Title: **Influence of Co-planting on Survival and Persistence of Escherichia coli in Hydroponic Systems**

Authors: **Victor Akao**, Florida A&M University
Cody Scales, Florida A&M University
Edwin Duke, Florida A&M University
Vijay Chhetri, Florida A&M University

Introduction: Co-planting hydroponics is a soilless farming method that involves growing two or more crops together in a nutrient solution. There is limited data on the influence of this strategy on microbial pathogen dynamics.

Purpose: This study examined the effects of co-planting lettuce, mint, and basil on the persistence and distribution of generic *E. coli* in hydroponic systems.

Methods: Lettuce, mint, and basil were co-planted in Nutrient Film (NFT) Technique and Deep-Water Culture (DWC) systems (n=6, 3 replicates). Nutrient solutions were inoculated with *E. coli* ATCC 25922 (5 log CFU/mL). The samples, including nutrient solutions and plant parts (roots, stems, and leaves), were collected over 24 days, and the *E. coli* population and physicochemical changes in the systems were determined. Tukey's honestly significant difference test was used to determine significant differences ($P<0.05$) in *E. coli* levels based on treatments.

Results: The *E. coli* population decreased significantly ($P < 0.05$) within 24 h in both NFT and DWC systems. *E. coli* survival rate was variable with the co-planting treatments. The greatest reduction was observed in the mint and basil systems, dropping from around 5 log CFU/mL to below the detection limit (1 log CFU/g) on day 24. Furthermore, *E. coli* die-off on the leaves was highest in basil compared to mint and lettuce.

Significance: This study highlights the potential benefits of companion planting strategies in reducing food safety risks in hydroponic systems.

Title: **Nanomaterial Interactions with Agri-Food Microbiome: Impacts on Microbial Communities and Pathogen Dynamics**

Authors: **Sherry Bansal**, University of Florida
Yu Wang, University of Florida
Boce Zhang, University of Florida

Introduction: The agri-food microbiome plays a vital role in nutrient cycling, nitrogen fixation, and overall soil health, but its integrity is increasingly challenged by emerging environmental stressors such as nanomaterials (NMs). The widespread use of NMs in agriculture, in addition to their mobility and persistence in soil, raises concerns about potential unintended impacts on microbial communities and pathogen dynamics.

Purpose: This study investigates how commonly used metallic NMs influence microbial community composition and persistence of pathogens in agri-food environments.

Methods: Seven metallic NMs commonly used in agrochemicals, i.e., Ag, CuO, CeO_2, Fe_2O_3, MgO, TiO_2, and ZnO were applied to soil collected from a site classified under the Apopka soil series. Eight replicate pots per treatment, including an untreated control, were maintained under greenhouse conditions for 12 weeks with periodic irrigation to simulate rainfall. Soil samples were collected cross sectionally at two depths, at time points: zero, 4 weeks, 8 weeks, and 12 weeks. Aerobic plate counts (APC) were used to access bacterial responses to NM exposure and migration over time. Samples will undergo marker gene, multi-omics, and machine-learning analyses to evaluate the broader effects on native microflora and foodborne pathogens.

Results: Silver (Ag) NMs caused a pronounced decline in bacterial abundance at both depths, with significant reductions in the middle ($p < 9.31 \times 10^{-10}$) and bottom ($p < 0.000128$) layers relative to controls. Within the Ag treatment, no significant temporal variation in bacterial counts was observed at either depth (middle: $p = 0.39$; bottom: $p = 0.508$; 95% confidence intervals).

Significance: These findings highlight the potential for Ag NMs to alter microbial populations and influence pathogen persistence, providing insights into their environmental safety, food security implications, and responsible end-of-life management.

Title: **Development and Validation of an RNase H-Dependent PCR Assay for Differentiating Pleoticus robustus and Pleoticus muelleri in U.S. Shrimp Markets**

Authors: **Samuel Kwawukume**, Florida State University
Nethraja Kandula, Florida State University
Leqi Cui, Florida State University
Prashant Singh, Florida State University

Introduction: Seafood mislabeling remains a persistent food-fraud issue in the United States, with up to 30% of shrimp products sold under incorrect species names. Royal red shrimp (*Pleoticus robustus*) are frequently substituted with the less expensive Argentine red shrimp (*Pleoticus muelleri*), undermining consumer trust and harming domestic fisheries. Accurate, rapid species authentication tools are needed to support regulatory and industry enforcement.

