

Through the bugs' lens: Insights into bacterial persistence and dynamics on fruit and vegetables

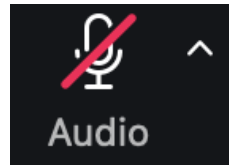
With Dr. Shirley Micallef



Instructions

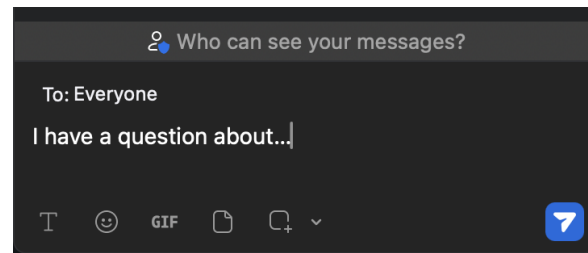
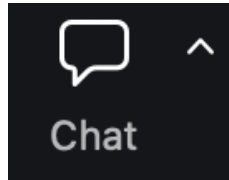
1

- All participants are muted
- There will be time for questions and discussion at the end of the presentation



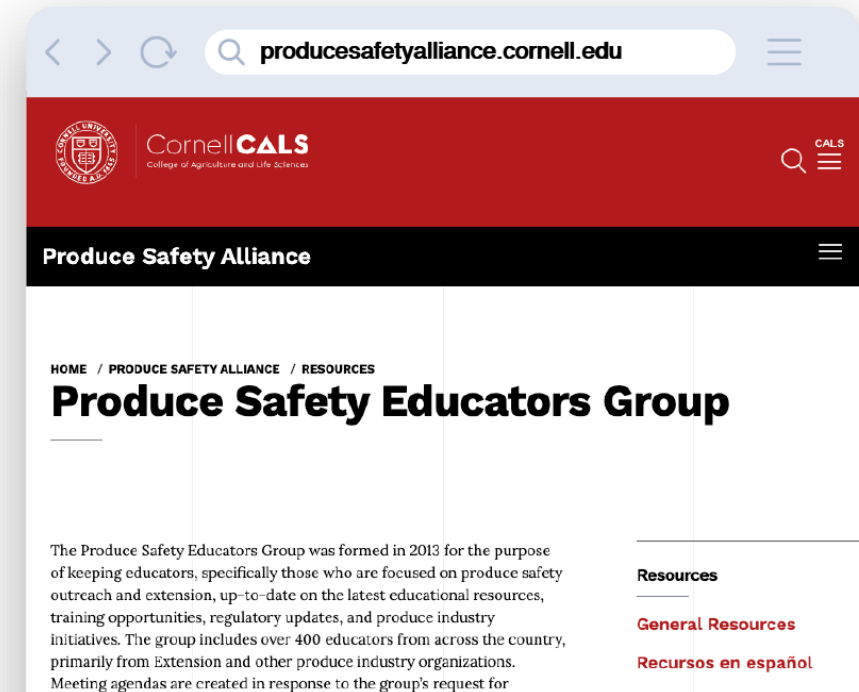
2

- Feel free to use the chat box to ask questions as well



3

- We are recording this session and will share the presentation via the email list and our website after the call



Welcome Back!

- We are very excited to be able to continue these webinars in both English and Spanish!
- Funding for this series of English Educators Calls is provided by the Local Food Safety Collaborative
- We really appreciate all your engagement AND the participation of content experts so we can discuss topics important to you



Agenda



Introductions



Dr. Shirley Micallef. *Through the bugs' lens: Insights into bacterial persistence and dynamics on fruit and vegetables*



Q&A session



PSA updates and upcoming events

Dr. Shirley Micallef

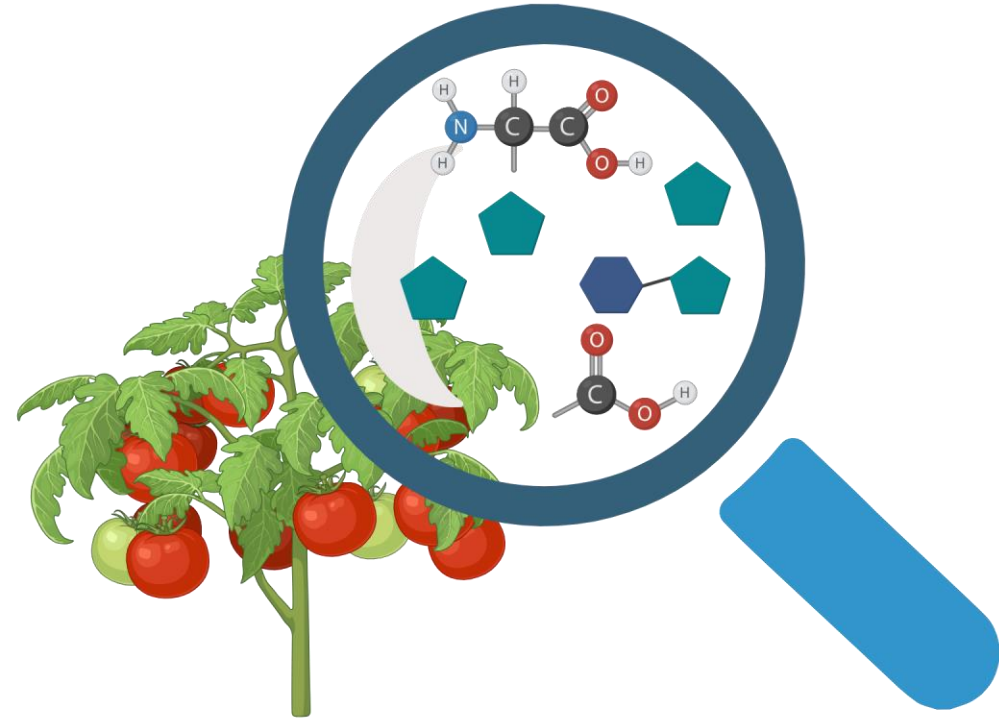
- Professor, University of Maryland

- Research focus:

- Microbiological produce safety
 - Pre-harvest plant associations with *Salmonella* and *E. coli* O157:H7
- Impact of cropping practices and environmental/climatic conditions on the persistence and dispersal of foodborne enteric pathogens in the agricultural environment
 - Cover crops, soil amendments, mulches, weather events
- Reciprocal responses in enteropathogens - plant associations
- Environmental metabolomics of plant surfaces to explain enteric pathogen colonization of plants
- Beneficial microbes, plant-growth promoting bacteria and their role in plant resilience to abiotic and biotic stresses
- Crop microbiomes
- Safety of harvested rainwater
- Crop nutritional quality



Through the bugs' lens: Insights into bacterial persistence and dynamics on fruit and vegetables



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Micallef, S. (2026)

Shirley Micallef, Ph.D.

Department of Plant Science & Landscape Architecture

Center for Food Safety and Security Systems

MD Food Safety Network

The plan



- 🦠 Bacterial growth and requirements
- 🦠 Bacterial attachment
- 🦠 Plants as a niche for bacteria - microbiomes
 - 🍃 Crops as a habitat for enteropathogens: persistence, attachment, internalization
 - 🍃 Enteric bacteria on leafy greens
 - 🍃 Enteric bacteria on fruit
 - 🍃 Approaches and methods
 - 🍃 Influence of environmental conditions
 - 🍃 Biological variability



Photo: S. Micallef





Bacterial growth requirements

- Water
- Nutrients
 - Carbon
 - Nitrogen – amino acids
 - Phosphorous – DNA/protein synthesis
 - Elements/Trace elements – enzymatic reactions
- Physical conditions
 - Medium (surface/liquid), temperature, relative humidity, pH, osmotic pressure

**Physicochemical
properties**



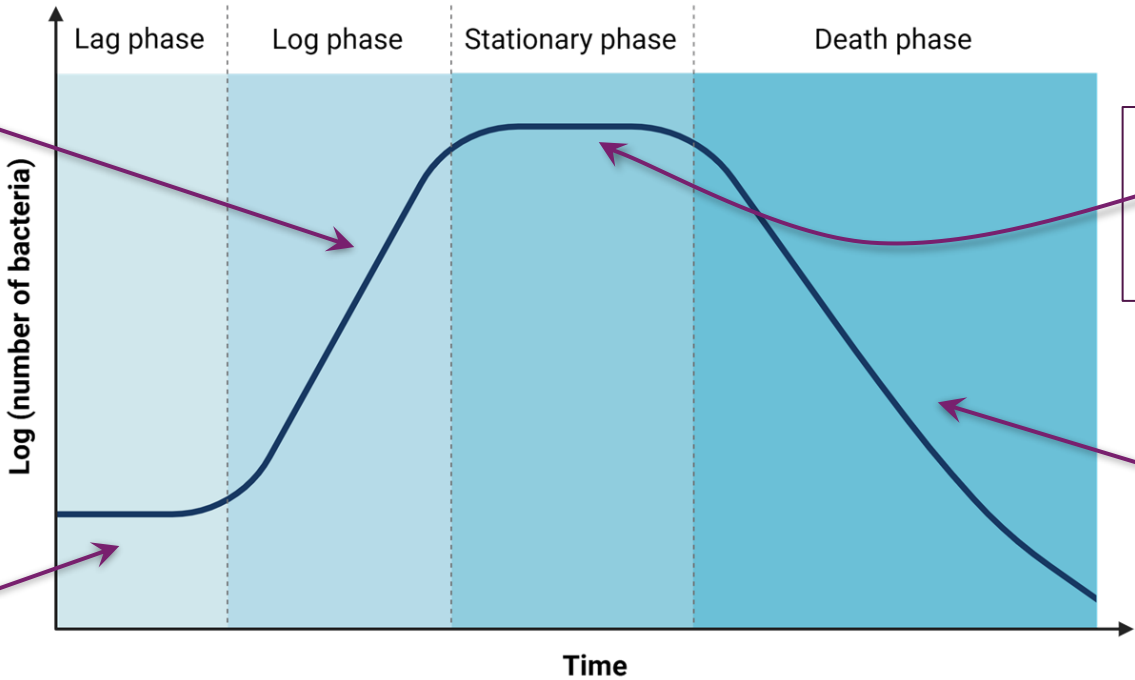
Bacterial growth

Bacterial Growth Curve



Bacteria are dividing (doubling every generation)

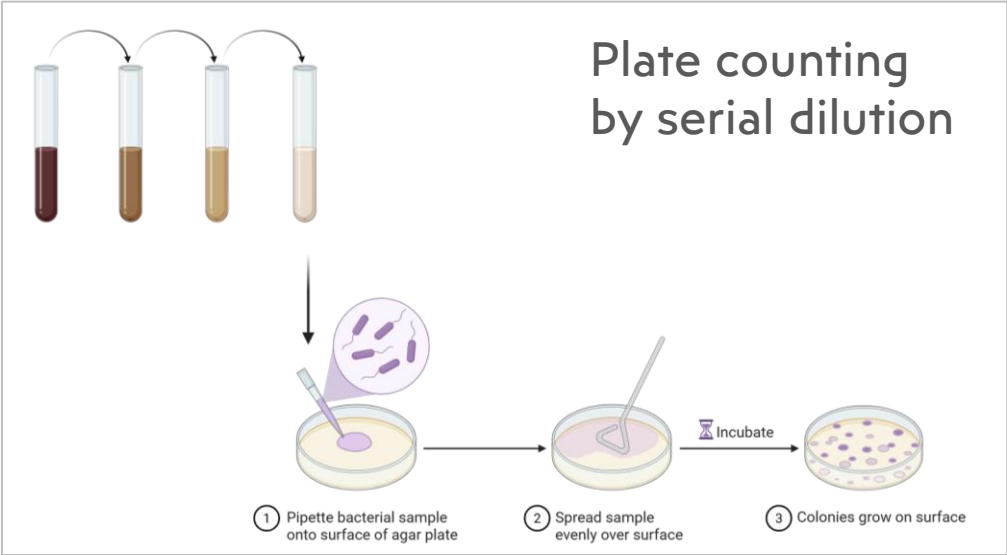
Bacteria are adapted to the environment



Bacteria are dividing at the same rate as they are dying

Bacteria are dying at a faster rate as they are dividing

Plate counting by serial dilution



Measuring bacteria

- **CFU/unit** = Recovered cell count, cell concentration, population size
 - Frequently log transformed

