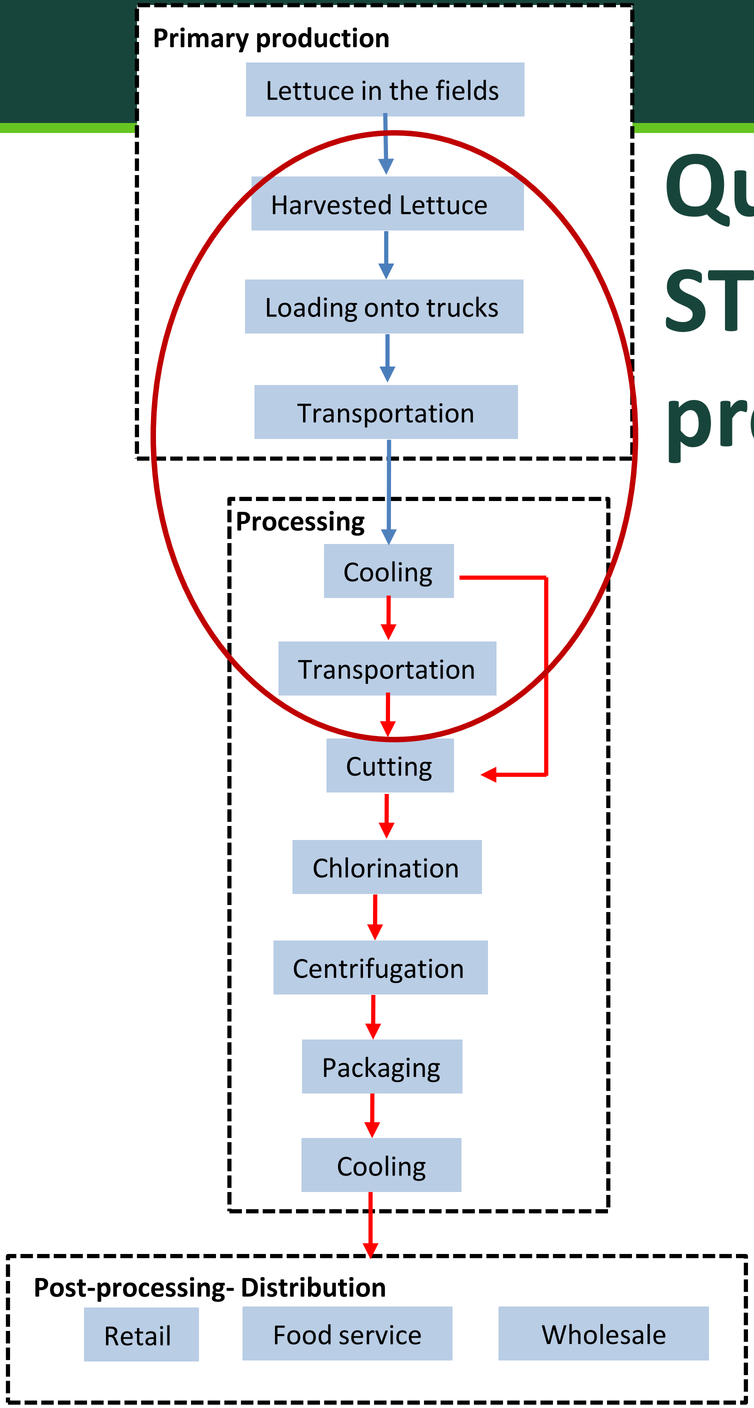
- Variability in extent of persister formation among strains
- 10 to 20% of the population in persister state after 1 week in water

Impact of persister and VBNC formation on STEC in the lettuce supply

- Bi-phasic decay on plants, remaining population likely to be in persister state (Brandl et al., 2022)
- Persister cells have enhanced survival to low pH, antimicrobial compounds and ROS (Gollan et al., 2019)
- VBNC cells form in the phyllosphere (Dinu et al., 2011) and in response to sanitizers used in lettuce processing (Truchado et al., 2023)

- Does this contribute to transmission and outbreaks? How?