Purpose: This study aimed to develop and validate a rapid RNase H-dependent PCR (rhPCR) assay capable of reliably distinguishing *P. robustus* from *P. muelleri* and other commercially available species in the US.

Methods: Species-specific primers targeting mitochondrial COI genes were designed, optimized, and converted into RNase H2-activated primers labeled with FAM and biotin for lateral-flow detection. Assay performance was evaluated using genomic and crude DNA extracted from 65 barcoded shrimp samples representing seven commonly traded species. Specificity and sensitivity were further assessed using 20 blinded retail samples marketed as royal red shrimp. Amplification and detection limits were recorded to assess field applicability. All tests were carried out in duplicates.

Results: The rhPCR assay demonstrated 100% specificity, correctly identifying *P. robustus* and *P. muelleri* and excluding all non-target species. Detection sensitivity reached 0.1 ng/µL for *P. robustus* and 0.01 ng/µL for *P. muelleri*. The complete workflow, including DNA extraction, amplification, and lateral-flow readout, required approximately 150 minutes for 20 samples. Retail testing revealed species substitutions consistent with known market trends.

Significance: This assay provides a rapid, accurate, and field-deployable method for detecting shrimp species substitution, supporting regulatory enforcement and protecting domestic shrimp producers. Adoption of this tool may strengthen seafood traceability systems and reduce economic fraud in U.S.

Title: **Aggregation behavior of Tulane virus, a human norovirus surrogate, under various ionic conditions: Implications for thermal tolerance**

Authors: **Razieh Sadat Mirmahdi**, University of Florida
Ananya Pradhan, University of Florida
Kelani Saez Montoya, University of Florida
Alireza Abbaspourrad, Cornell University
Razieh Farzad, University of Florida
Andrew J MacIntosh, University of Florida
Arie H. Havelaar, University of Florida
Naim Montazeri, University of Florida

Introduction: Human norovirus is a major cause of foodborne illness; however, its behavior and thermal tolerance in aggregated forms remain unclear. Similarly, the aggregation behavior of Tulane virus, a common surrogate for human norovirus, under varying ionic conditions is not characterized.

Purpose: To examine how ionic strength, pH, and aggregation affect the characteristics and thermal stability of Tulane virus, providing insights into the fate of human norovirus in food and environment.

Methods: Tulane virus was propagated in LLC-MK2 cells and purified via iodixanol density gradient ultracentrifugation. Virus stocks were sonicated and filtered to disperse aggregates, then suspended in Tris–EDTA buffer. Viral particles were characterized using dynamic light scattering and transmission electron microscopy. Zeta potential and isoelectric points were measured across pH 3.3-10 and $CaCl_2$ concentrations of 1, 10, and 100 mM. Aggregation effects on viral titers were assessed via plaque assay after 5 h of exposure to different ionic and pH conditions. Thermal stability of samples was evaluated at 60°C for 2.5 min. Experiments were performed in triplicate, except heat-treatment plaque assays, which were conducted in quadruplicate.

Results: In Tris-EDTA buffer (pH 7.2), Tulane virus was primarily monodispersed, with diameters of 39-41 nm and intact morphology. Zeta potential measurements indicated an isoelectric point of 4.5-6.3, with near-zero surface charge favoring aggregation. Higher $CaCl_2$ concentrations promoted larger aggregates, with hydrodynamic diameters up to ~2,917 nm at 100 mM $CaCl_2$ (pH 6.3). Plaque assay showed aggregation reduced viral titers by up to ~4 $\log_{10}$ under specific pH and ionic conditions ($p < 0.05$). No significant differences in thermal tolerance were observed between aggregated and dispersed particles after treatment at 60°C for 2.5 min ($p > 0.05$).

