Station News

The Connecticut Agricultural Experiment Station
Volume 14 Issue 9 | September 2024

The mission of The Connecticut Agricultural Experiment Station is to develop, advance, and disseminate scientific knowledge, improve agricultural productivity and environmental quality, protect plants, and enhance human health and well-being through research for the benefit of Connecticut residents and the nation. Seeking solutions across a variety of disciplines for the benefit of urban, suburban, and rural communities, Station scientists remain committed to "Putting Science to Work for Society", a motto as relevant today as it was at our founding in 1875.

CAES

The Connecticut Agricultural Experiment Station

Putting Science to Work for Society since 1875

This Issue

ADMINISTRATION

JASON C. WHITE, PH.D. met by Teams with staff at Levo International to discuss a collaboration on urban agriculture (August 2); met by Zoom with program staff at NSF to discuss an upcoming Engineering Research Center submission (August 2); met by Zoom with USDA program staff to discuss a conference grant on the Closer-2-Zero initiative (August 6); along with Nubia Zuverza-Mena, Ph.D. participated in a Zoom meeting with collaborators at Yale University and the University of Minnesota for a joint NIEHS grant (August 6); along with **CHRISTIAN DIMKPA, PH.D.** met by Zoom with collaborators at Johns Hopkins University and Stonybrook University to discuss ongoing experiments and a collaborative USDA grant submission (August 6, 13); met in person with staff from Taylor and Francis to discuss the *International Journal of Phytoremediation* (August 6); met with collaborators at the University of Minnesota and Convergent Bioscience to discuss joint experiments (August 8, 21); met by Teams with staff from the CT Department of Consumer Protection Drug Control Division to discuss the state Adult Use Cannabis program (August 8); hosted the monthly CAES j-visa recipient meeting (August 9); hosted a Zoom meeting with collaborators at Johns Hopkins University to discuss collaborative research (August 9, 16, 26); hosted a Amity High School student and her mother to discuss a mentorship opportunity with CAES (August 9); met by Zoom with colleagues at the University of Minnesota to discuss a collaborative grant proposal (August 12); hosted and gave a tour to a group agriscience educators from Housatonic Valley Regional High School and other locations (August 13); participated in the weekly NSF Center for Sustainable Nanotechnology (CSN) annual strategic planning meeting by Zoom (August 13); attended the Joint Conference of ISEH ICEPH & ISEG on Environment and Health at the University of Galway in Galway Ireland and gave a presentation entitled "Nanobiotechnology-based Strategies for Enhanced Crop Resilience" (August 14-18); attended the American Chemical Society (ACS) Fall 2024 Meeting in Denver Colorado and gave a presentation entitled "Nanobiotechnology-based Strategies for Climate Resilient Crops" (August 18-21); gave a remote presentation at the AgroFood 2024 5th International Conference on Agriculture, Food Security, and Safety in Colombo, Sri Lanka and gave a presentation entitled "Nano-enabled Agriculture: A Path to Global Food Security in a Changing Climate" (August 22); hosted faculty and REU students from the Stockbridge School of Agriculture University of Massachusetts Amherst for a symposium on CAES research programs (August 26); participated in a Board meeting for the International Phytotechnology Society and discussed the upcoming conference in Kerala India (August 26); spoke by phone with reporter Natalie Demaree about plastics contamination in the environment (August 27); participated in CAES Teams calls concerning a possible Oak Wilt detection (August 27, 28); participated in a Zoom call with collaborators at FDA, the University of Iowa and the Aukland University to begin planning for the 2026 Gordon Research Conference on Food and Agriculture (August 29); met by Zoom with collaborators at UConn and the University of Hawaii to discuss a joint grant application (August 29).

PUBLICATIONS:

1. Cao, X., Chen, X., Jiang, Y., Wang, C., Yue, L., Li, X., **White, J. C.,** Wang, Z., Xing, B. (2024). Selenium nanomaterials enhance sheath blight resistance and nutritional quality of rice: Mechanisms of action and human health benefit. *ACS Nano* 18, 20, 13084–13097.