CFU count/unit	Log CFU/unit
1 (1.01)	0 (0.004)
10	1
100	2
1,000	3
1 million	6

**Direct measure
**Sensitive to Limit of Detection (LOD)

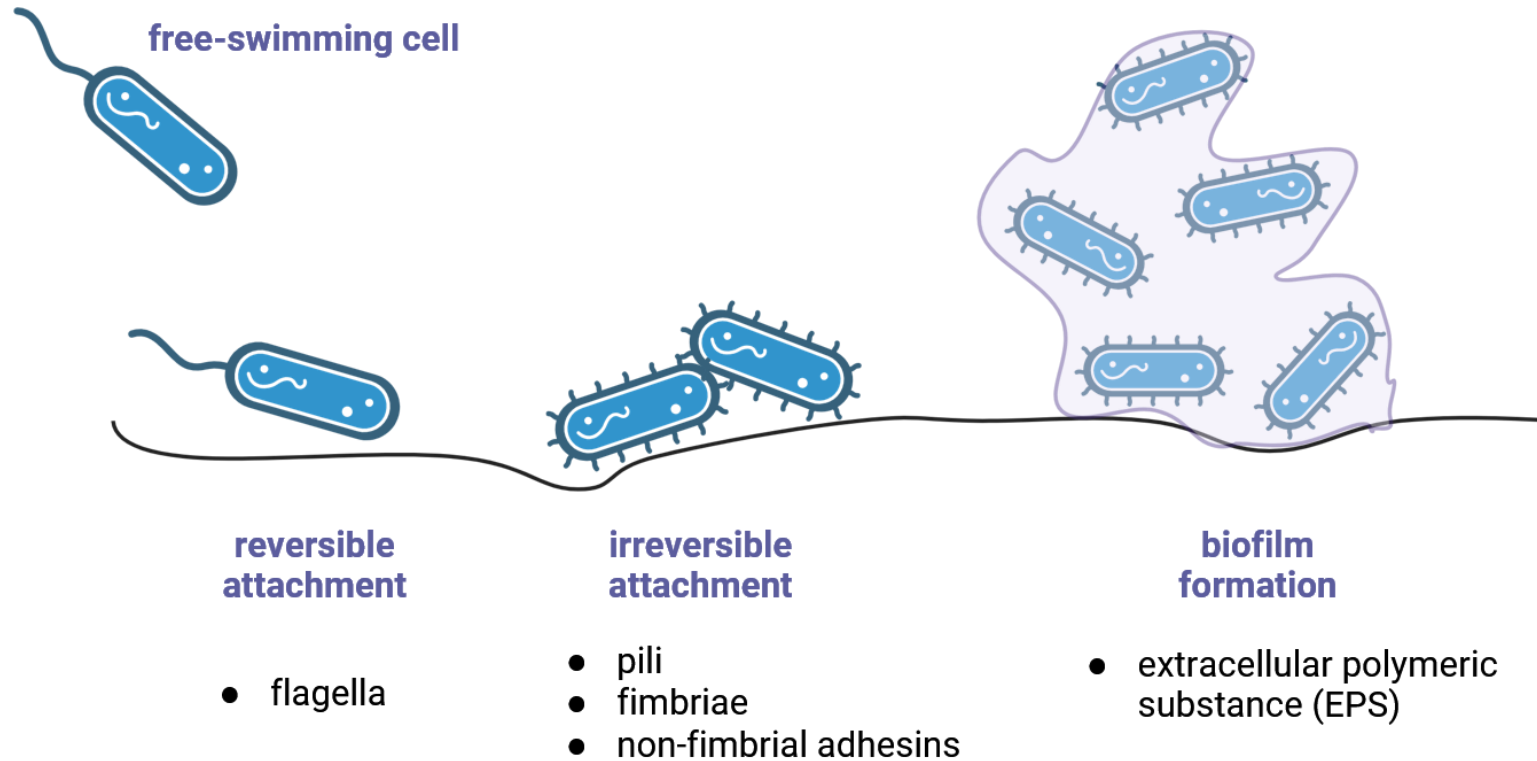
- **Log Reduction** = decline from initial (inoculum) concentration

Log reduction	CFU count	% Reduction
0	1 million	0
1	100,000	90
2	10,000	99
3	1,000	99.9
6	1	99.9999

**Not a direct count
**Actual cell concentration depends on starting (inoculum) level



Bacterial attachment: reversible → irreversible



Factors identified in enterobacterial attachment of non-host surfaces, e.g., plant cell wall models:

- Flagella
- Fimbriae
- Cellulose

Tan et al., 2016

Micallef, S. (2026) . Created in BioRender



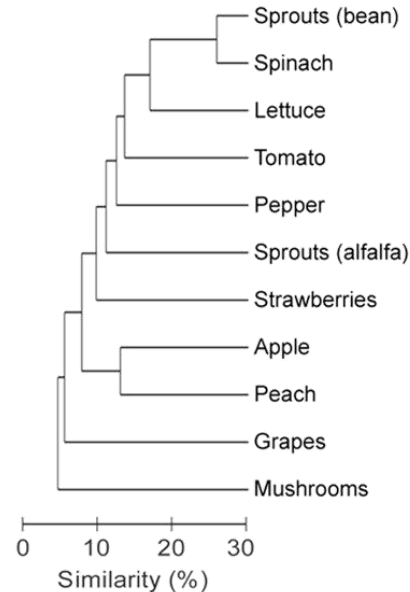


Crops as a habitat for microbial communities



Bacterial Communities Associated with Fresh Fruit and Vegetables

- Produce carries diverse bacterial communities (Enterobacteriaceae, Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria).
- Drivers for crop microbiomes:
 - ⊗ Soil type
 - ⊗ Plant species
 - ⊗ Crop characteristics
 - ⊗ Environmental conditions
 - ⊗ Agricultural inputs
 - ⊗ Crop management practices
 - ⊗ Native microbiota



Phylogeny

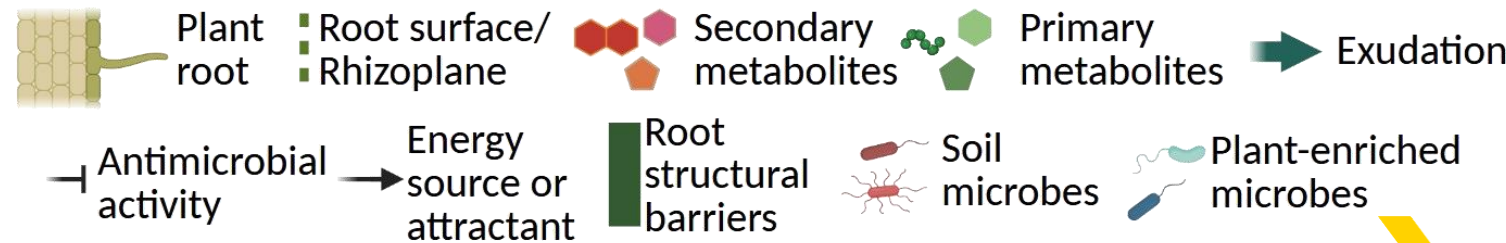
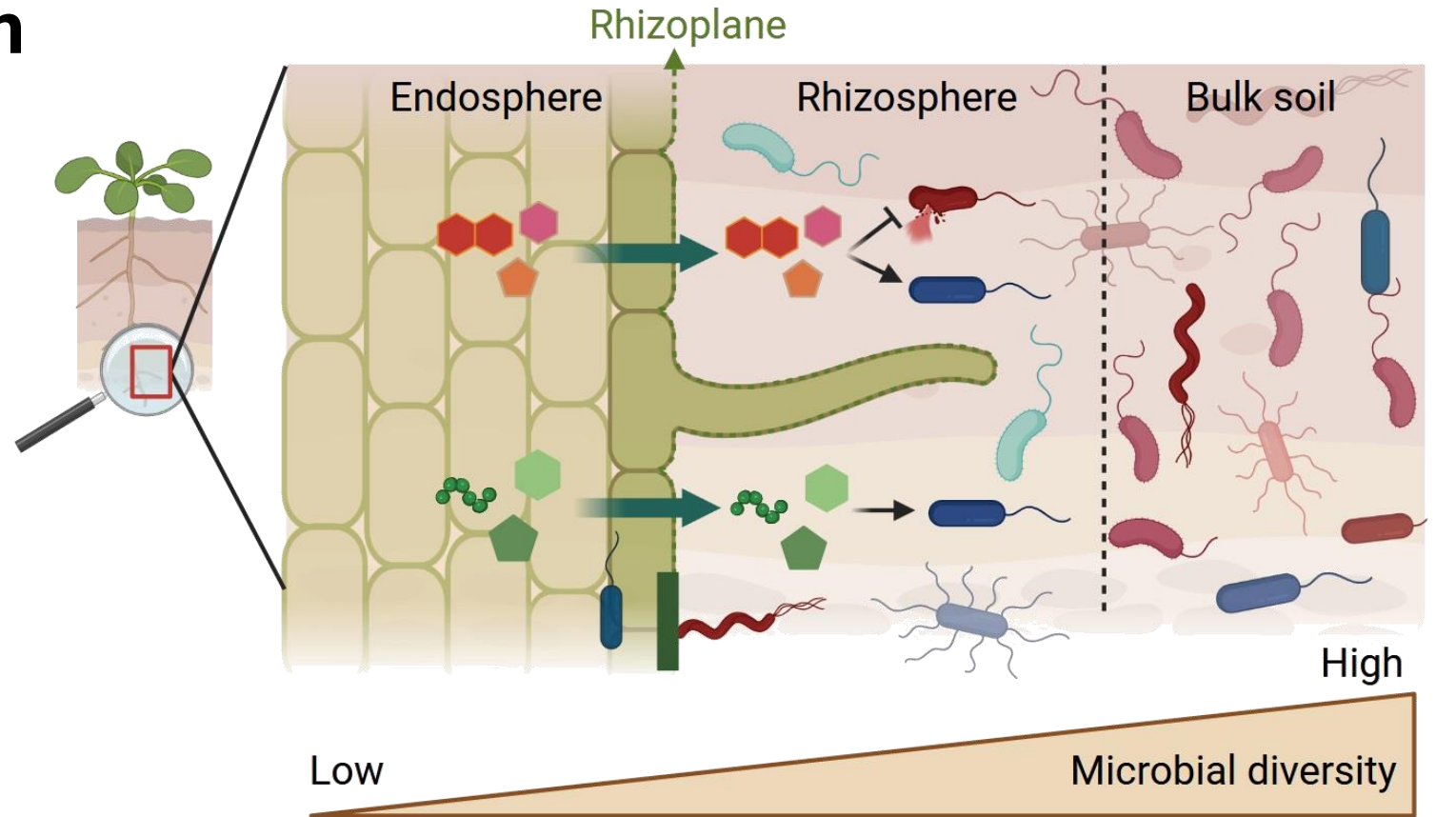
Actinobacteria				Bacteroidetes		Firmicutes					Proteobacteria														TM7								
Actinobacteria				Flavobacteria		Sphingobacteria		Bacilli					Alphaproteobacteria			Betaproteobacteria		Gammaproteobacteria							TM7-3								
Actinomycetales				Flavobacteriales		Sphingobacteriales		Bacillales		Exiguobacteriales		Lactobacillales		Rhizobiales		Rhodospirillales		Sphingomonadales		Burkholderiales		Alteromonadales		Enterobacteriales		Pseudomonadales		Vibrionales		Xanthomonadales		EW055	
Microbacteriaceae *	Micrococcaceae *	Nocardioidaceae *	unclassified *	Flavobacteriaceae *	Sphingobacteriaceae *	Bacillaceae *	unclassified *	Exiguobacteraceae *	Leuconostocaceae *	Rhizobiaceae *	Acetobacteraceae *	Sphingomonadaceae *	Comamonadaceae *	Oxalobacteraceae *	Shewanellaceae	Enterobacteriaceae *	Moraxellaceae *	Pseudomonadaceae *	Vibrionaceae	Xanthomonadaceae *	unclassified *	unclassified *	unclassified *	unclassified Bacteria *									
0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.5	0.2	1.2	0.0	87.6	0.3	0.3	0.0	0.0	5.0	2.5	0.0	0.6	1.6								
0.2	0.5	0.1	0.4	2.1	1.1	1.0	1.1	4.2	0.0	0.3	0.0	0.1	0.0	2.6	2.2	57.7	3.1	4.1	0.1	0.2	6.5	0.9	0.0	5.4	6.6								
0.5	0.2	0.0	0.2	1.2	1.1	1.3	1.3	3.1	7.4	0.3	0.0	0.4	0.8	9.9	0.1	38.1	2.9	4.6	0.7	12.5	3.4	0.6	0.0	5.0	4.5								
3.1	1.5	0.4	2.2	0.7	0.3	1.1	1.2	0.3	4.1	1.0	1.4	0.7	1.3	1.3	3.6	46.2	4.8	0.8	0.2	1.2	2.4	0.1	0.9	1.8	17.1								
6.0	5.0	1.4	6.7	2.9	0.7	2.1	1.6	0.5	0.5	0.7	0.4	1.4	4.2	5.8	0.5	21.3	2.7	0.9	0.7	1.8	2.0	0.3	1.5	1.9	26.5								
0.1	0.0	0.0	0.0	9.4	4.3	0.0	0.0	0.0	0.1	0.4	0.0	0.2	6.0	7.6	0.0	20.4	12.0	0.4	0.0	1.2	9.7	7.0	0.1	11.3	10.3								
0.9	0.6	0.3	0.4	0.1	0.1	17.8	1.4	0.0	0.3	0.1	0.5	5.5	0.4	1.0	0.4	52.8	0.4	0.1	1.7	0.6	1.7	0.3	0.1	2.5	10.2								
6.9	1.9	4.0	3.8	0.7	0.9	1.4	0.4	0.2	0.2	0.8	0.7	8.1	2.3	4.4	0.4	4.8	3.4	1.0	5.8	0.6	1.1	0.1	2.1	3.9	40.2								
16.4	1.6	1.1	3.3	4.4	1.0	1.4	2.7	0.1	0.5	0.8	1.1	6.0	5.4	4.4	0.4	5.3	2.0	0.2	1.1	0.8	0.7	0.3	5.1	2.4	31.7								
1.0	2.1	0.6	2.0	0.3	0.4	24.0	10.0	0.0	0.0	0.3	9.0	1.0	1.3	3.6	0.2	1.9	0.8	0.3	1.3	0.5	2.3	0.4	0.1	4.7	31.8								
1.1	8.9	0.3	1.3	6.8	20.8	0.3	0.2	1.3	0.0	3.3	0.0	0.3	2.8	2.7	0.1	1.4	0.6	14.2	0.8	1.3	1.8	1.1	0.2	5.4	23.0								
All clades comprising < 3% of sequences in any produce type																																	