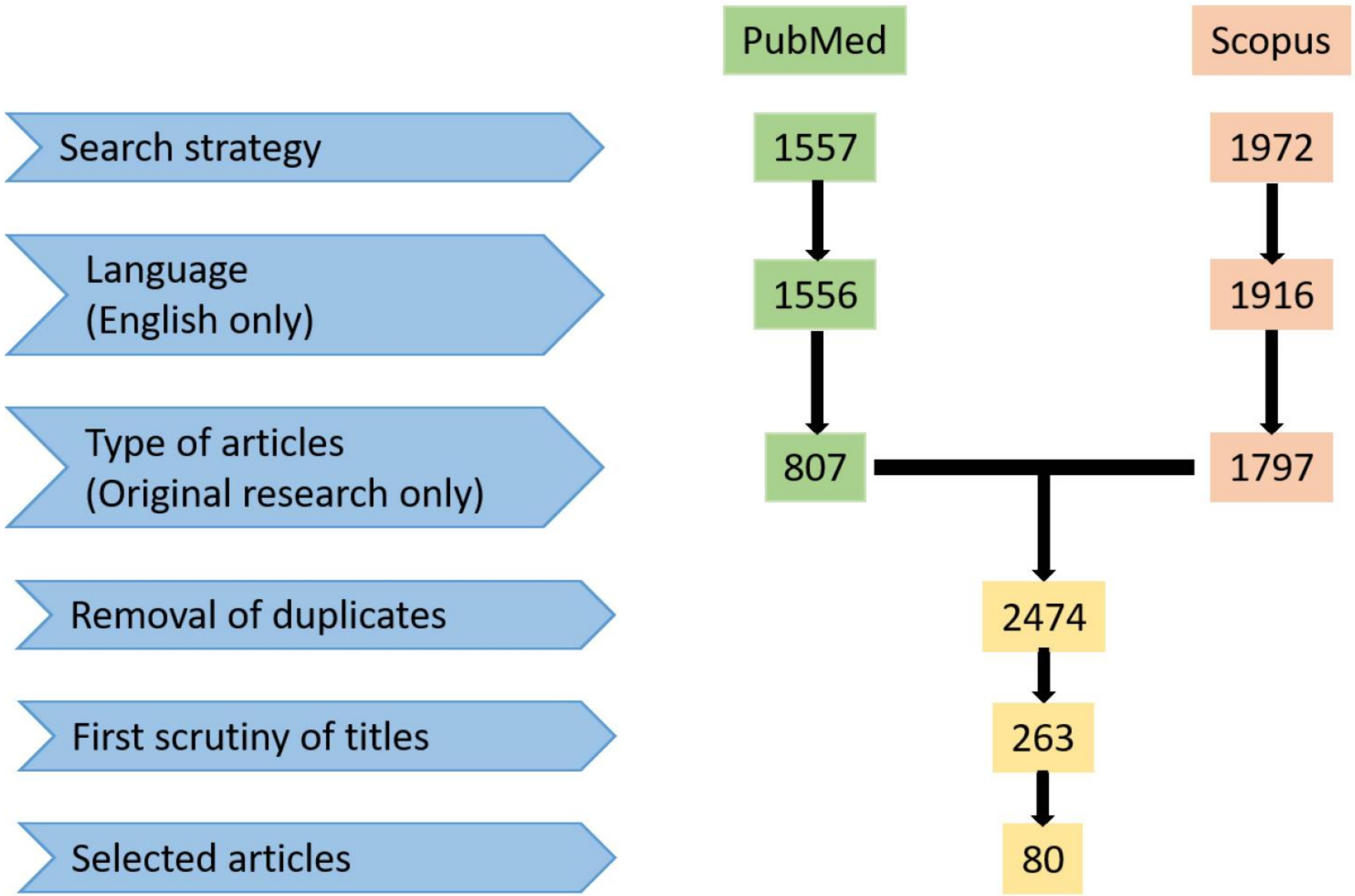
Quantifying risk associated with changes in STEC physiology during post-harvest pre-processing stages of leafy green production