Significance: Tulane virus aggregates under ionic conditions typical of food and environmental systems, enhancing understanding of adsorption processes and stability in human norovirus surrogate models.

Title: **Effect of Plasma-Activated Water Treatment on Microbial Survival and Cilantro Growth in Deep Water Culture (DWC) Hydroponic Systems**

Authors: **Cody Scales**, Florida A&M University
Madison Williams, Florida A&M University
Victor Akao, Florida A&M University
Afef Janen, Florida A&M University
Edwin Duke, Florida A&M University
Vijay Chhetri, Florida A&M University

Introduction: The numerous reported foodborne outbreaks involving hydroponic leafy greens have raised concerns about the safety of Controlled Environment Agriculture (CEA). However, reducing food-borne pathogens while ensuring optimal plant growth remains a consistent challenge. Plasma-activated water (PAW) has gained popularity in hydroponic production for its dual role as a nitrogen source and an antimicrobial, potentially enhancing plant growth while mitigating food safety risks.

Purpose: This study investigated the effectiveness of PAW treatments on the microbial survival and the growth of Cilantro (*Coriandrum sativum*) in Deep Water Culture (DWC) Hydroponic systems.

Methods: Plasma-activated water with a nitrate level of 100 ppm was produced using a PlasmaLeap100 direct bubble reactor. Cilantro (*Coriandrum sativum*) was grown for 16 days using PAW, pH-adjusted PAW, a combination of PAW and Nutrient solution (NS), and NS only. The systems were inoculated with *E. coli* ATCC 25922 and *Listeria innocua* ATCC 33090 in the second week of treatment. Hydroponic treatment solutions and cilantro plants (roots and leaves) were collected, and the microbial population and plant growth were determined.

Results: The *Listeria innocua* and *E. coli* populations decreased significantly ($P < 0.05$) after 72 hours in all treatment systems, with the highest reduction of 5.6 log CFU/mL in pH-unadjusted PAW systems. *Listeria* appeared to be more susceptible to the treatments compared to *E. coli*. The combination of PAW and NS yielded the highest plant growth, while plant growth was significantly lower in pH-unadjusted treatments.

Significance: These findings suggest that adjusting the concentration and pH of PAW is an effective strategy for controlling the growth of foodborne pathogens while ensuring the plant's growth.

Title: **Impact Of Real-Time Feedback on Cleaning and Sanitation Efficacy on Microbiological Loading on a Leafy Green Harvest Machine**

Authors: **LaTaunya Tillman**, University of Florida
Clara Diekman, University of Florida
Dalton Zingali, University of Arizona
Trevor V. Suslow, Trevor Suslow Consulting, LLC
Justin Kerr, Factor IV Solutions, LLC
Channah M. Rock, University of Arizona
Michelle D. Danyluk, University of Florida

Introduction: Harvest equipment has been identified as potential sources of microbiological contamination; support to identifying opportunities to improve cleaning and sanitation (C/S) programs is needed.

Purpose: Assess efficacy of onsite feedback on leafy green harvester (LGH) C/S programs.

Methods: An assessment of C/S practices, as well as swabs of food contact and non-contact surfaces evaluating microbiological quality (total count, coliforms, *E.coli*, and *Listeria*) was performed using AOAC methods for a 3-point LGH before and after C/S practice feedback. Swabs (n=60) were collected at three timepoints: Post-Harvest(PH) Post-Detergent(PD) and Post-Sanitation(PS) with five sampling zones (A-E). Metadata were recorded for each event, including cleaning and sanitation chemistries and practices used. Feedback was provided in real time; T-tests and ANOVAs were performed (n=2 visits).

Results: The LGH was initially cleaned in the field with a non-chlorinated alkaline detergent and sanitized with chlorine. Following location and chemistry feedback, the LGH was moved from harvest area and sanitized with a quat. Considerable improvement was observed between samplings. Despite a similar volume of water (567 L) being used, notable differences were observed including: cleaning location change; additional staff; a top-down approach; addition of a hygienic brush; and an increased volume of detergent. Presumptive *Listeria* populations decreased from 51.7% of all swabs (n=60: 48%PH, 60%PD, 80%PS) at the first event, to 8.3% of all swabs (n=60: 8%PH, 8%PD and 10%PS). *E. coli* detection (≥10 CFU/swab) was infrequent and decreased from: 8%PH, 8%PD, 0%PS at the first event, to 4%PH, 0%PD, 0%PS. Total and coliform counts significantly decreased ($p < 0.05$) between PH and PD, and PH and PS.