Leff & Fierer, 2013

Belowground plant system

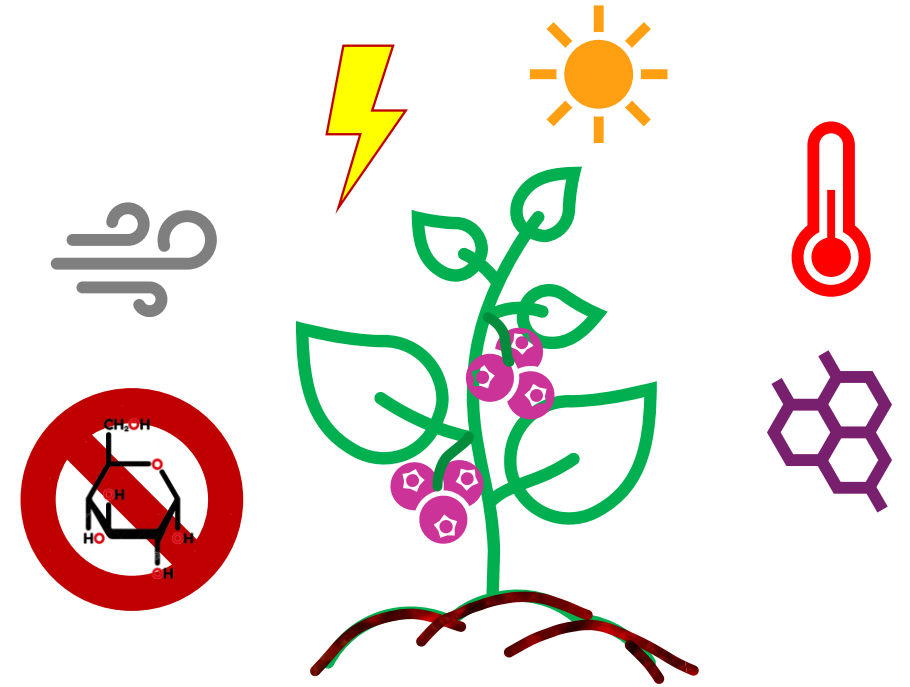
- **Endosphere:** Inside roots
- **Rhizoplane:** Root surface
- **Rhizosphere:** Soil under the influence of roots
- **Bulk soil:** Soil not under the influence of roots

❖ Soil seeds phyllosphere microbial communities



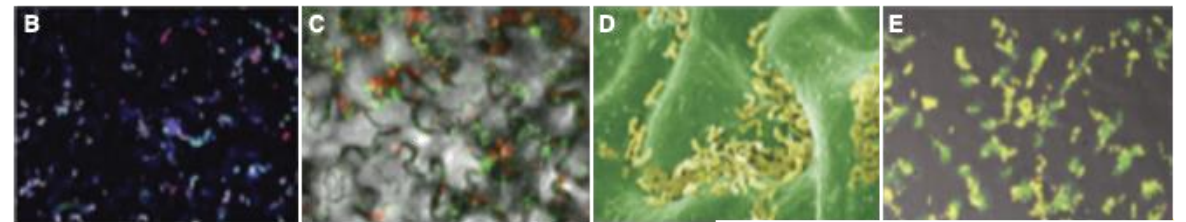
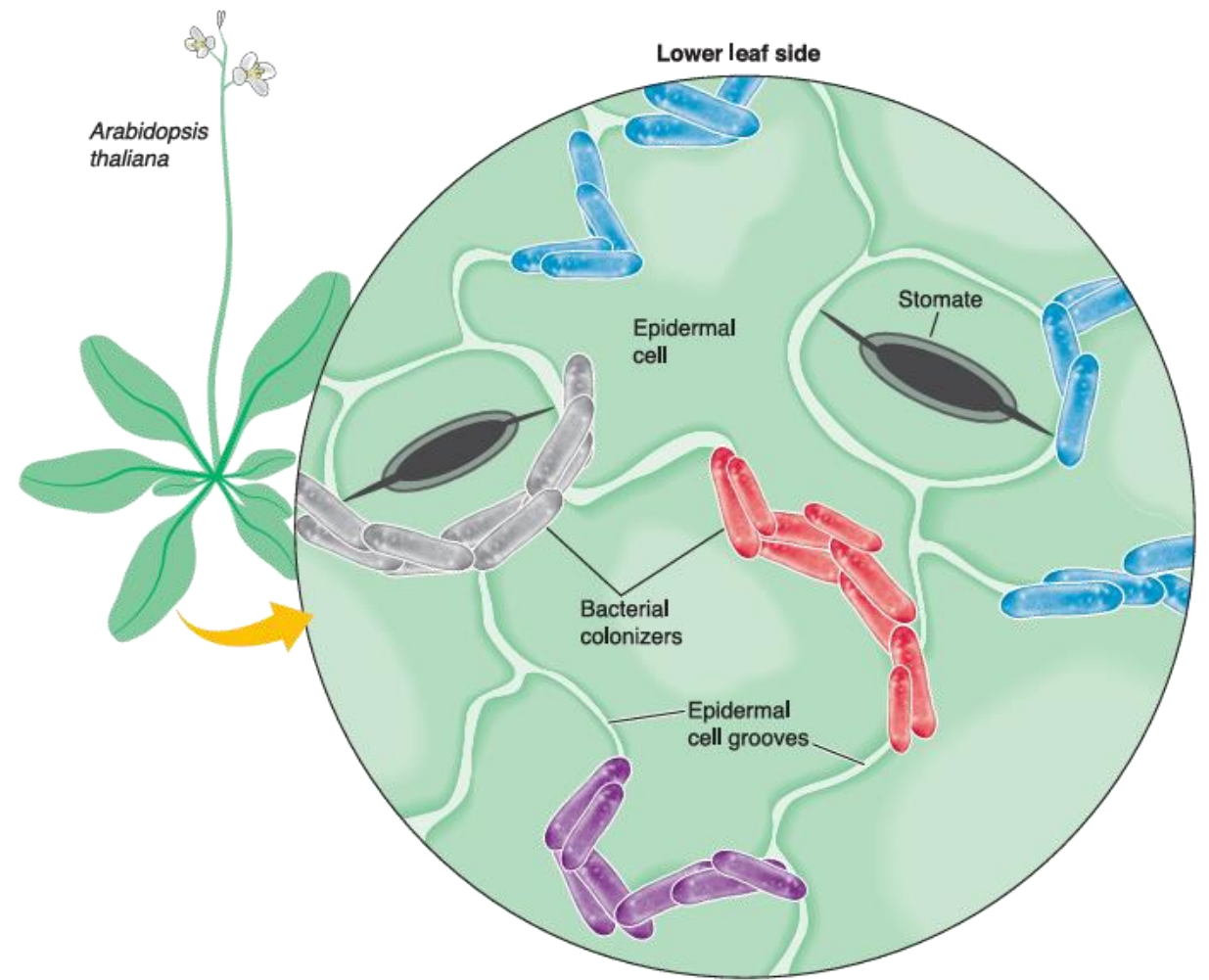
Above-ground plant system

- The phyllosphere is a harsh environment
 - UV irradiation
 - Extreme temperatures
 - Waxy cuticle
 - Lack of water
 - Lack of nutrients
 - Rain/humidity/dryness
- Leaf microbiota dominated by bacteria
- Up to $\sim 10^7$ bacteria per cm^2 of leaf surface
- Surface (epiphytic) diversity $\gg$ endophytic diversity
- Epiphytes have high stress tolerance traits
- Endophytes are highly specialized



Bacterial colonization of leaf

- The leaf is **heterogeneous** at the microscopic scale.
- Bacteria form **aggregates** that may be **microcolonies**.
- **Spatial distribution of bacteria/spp.** could be related to spatial distribution of **metabolites on leaf**.
- Most colonized leaf sites are:
epidermal cell grooves (depressions between epidermal cells), **veins**, **base of leaf hairs**, **stomatal areas**.



(A) Scheme of a plant leaf that is colonized by various bacterial species;





Enteric (foodborne) bacterial pathogens on leaves

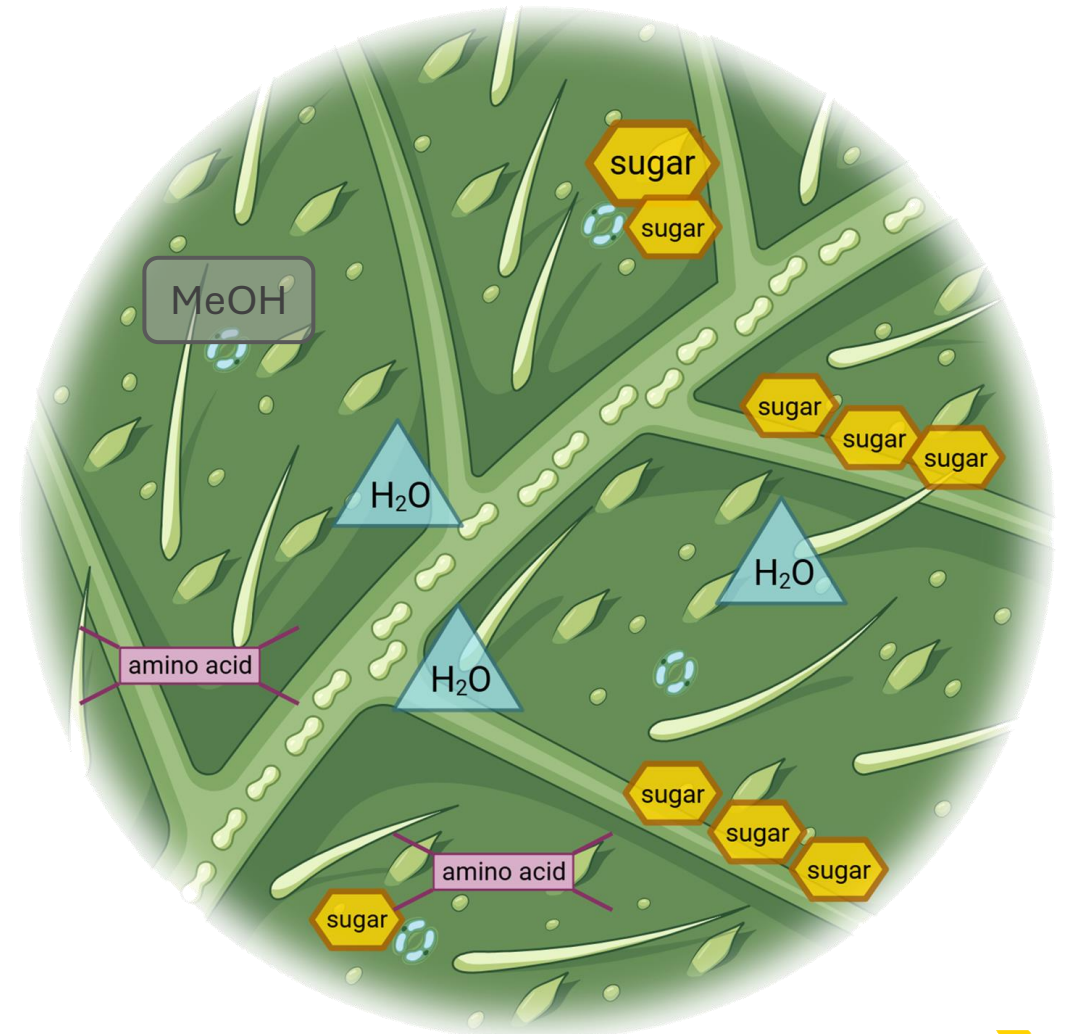


Enteric bacteria growth requirements

Physicochemical properties determine how, where and the nature of enteric bacterial association with plants.

Factors affecting colonization:

- Topography (creases, grooves, etc)
- Waxy cuticle
- Veins
- Hydrophobicity
- Stomata (openings)
- Trichomes (hairs)
- Moisture retention
- Nutrients
- VOCs
- Resident (native) microbes



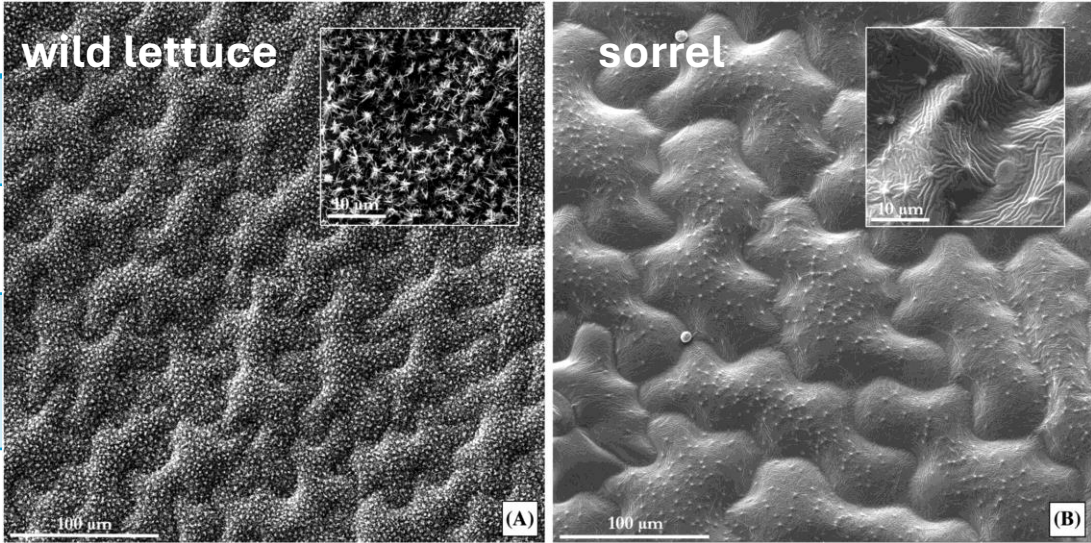
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Micallef, S. (2026)