- Effects of temperature and time on formation of persisters and VBNC cells on lettuce
- Changes in acid resistance and chlorine tolerance over time
- Changes in virulence over time
- Outputs: QMRA and online quantitative assessment tool for leafy green producers

Systematic review process

- Systematic review
  - O157 kinetics during each stage of romaine lettuce production
- 80 papers reviewed
- Data gaps identified
  - Harvest to cooling



Temperature at harvest

- Salinas at harvest
  - Median = 57.9F
  - Mean = 57.8±7.4F
- Yuma at harvest
  - Median = 53.6F
  - Mean = 54.2±8.2F
- Temp after cooling
  - Salinas median = 34.5F
  - Yuma median = 34.7F

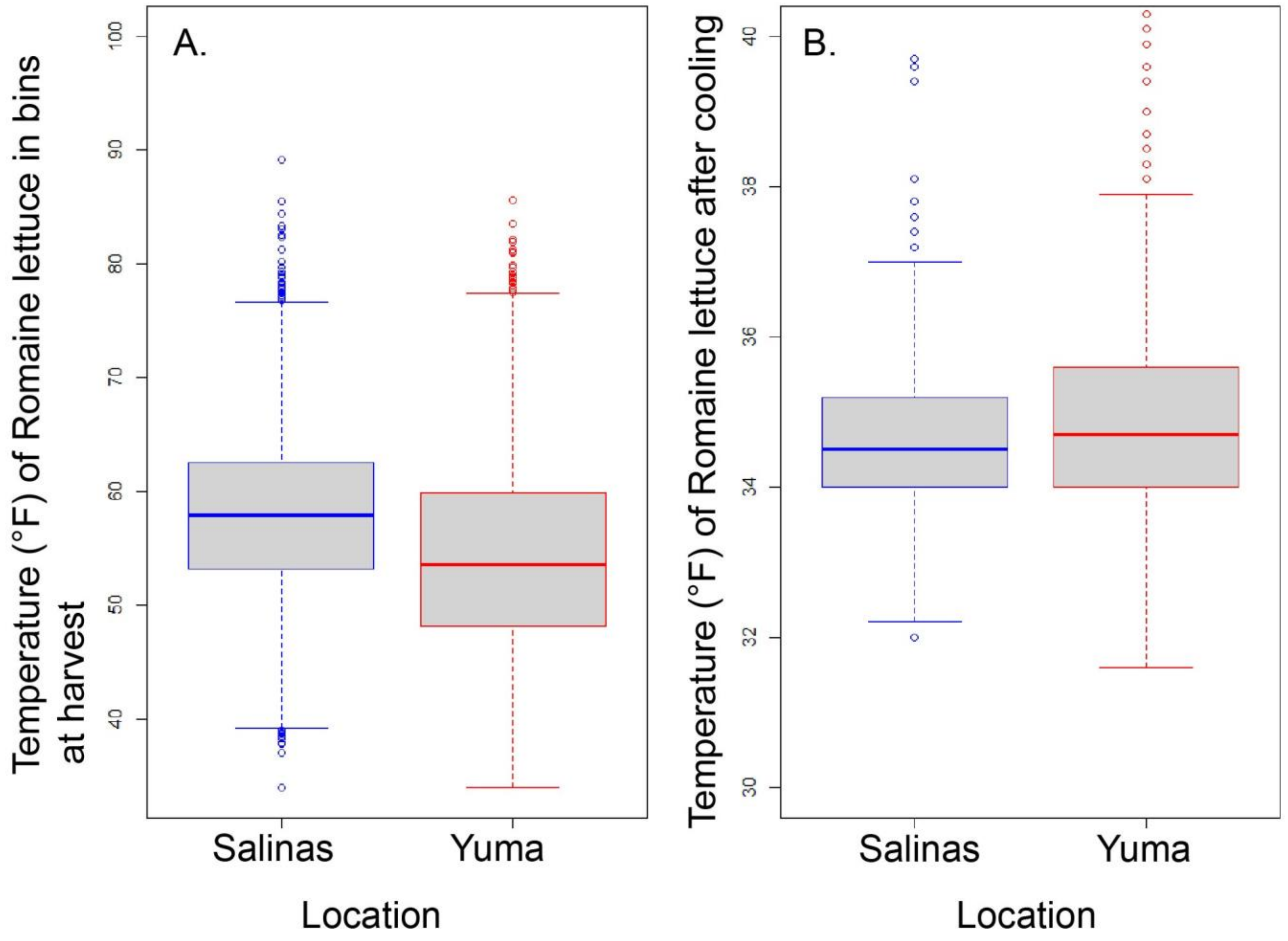


Figure 1. Temperature of Romaine lettuce in bins at harvest (A) and after cooling (B). Boxplots represent 5615 and 4623 datapoints collected from the Salinas and Yuma growing regions, respectively.

Cut to cool time

- Salinas = 163 min
- Yuma = 232 min
- Time to cool at cooling center
  - Salinas = 32 min
  - Yuma = 33 min