Significance: Simple changes after real time feedback can impact the effectiveness of LGH cleaning practices, resulting in significant improvements in microbiological quality of food contact and non-contact surfaces.

Title: **Microbial risks of groundwater under the influence of surface water used in Florida specialty crop production**

Authors: **Rory Wang**, University of Florida
Claudia Pegueros-Valencia, University of Florida
Michelle Danyluk, University of Florida

Introduction: In Florida, groundwater is the most used water source for fresh produce production and is generally considered safe due to natural filtration processes. However, the sandy soil in Florida allows hydraulic connections between surface water and groundwater, potentially increasing the microbial risks of groundwater.

Purpose: This study evaluated microbiological risks associated with groundwater potentially under the influence of surface water in six Florida wells and their adjacent canals.

Methods: From each well, 111L of water was collected for detection and enumeration of waterborne and foodborne pathogens including *Salmonella*, Shiga toxigenic *Escherichia coli* (STEC), *Giardia* and *Cryptosporidium*, as well as for enumeration of microbial quality indicators, generic *E. coli* and total coliforms. From adjacent canals, 1L of water was collected for detection and enumeration of generic *E. coli* and coliforms as well.

Results: *Salmonella*, STEC, and parasites were not detected in any well sample. Generic *E. coli* levels in well water (<0.005 to -0.70 log MPN/100mL) were also much lower than in canal water (<1.00 to 2.14 log MPN/100 mL).

Significance: The absence of foodborne pathogens and lower levels of total coliforms and generic *E. coli* in groundwater indicate minimal surface water influence in Florida and supports the assumption that groundwater use on specialty crops poses lower microbial risks than surface water.

Title: **Microbiological and Physicochemical Dynamics During Black Tea Kombucha Fermentation and the Survival of *Salmonella enteritidis***

Authors: Philip J. Yeboah, University of Florida
Tingting Gu, University of Florida
Andrew J. MacIntosh, University of Florida
Katherine Thompson-Witrick, University of Florida
Boce Zhang, University of Florida

Kombucha tea, a fermented beverage, is widely consumed and gaining global popularity for its perceived functional properties. The kombucha microbial community comprises bacteria and yeast that can produce antimicrobial metabolites during fermentation to suppress foodborne pathogens. However, this aspect of kombucha's functionality remains only partially characterized under controlled conditions. This study, therefore, investigated the survival of *Salmonella enteritidis* and the associated physicochemical parameters during 14 days of black tea kombucha fermentation. The study also assessed changes in the indigenous microbiota responsible for fermentation and the kombucha microbiome during the 14-day fermentation. Kombucha was prepared using 1.6% (w/v) black tea, 10% (w/v) sucrose, and a commercially available starter culture. The kombucha was inoculated with 7.2 log CFU/mL of *Salmonella enteritidis* and fermented at room temperature for 14 days. Pathogen populations, lactic acid bacteria (LAB), total yeast and mold (TYM) counts, microbiome composition, pH, titratable acidity, and total phenolic content were monitored over 14 days. The initial *Salmonella* population (7.24 ± 0.44 log CFU/mL) decreased by 5 log units by day 10 (2.01 ± 0.1 log CFU/mL) and continued to reduce to 1.4 ± 0.3 log CFU/mL by day 14, whereas the *Salmonella* population in the black tea increased by day 1 and remained stable throughout the incubation. The *Salmonella* inactivation coincided with increased titratable acidity, reduced pH, and increased total phenolic content. Growth in the populations of LAB and TYM increased, corresponding to acidification and associated with the inhibition of pathogens. Results from this study demonstrate that kombucha fermentation can suppress *Salmonella enteritidis*, thereby substantially reducing pathogen growth and the risk of foodborne illness.