Study: A focus on *Salmonella* association with 30 baby-leaf salads



Leaf feature	Significantly associated (✓) with bacterial attachment
Contact angle (hydrophobicity)	✓ ↓
Waxiness	✓ ↓
Wax components (alcohols, fatty acids)	✓ ↓
Surface roughness	✗ ?
Water content	✗ ↑



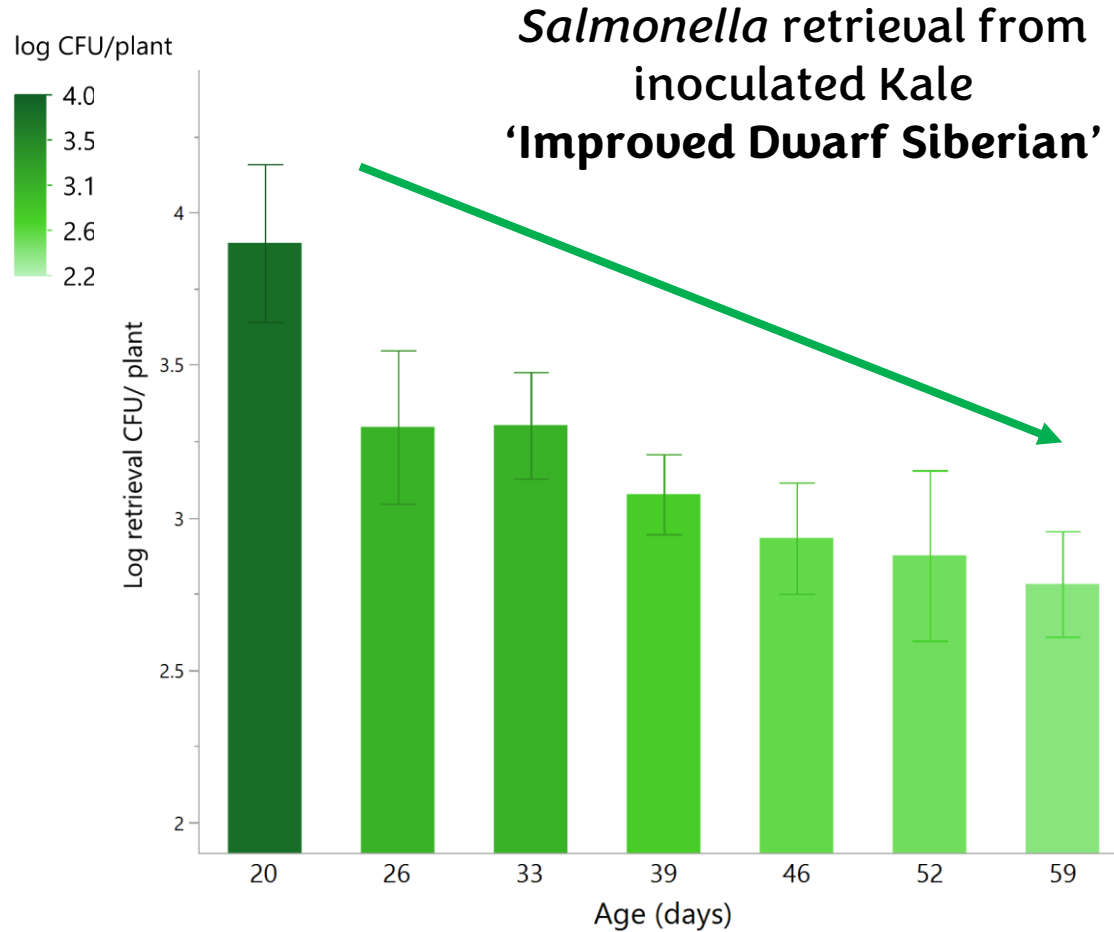
SEM image (800×, 8000×) of adaxial leaf surface of wild lettuce (A) and sorrel (B) with the highest and lowest concentration of waxes, and the lowest and highest susceptibility for *Salmonella*, respectively.



Pathogen susceptibility may diminish with leaf age



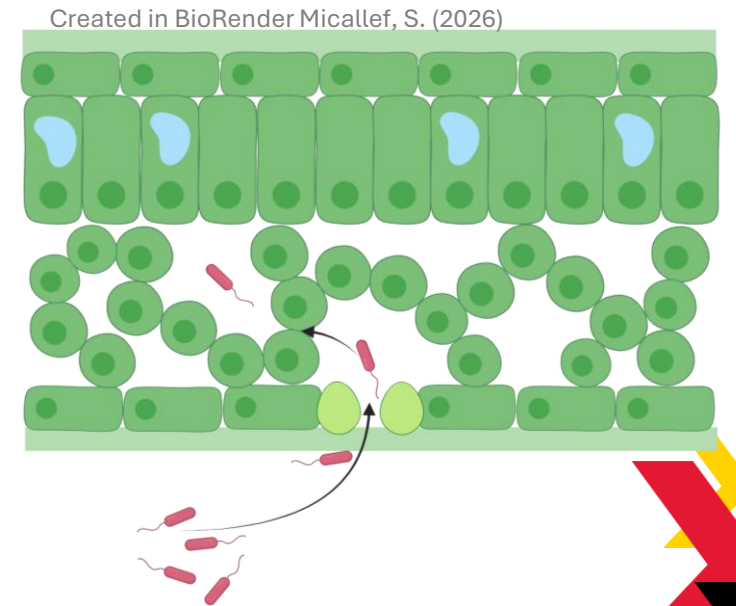
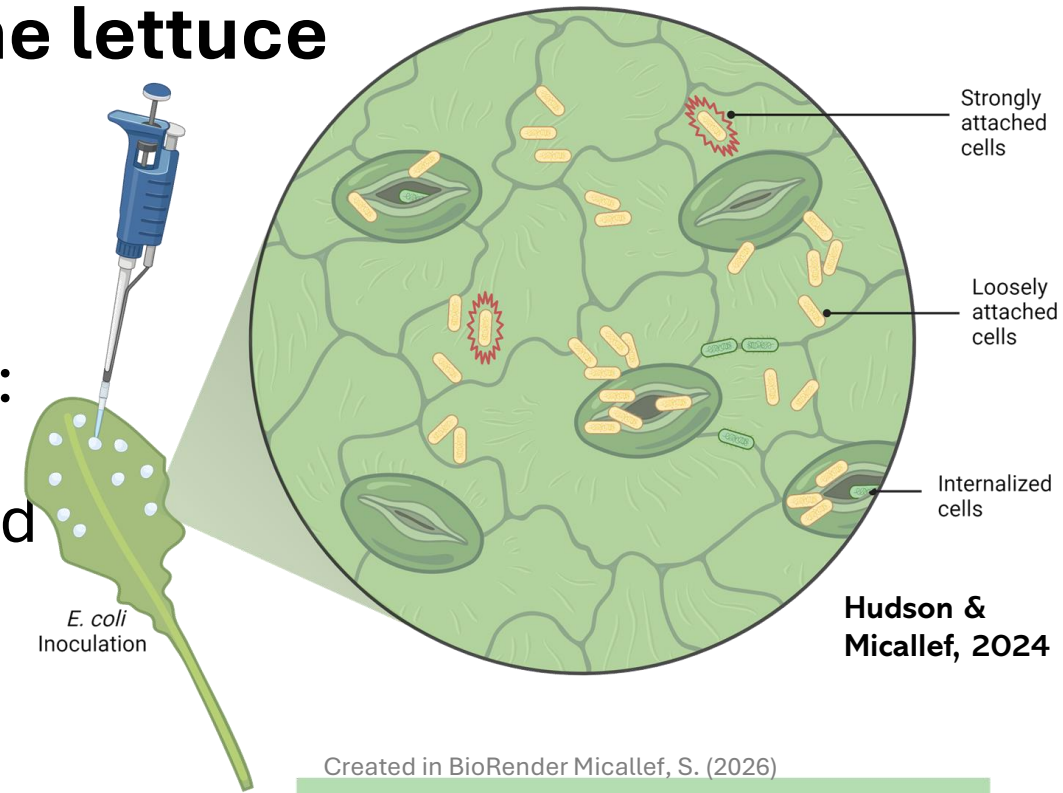
(e.g., baby leaf vs. mature leaves)



E. coli O157:H7 association with romaine lettuce

We hypothesized that *E. coli* O157:H7 would associate with romaine leaves in different ways:

- **Loosely attached cells** – reversibly attached epiphytic cells
- **Strongly attached cells** – cells irreversibly attached to the leaf surface
- **Internalized cells** – cells that make their way through the epidermis, entering in between plant cells (e.g., sub-stomatal or intracellular spaces)



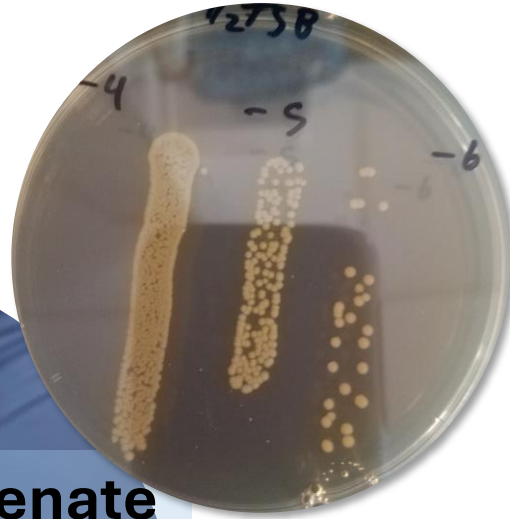
Wash = epiphytic fraction



Photo: C. Hudson

**Homogenate
= whole
fraction**

Direct plate counting approach



This method ensures we are detecting viable cells but limit of detection is:

Wash = $2.4 \log \text{ CFU mL}^{-1}$

Homogenate = $1.7 \log \text{ CFU mL}^{-1}$

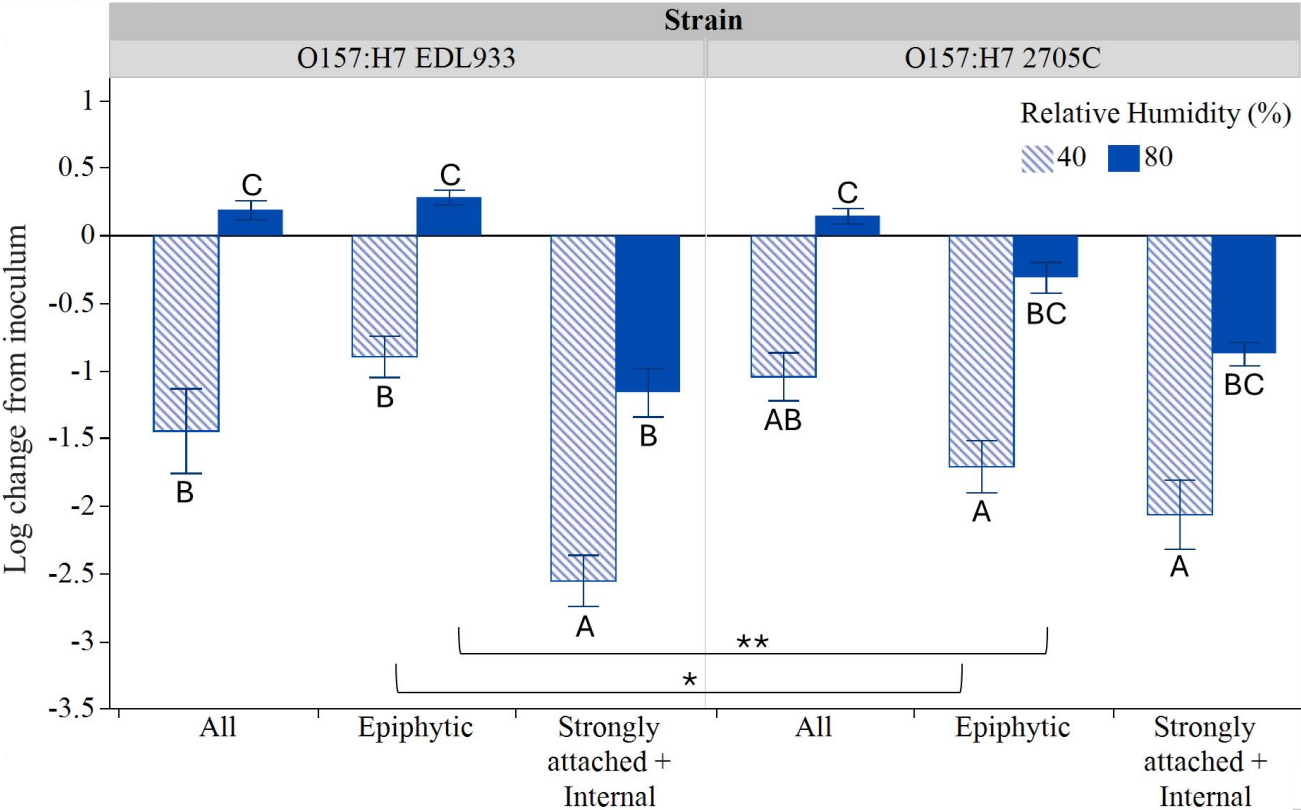


Photo: S. Micallef

Romaine lettuce 'Carlsbad'