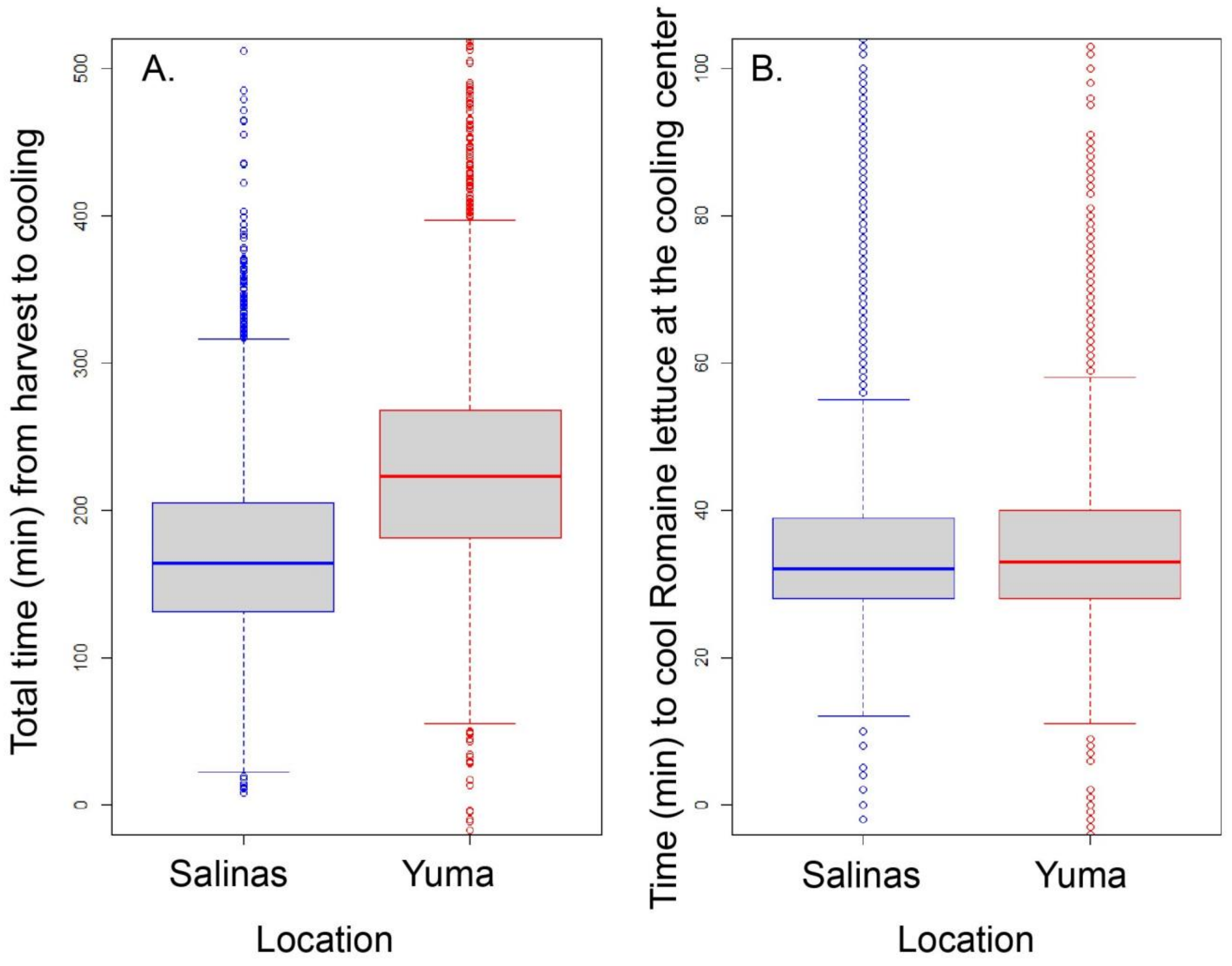


Figure 2. Cut to cool time for Romaine lettuce in bins (A) and time to cool Romaine lettuce at the cooling center (B).Boxplots represent 5615 and 4623 datapoints collected from the Salinas and Yuma growing regions, respectively.

EHEC O157:H7 strain set

- Obtained from patient isolates associated with Romaine outbreaks
    - Yuma 2018
    - Central coast, CA 2018
    - Salinas 2019
- All strains selected for rifampicin resistance