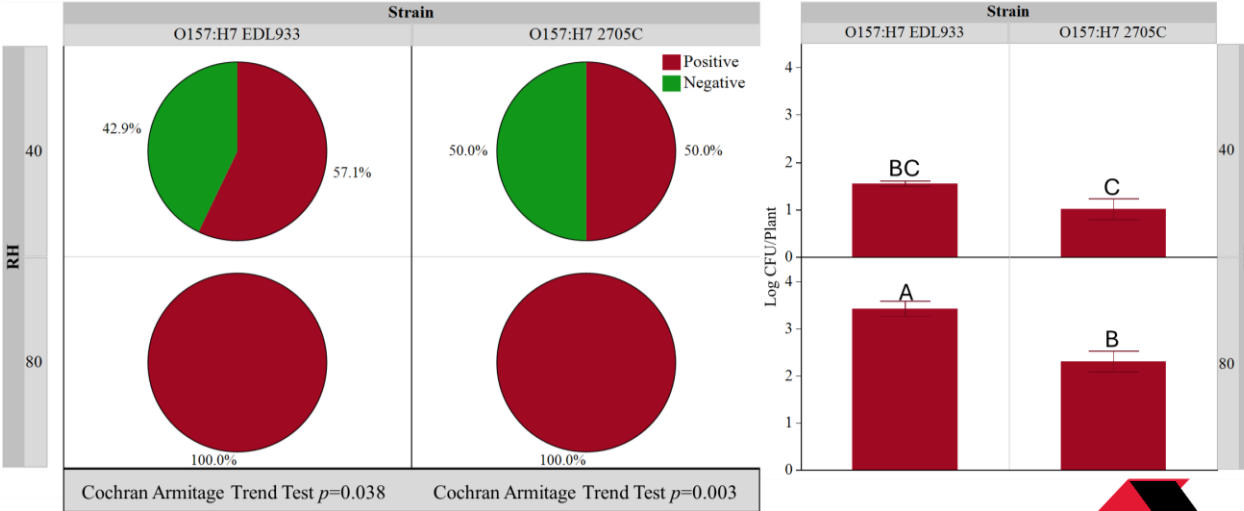
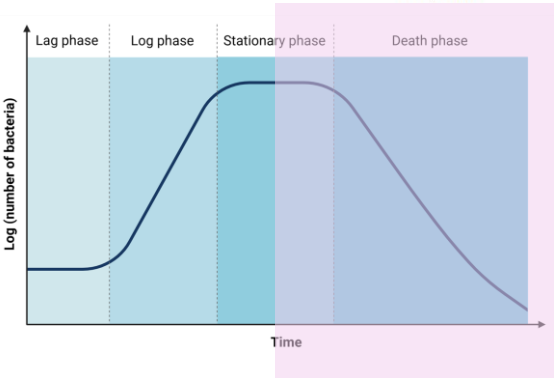


Pre-harvest: Attachment

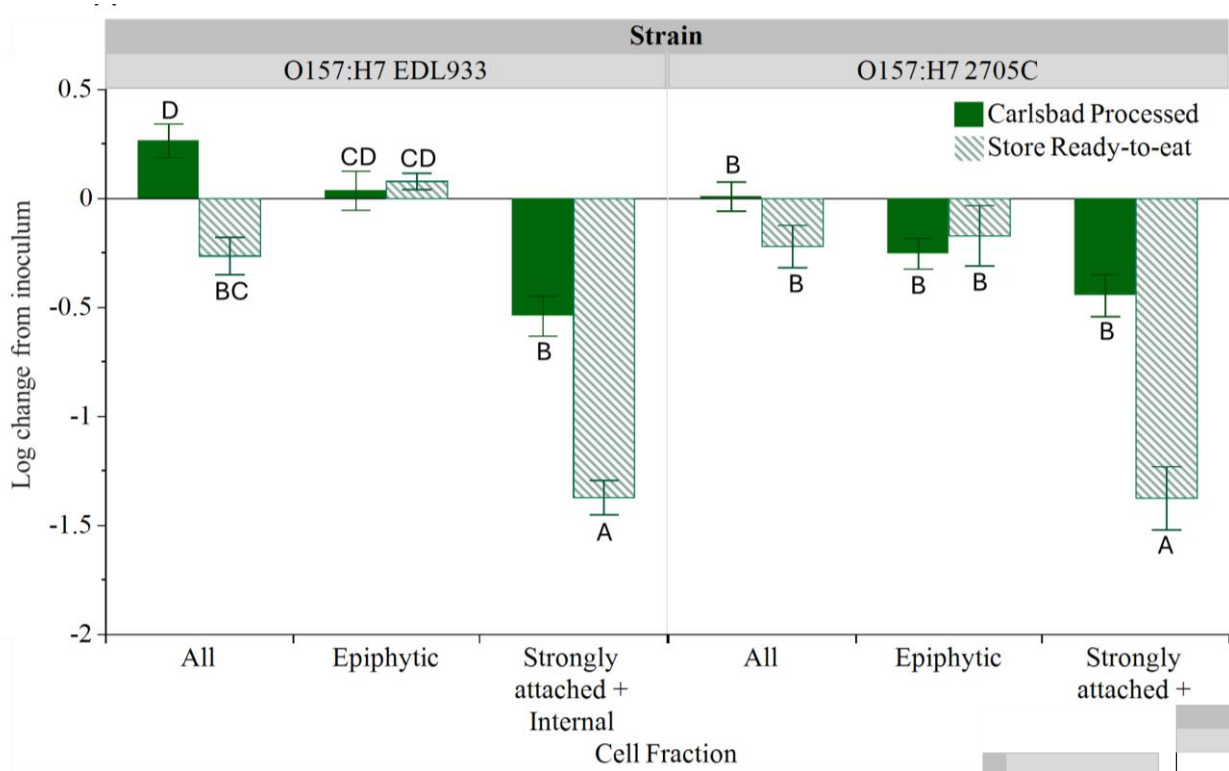


- Ec O157 found in all fractions (All = Epiphytic > SA + Internal)
- At 40% RH, epiphytic and internalized Ec O157 declined
- At 80% RH, epiphytic Ec O157 grew
- Internalization rate increased with higher RH (100% at 80% RH)

Internalization

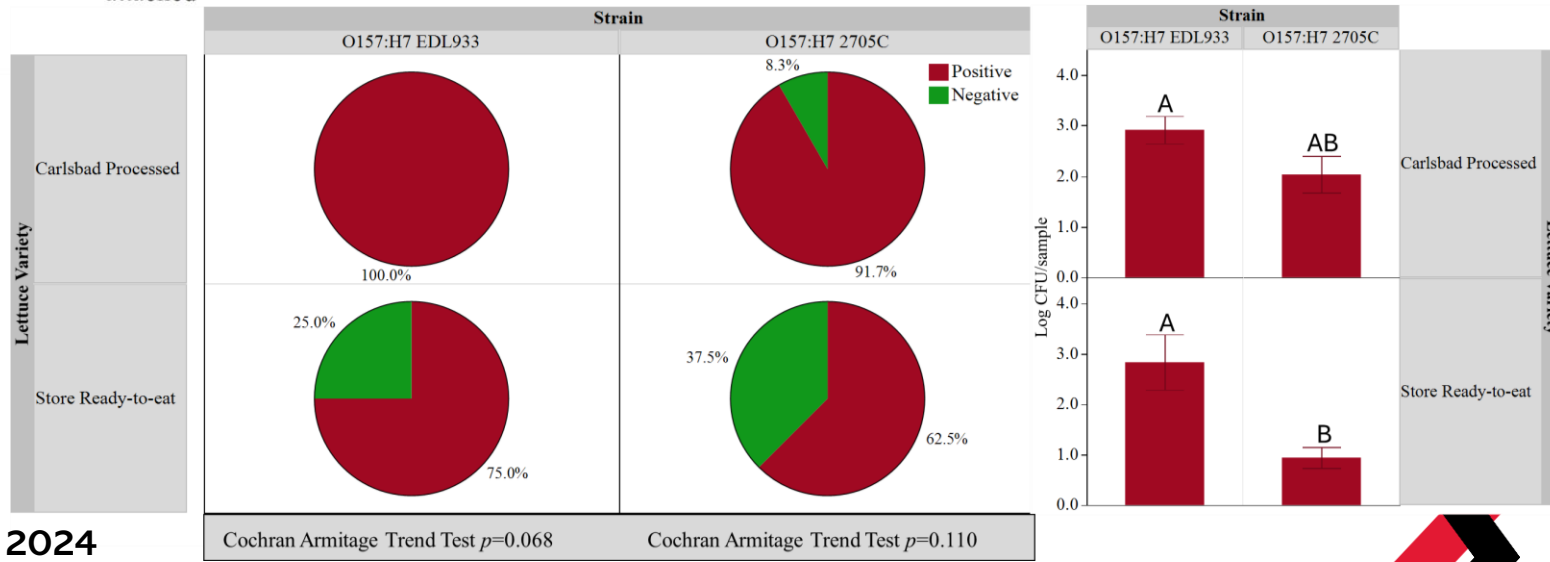


Post-harvest: Attachment

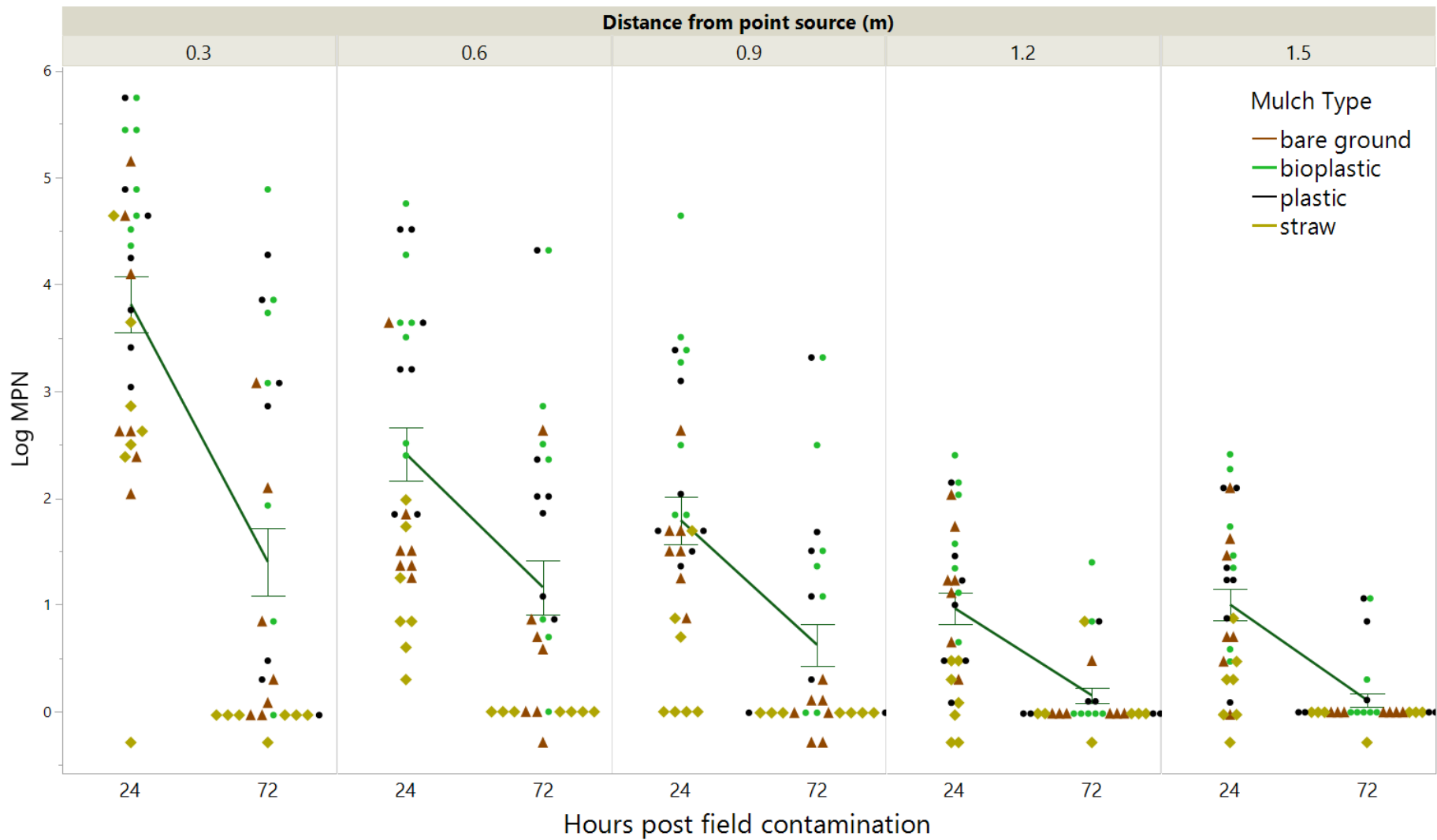


- Ec O157 log reductions were less remarkable post-harvest
- Internalization was very high post-harvest
- Strain EDL933 (beef) internalized at a higher rate than the lettuce outbreak strain 2705C and attained higher population levels.