- MICs for ciprofloxacin determined
    - Necessary for persister assay

Selected harvest temperatures and time

- 75th percentile for cut to entry to cooling center time (240 min, 4 hours)
- 25th and 75th percentile for harvest temperature 48.2F (9C) and 62.6F (17C)

Total time (min) from harvest to entry at the cooling center

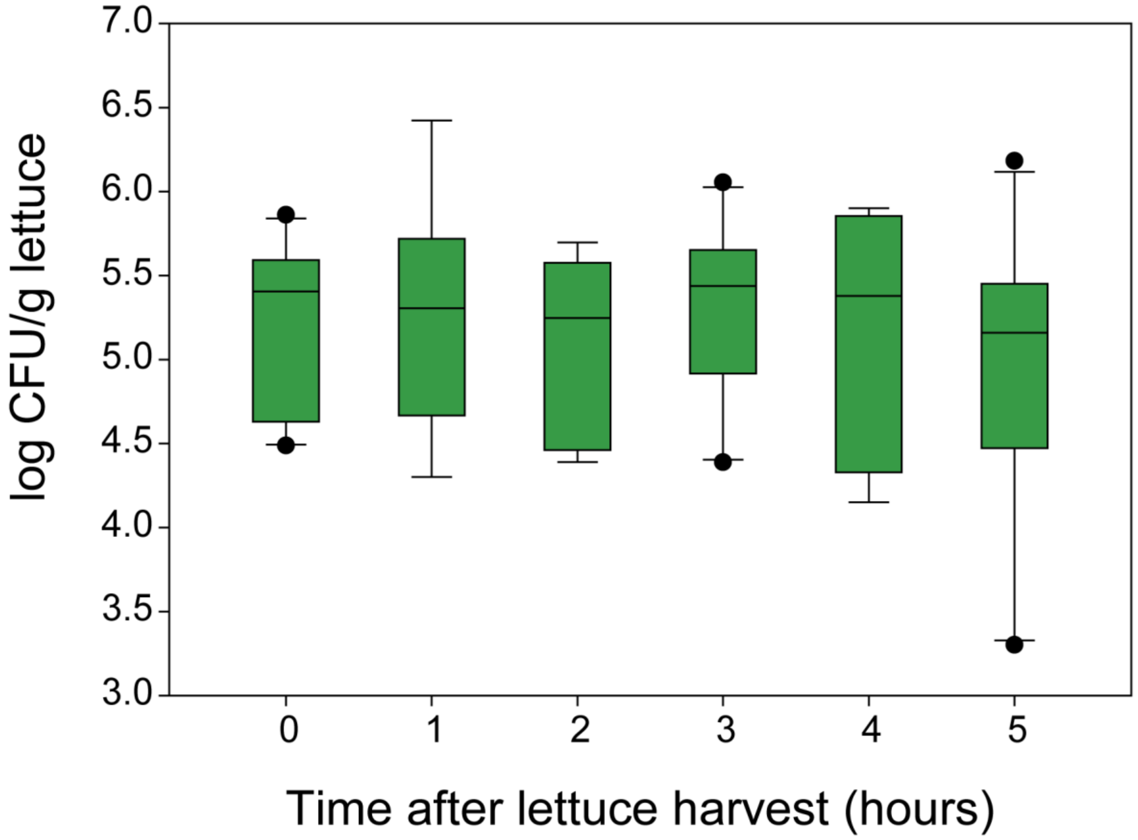
Location	Median	25th Percentile	75th Percentile
Salinas	129	95	169
Yuma	186	144	232

Average temperature (°F) of the bin at harvesting

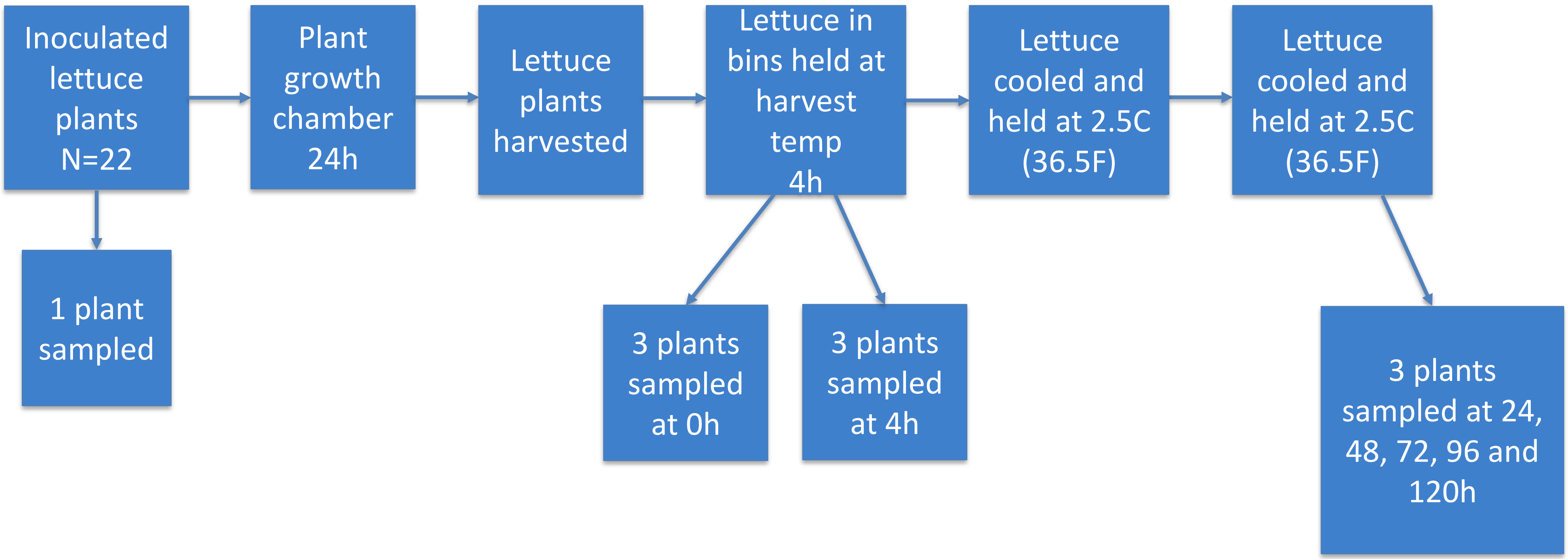
Location	Mean	Median	25th Percentile	75th percentile
Salinas	57.8	57.9	53.2	62.6
Yuma	54.2	53.6	48.2	59.9