Internalization



Bacterial die-off



Lettuce cv. 'Magenta'



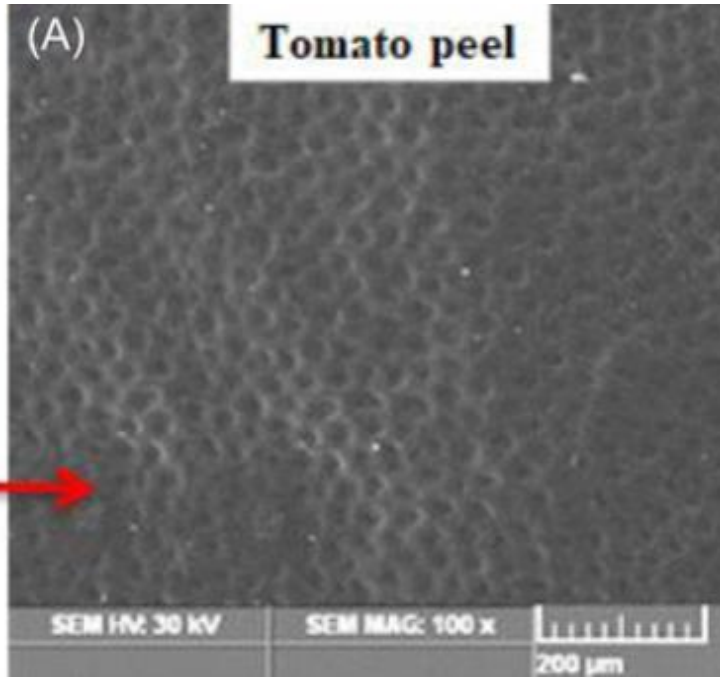
Lettuce trial with *E. coli* TSV353 at Wye REC, MD



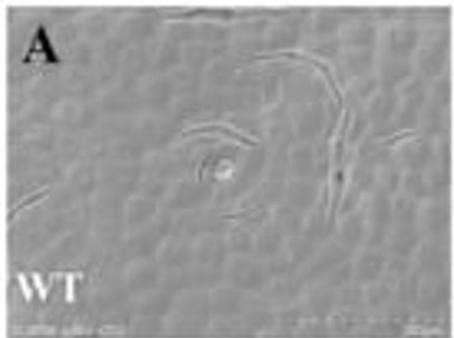
Enteric (foodborne) bacterial pathogens on fruit



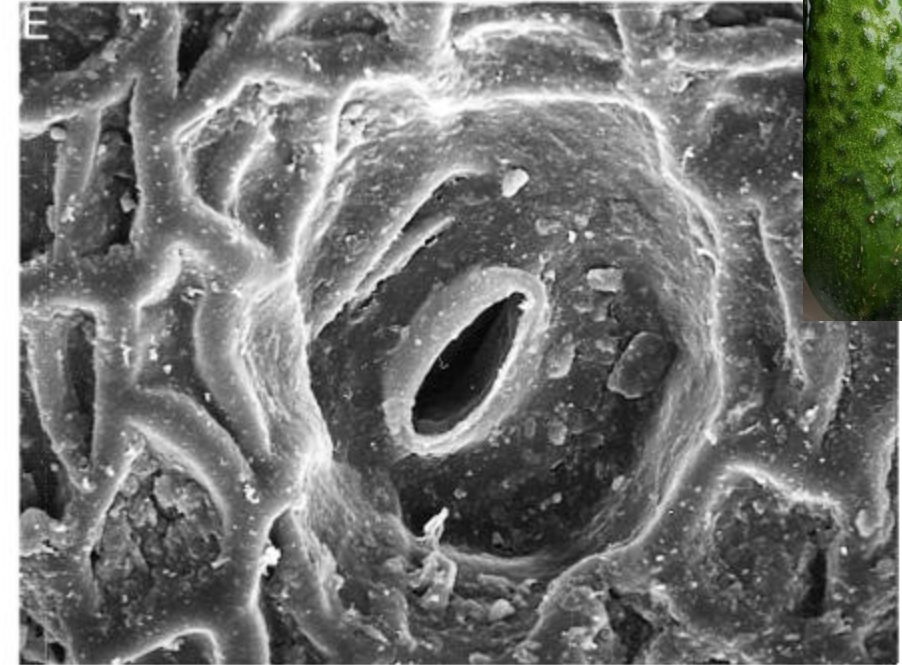
Fruit exocarp (peel)



SEM tomato peel
Korumilli *et al.*, 2022



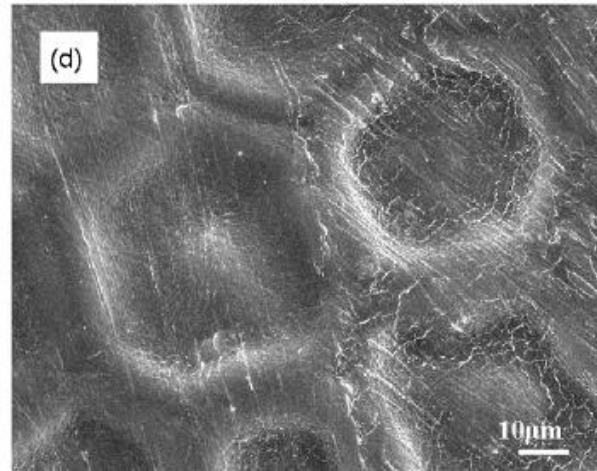
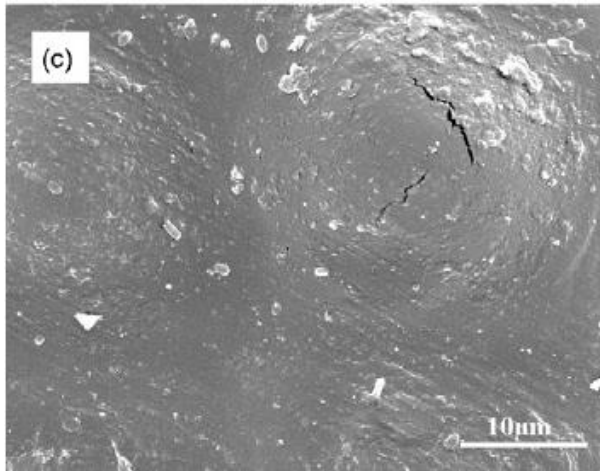
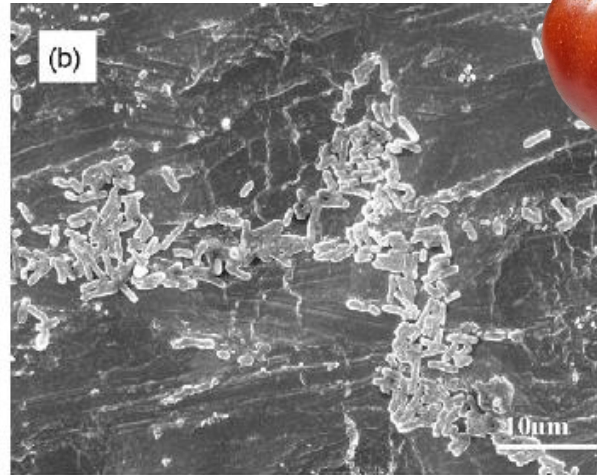
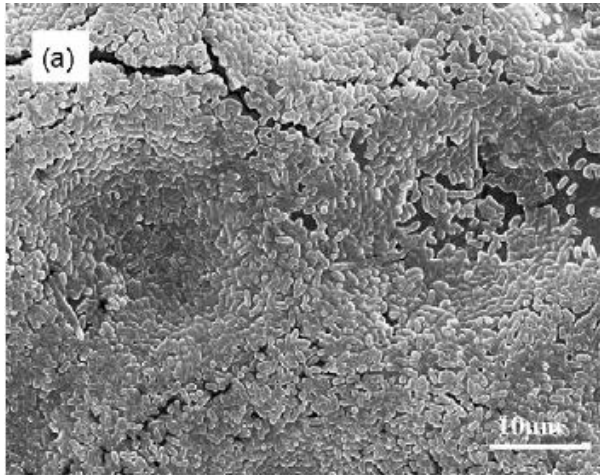
SEM tomato peel cv.
Ailsa Craig showing
microcracks.
Liang *et al.*, 2024



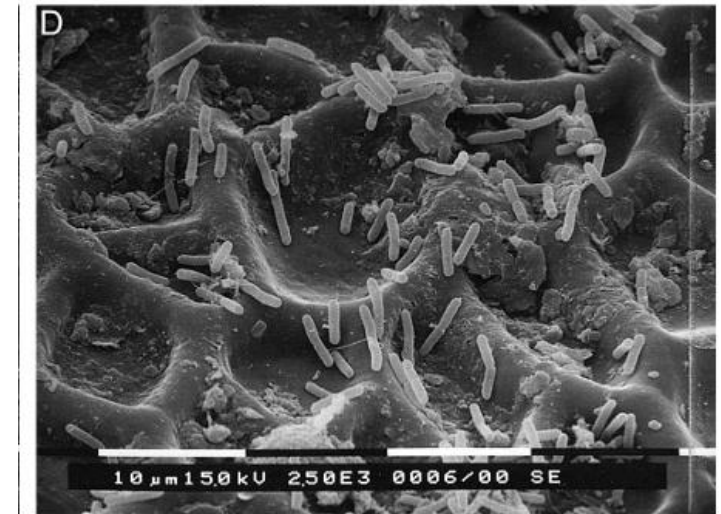
Surface of pickling cucumbers, Calypso
variety, stomata open
Reina *et al.*, 2002. JFP, 65: 1881–87



Enteric bacteria on fruit surface

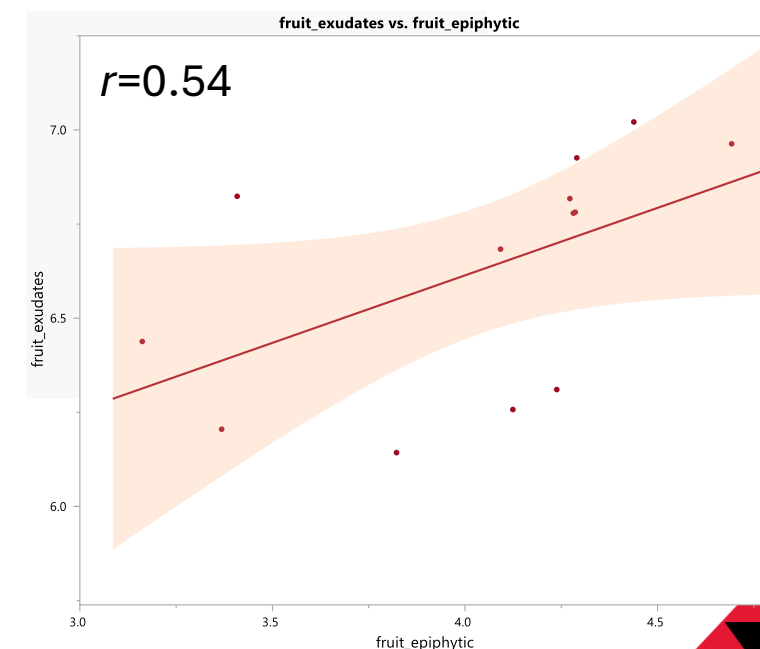
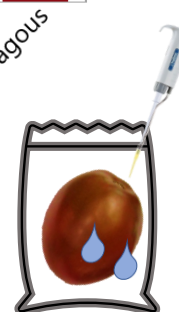
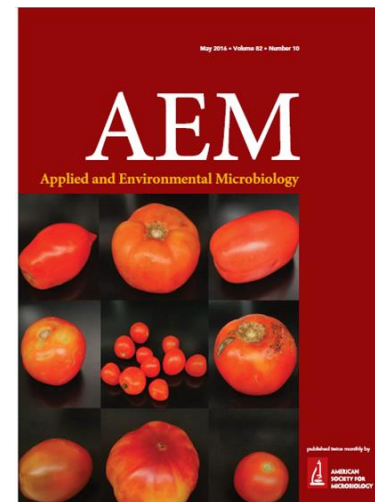
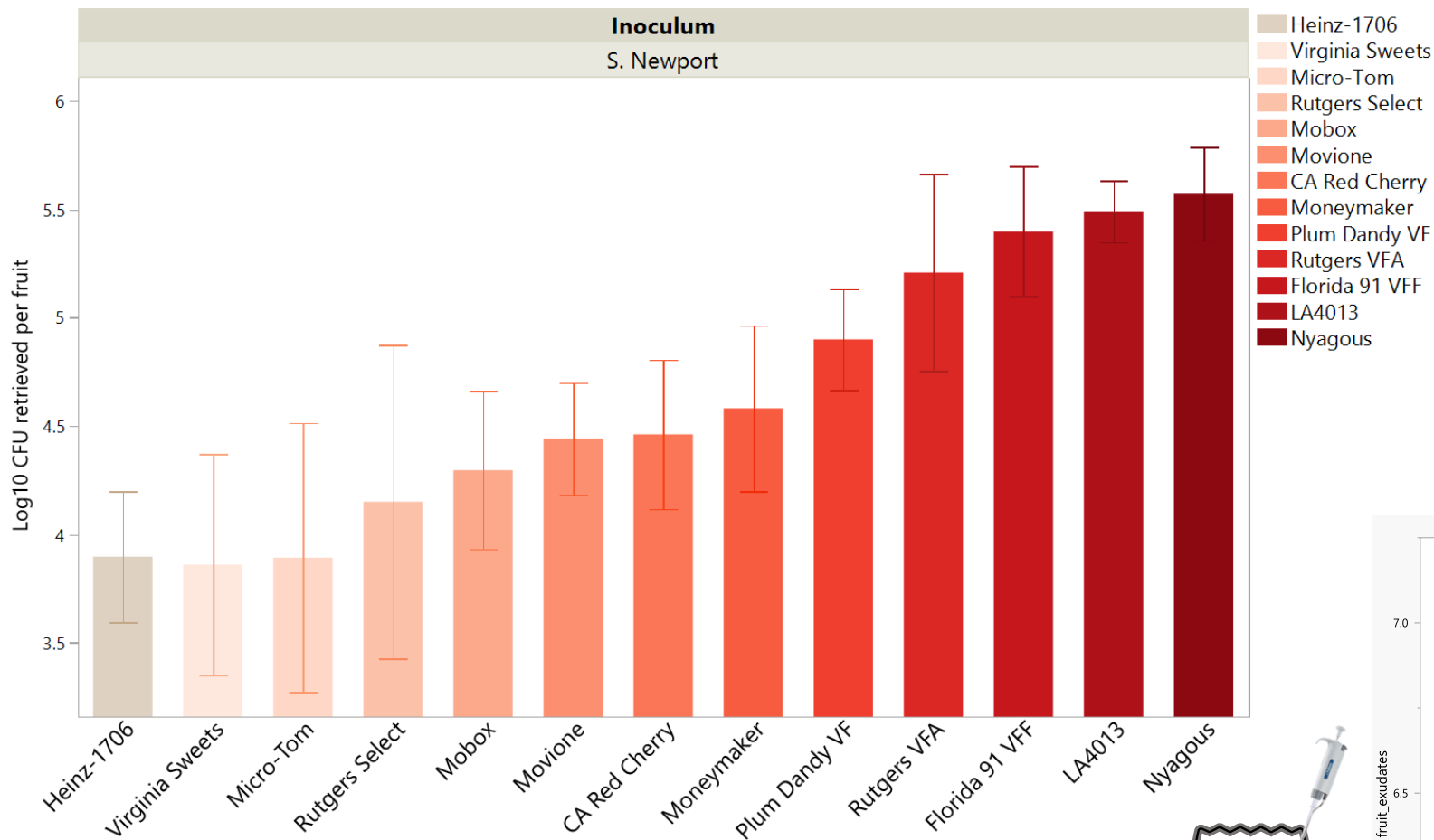


SEM tomato surfaces spiked with *Salmonella* concentrations of (a) 5×10^8 CFU ml⁻¹, (b) 5×10^6 CFU ml⁻¹, (c) 5×10^3 CFU ml⁻¹ (d) fresh tomato surface. Li *et al.*, 2009

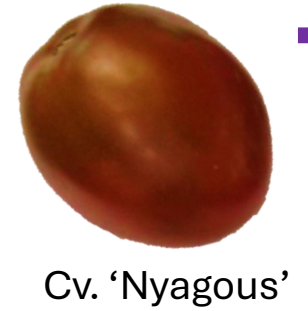
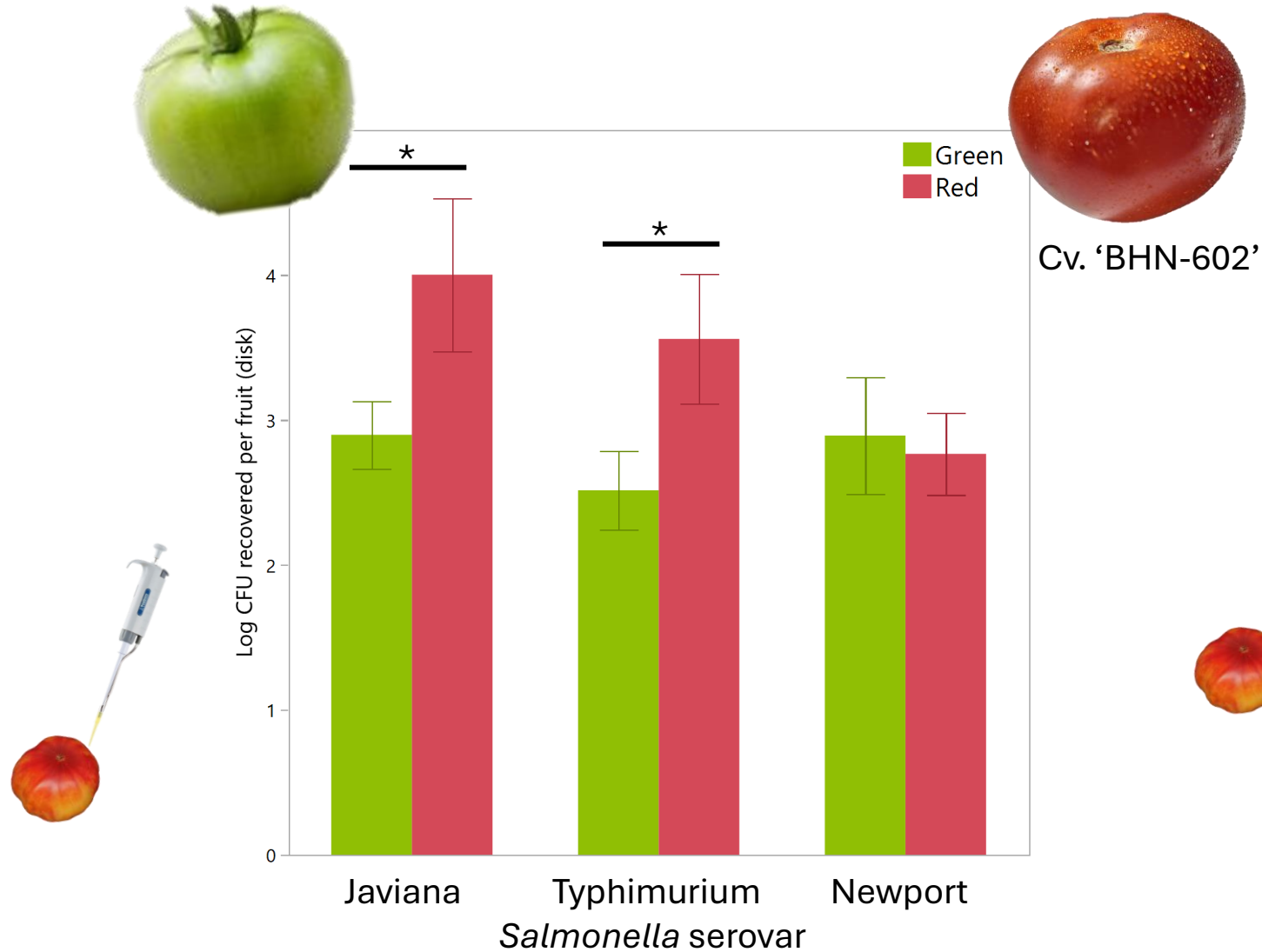


Adhesion of bacteria to the surfaces of pickling cucumbers (Calypso) with wax. (C) *Listeria monocytogenes*, (D) *Salmonella* Typhimurium. Reina *et al.*, 2002

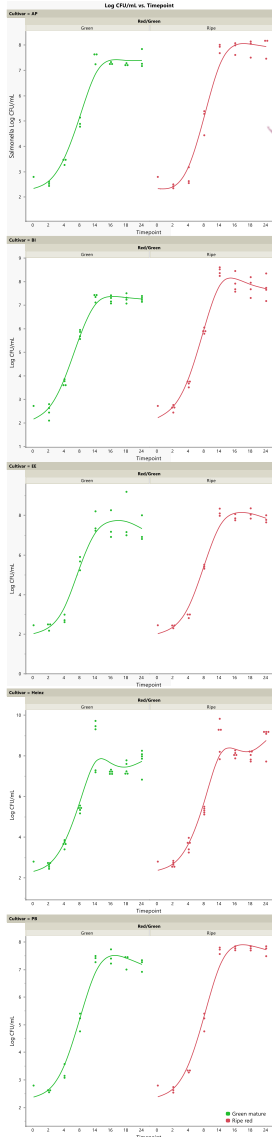
Differential association of *Salmonella* with tomato fruit



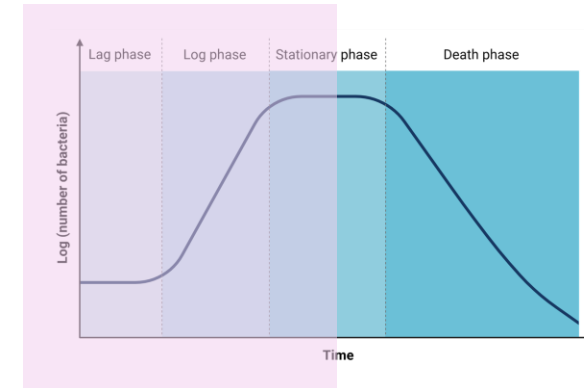
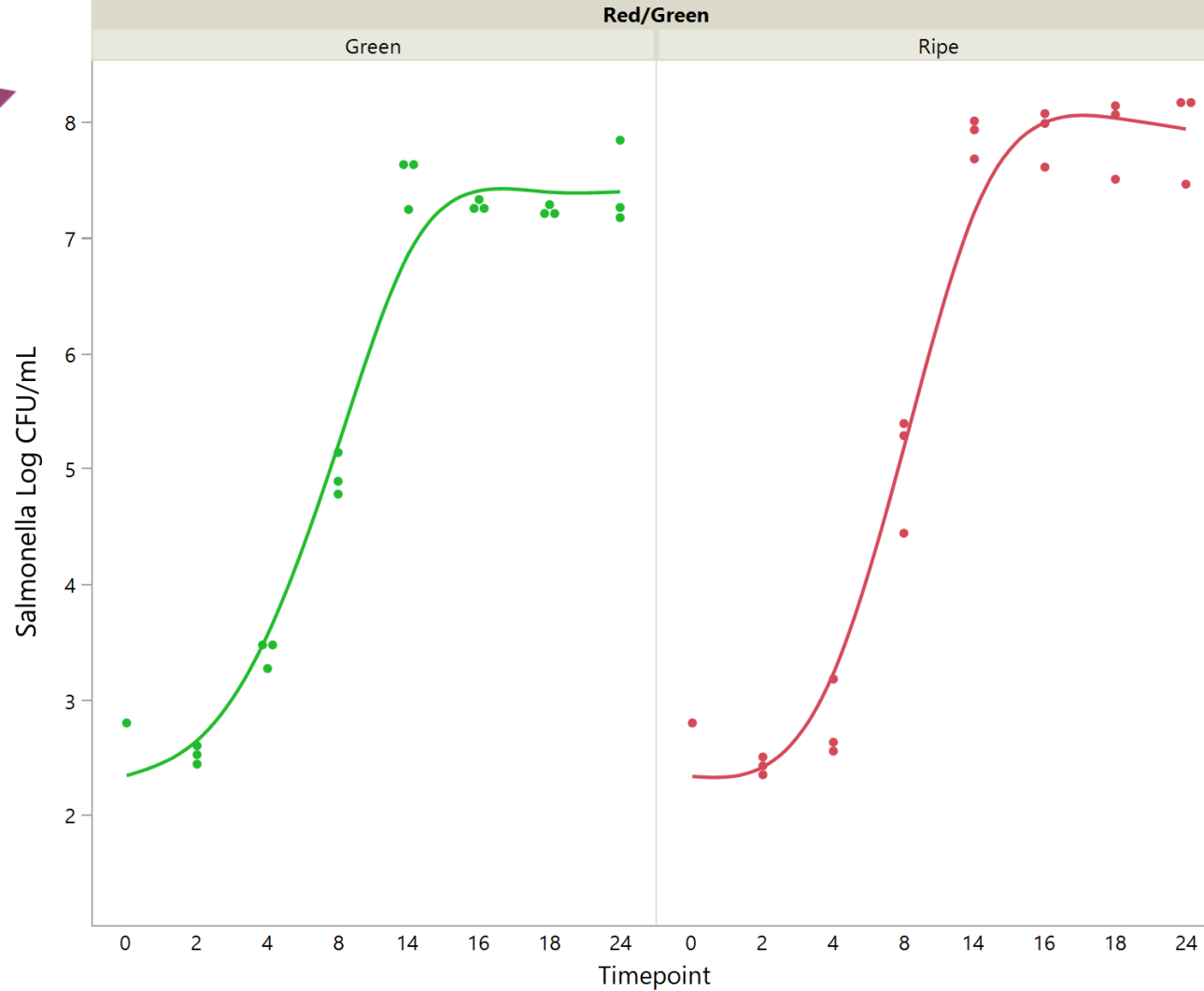
Salmonella persists better on red fruit