Minimal change in *E. coli* O157 density on lettuce after harvest at 17C

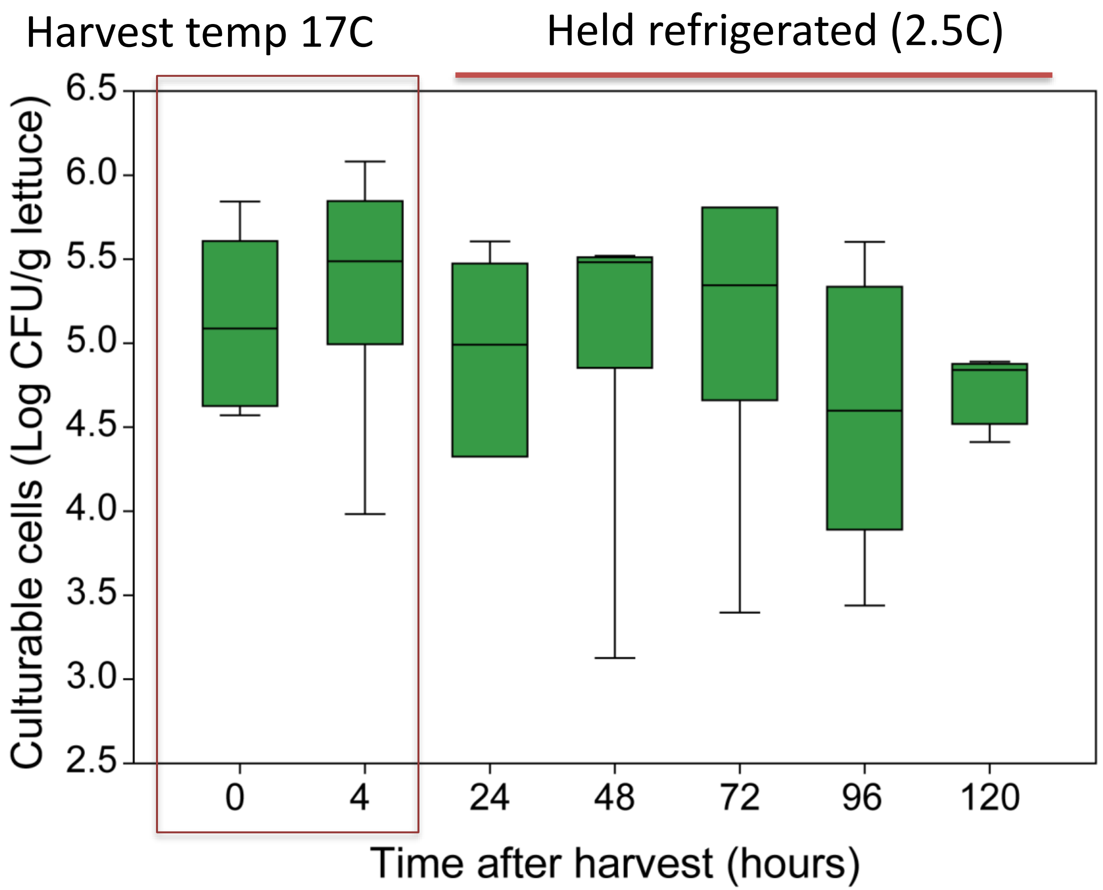
- Plants inoculated and held in growth chamber 24h before harvest