Salmonella growth in fruit washes



Cultivar = AP



- Green mature
- Ripe red



UNIVERSITY OF MARYLAND

FEARLESSLY FORWARD

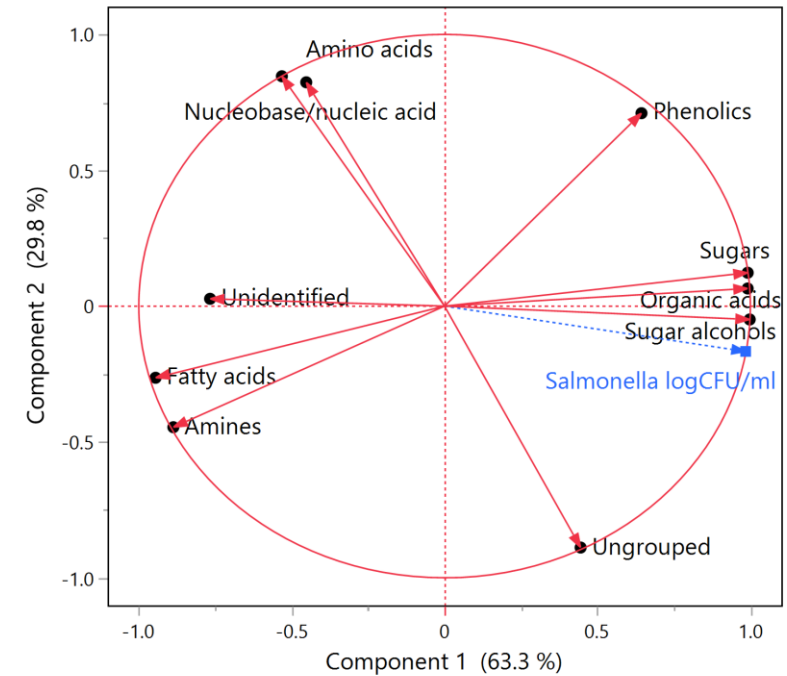
Hopper & Micallef, unpublished



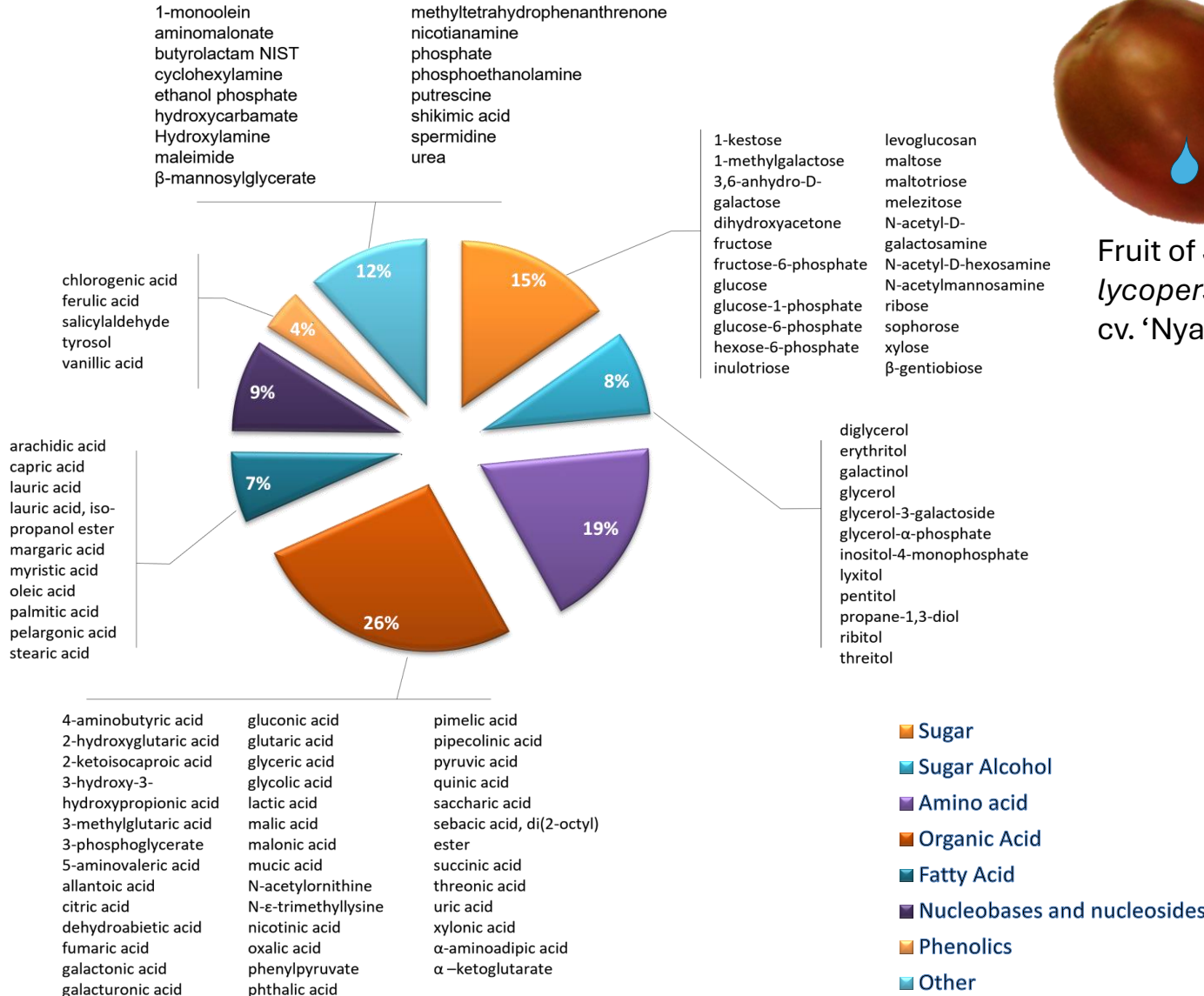
What metabolites are present on the fruit surface?



Fruit of *Solanum lycopersicum* cv. 'Nyagous'



Analysis showed that sugars, organic acids, sugar alcohols support *Salmonella*; fatty acids, phenolics correlate negatively.



Final thoughts ...



Photo: S. Micallef



- Fresh crops harbour rich microbial communities.
- Bacteria thrive where they find **surfaces** for attachment that provide **nutrients** and **moisture**. *This includes edible plant parts (pre- and post-harvest) and contact surfaces.*
- Despite harsh conditions in the phyllosphere, heterogeneity at the microscopic scale and physicochemical properties support diverse bacterial taxa, including enteric pathogens.
- Crop–human pathogen studies are complex, with **inherent biological variation**; with sound experimental design *we can apply results from controlled experiments to the field.*
- Bacterial numbers are not absolute, they only give a partial picture of population levels that are present *but indicate dynamics over time.*
- Bacterial decline does not mean complete die-off.
- Pathogen persistence at low levels can still pose food safety risk.



Acknowledgements

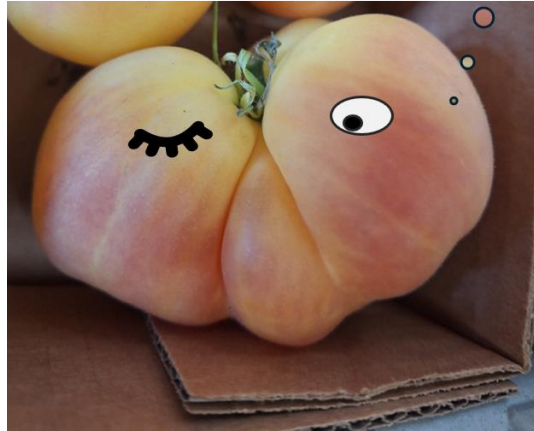


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Dr Angela Ferelli Gruber, Ph.D.
Dr Qiao Ding, Ph.D.
Adam Hopper, B.S.
Diksha Klair, M.Sc.
Wesley Deaver, M.S.



Many undergraduates
Collaborators

Thank you!



MICALLEF
Crop^{Micro} LAB



PSA Updates

- Working on PSA plans for next year
 - Funding ensured, working plans in the making
 - PSA high-priority requests are launching V2 and LTA portal reopening
 - Please add any other priorities you would like to see in chat or unmute to share
- Save the date for our **2026 Annual PSA Trainer Update** webinars on:
 - **November 17 at 2:00 pm ET, in English**
 - **December 3 at 2:00 pm ET, in Spanish**
 - Registration links will be shared through our PSA Trainers contact lists

Our Next Educators' Call

Produce Safety in Practice: Foundational Best Practices and Region-Specific Considerations



- Sonia Salas, Associate Vice President, Food Safety and Regulatory Affairs, Western Growers Association
 - October 8, 2026, at 2 pm ET, in Spanish
 - October 20, 2026, at 2 pm ET, in English
- Registration link will be shared through our contact list and social media channels

Join our
**Produce Safety
Educators' group!**

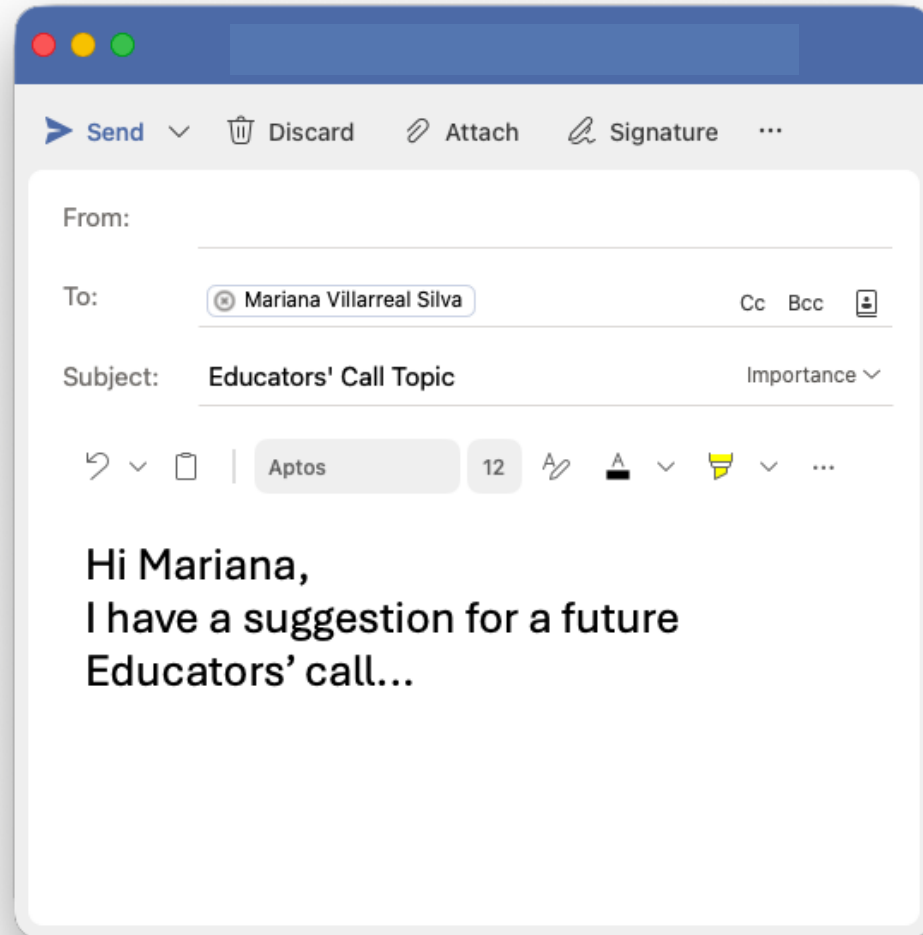
English



Spanish



Input welcome on future Educators' Call topics



Is there a webinar topic that you would like to see? Please email:

- Mariana Villarreal Silva
- mv378@cornell.edu

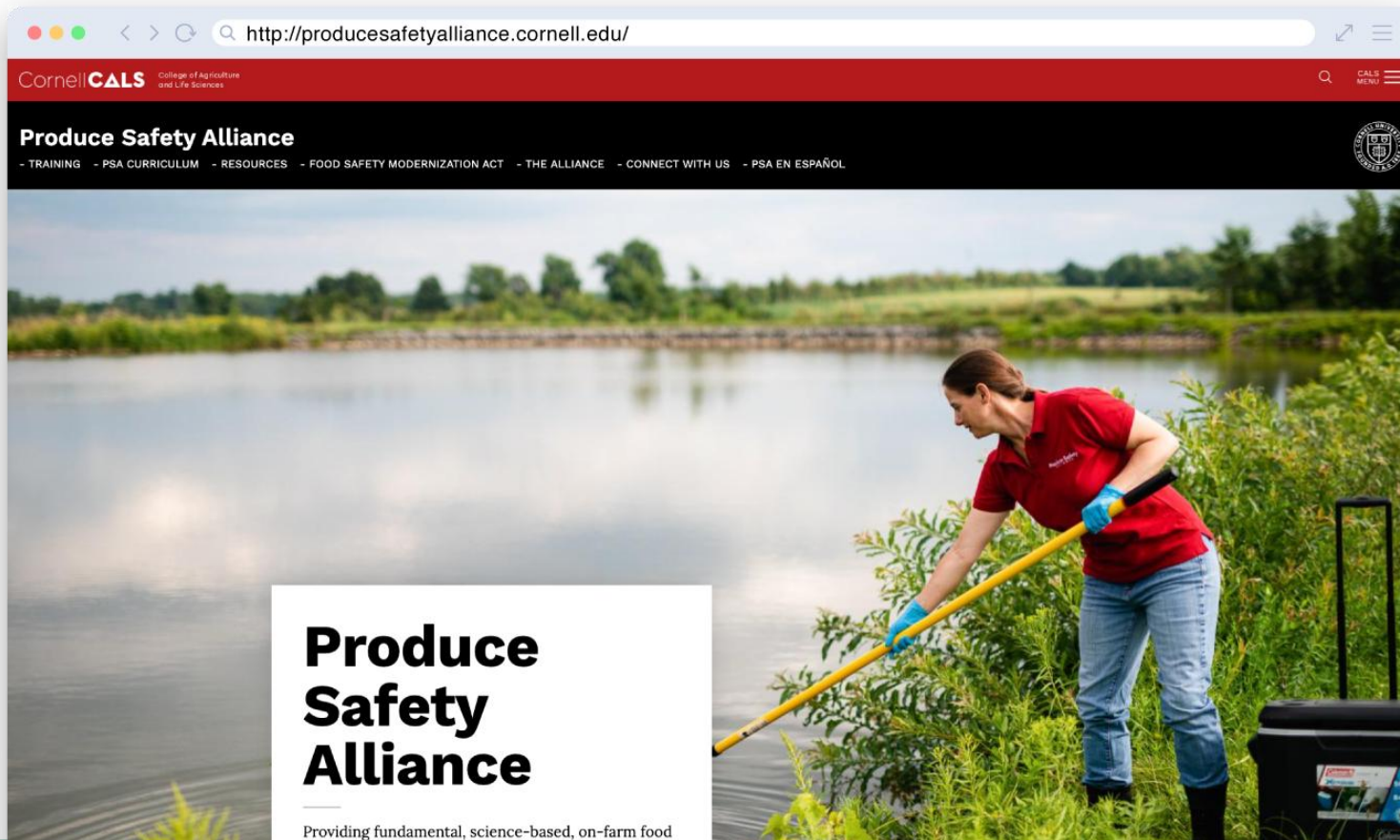
Thank you!

- To Dr. Shirley Micallef, for sharing her valuable time and expertise to enrich today's session
- To the PSA Team, for their support in planning, preparing, assisting, and hosting these PSA Educators Calls
- To our attendees, for your engagement and ongoing commitment to produce safety education



PSA Website

- English: <http://producesafetyalliance.cornell.edu/>
- En español: <http://es.producesafetyalliance.cornell.edu>



Follow along
@producesafetyalliance