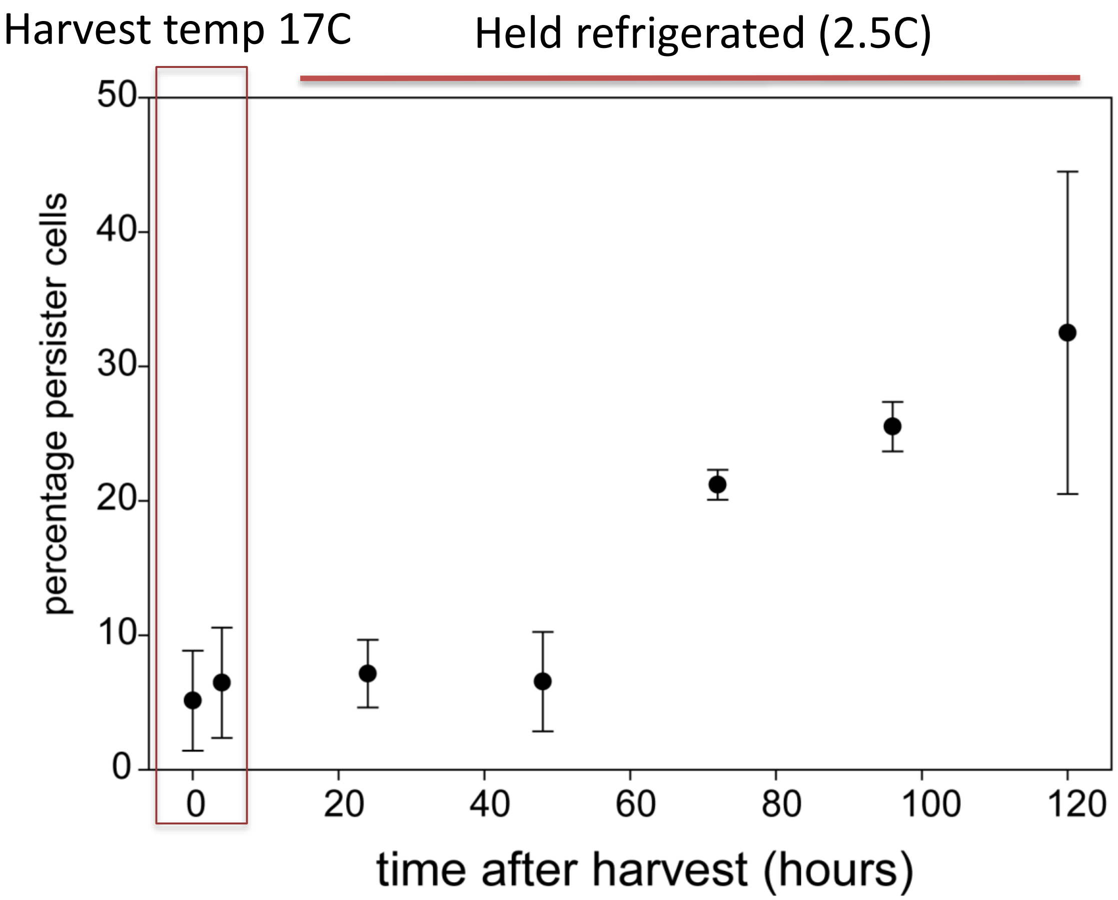


Experimental design – sample collection



Increase in persisters over cold storage





Stay tuned…

- Determine associations between increase in persister cells and stress tolerance
  - Acid resistance, chlorine tolerance
- Incorporate in QMRA for EHEC O157 on Romaine lettuce
- Identify handling and/or storage practices that reduce risk of O157 transmission

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- Center for Produce Safety
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- FDA 2R44FD006465-02

USDA United States Department of Agriculture National Institute of Food and Agriculture

CPS CENTER for PRODUCE SAFETY

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March 28, 2023	Food Toxicology Webinar-Food Chemical Safety and Current Tools and Methods
May 8, 2023	Is it a Listeria sensu stricto or sensu lato species? Why understanding the difference is important
May 16, 2023	Introduction to Toxicology Part II: New Methodologies: Application in Food Safety and International Trade
June 14, 2023	Dry Cleaning: Is Water Friend or Foe in Food Safety and Sanitation?
June 15, 2023	Tech-Enabled Traceability: Get Ready For FSMA 204 With GS1 Standards

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