Abstract: In the current work, foliar application of selenium nanomaterials (Se0 NMs) suppressed sheath blight in rice (Oryza sativa). The beneficial effects were nanoscale specific and concentration-dependent. Specifically, foliar amendment of 5 mg/L Se0 NMs decreased the disease severity by 68.8% in Rhizoctonia solani infected rice; this level of control was 1.57- and 2.20-fold greater than the Se ions with equivalent Se mass and a commercially available pesticide (thifluzamide). Mechanistically, (1) the controlled release ability of Se0 NMs enabled a wider safe concentration range and greater bioavailability to Se0 NMs; (2) transcriptomic and metabolomic analyses demonstrated that Se0 NMs simultaneously promoted the salicylic acid- and jasmonic acid-dependent acquired disease resistance pathways, antioxidative system, and flavonoid biosynthesis. Additionally, Se0 NMs improved rice yield by 31.1%, increased the nutritional quality by 6.4-7.2%, enhanced organic Se content by 44.8%, and decreased the arsenic and cadmium content by 38.7 and 42.1% in grains as compared with infected controls. Human simulated gastrointestinal tract model results showed that the application of Se0 NMs enhanced the bioaccessibility of Se in grains by 22.0% and decreased the bioaccessibility of As and Cd in grains by 20.3 and 13.4%, respectively. These findings demonstrate that Se0 NMs can serve as an effective and sustainable strategy to increase food quality and security.

2. Deng, C., Wang, Y., Castillo, C., Zhao, Y., Xu, W., Lian, J., Rodrigues-Otero, K., Brown, H., Cota-Ruiz, K., **Elmer, W. H.**, **Dimkpa, C. O.,** Giraldo, J. P., Hernandez, R., Wang, Y., **White, J. C**. (2024). Nanoscale iron (Fe3O4) surface charge controls Fusarium suppression and nutrient accumulation in tomato (*Solanum lycopersicum* L.). *ACS Sus. Chem. Engin.* 12, 35, 13285–13296.

Abstract: With the growing recognition that conventional agriculture will be unable to meet food production demands, innovative strategies to reach food security are imperative. Although nanoscale fertilizers are attracting increased attention as a sustainable platform for agricultural applications, limited data exists on how surface charge influences overall efficacy relative to disease suppression and nutrient accumulation. This study investigated the effect of positively and negatively charged iron oxide nanoparticles (Fe3O4 NPs) on the growth of tomato (Solanum lycopersicum L.) plants and their disease resistance against the pathogen Fusarium oxysporum f. sp. lycopersiciat both the greenhouse and field scale. In addition, a theoretical model of the bio-interface was employed for mechanistic understanding of the interaction and attachment efficiency between NPs and tomato leaves after foliar exposure. In the greenhouse, both positively and negatively charged Fe3O4 NPs significantly suppressed Fusarium wilt by 41.4% and 44.6%, and increased plant shoot biomass by 327.6% and 455.0%, respectively, compared to the diseased control. The impact of NP surface charge was apparent; positively charged Fe3O4 NPs demonstrated superior efficacy compared to their negatively charged counterparts in mitigating disease damage and regulating nutrient (Na, Si, and Cu) accumulation. Computationally, positively charged Fe3O4 NPs consistently migrate toward lipid layers, indicative of a pronounced affinity between these entities compared to the negatively charged particles, which aligns with the experimental data. The findings highlight the importance and tunability of nanomaterials properties, especially the surface charge, in optimizing the use for disease suppression and nutrient modulation, which offers a great potential for sustainable agriculture.

3. Jiang, X., **White, J. C.,** He, E., Van Gestel, C. A. M., Qiu, H. (2024). Foliar exposure of deuterium stable isotope labeled nanoplastics to lettuce: Quantitative determination of foliar uptake, transport, trophic transfer in a terrestrial food chain. *Environ. Sci. Technol*. 58, 35, 15438–15449.

Abstract: Nanoplastics (NPs) are widely detected in the atmosphere and are likely to be deposited on plant leaves. However, our understanding of their foliar uptake, translocation, and trophic transfer profiles is limited due to a lack of quantitative analytical tools to effec-

tively probe mechanisms of action. Here, using self-synthesized deuterium (2H) stable isotope labeled polystyrene nanoplastics (2H-PSNPs), the foliar accumulation and translocation of NPs in lettuce, as well as the dynamics of NPs transfer along a lettuce-snail terrestrial food chain, were investigated. Raman imaging and scanning electron microscopy demonstrated that foliar applied NPs aggregated on the leaf surface, entered mesophyll tissue via the stomatal pathway, and were eventually translocated to root tissues. Quantitative analysis showed that increasing levels of foliar exposure to 2H-PSNPs (0.1, 1, and 5 mg/L) enhanced NPs accumulation in leaves, with concentrations ranging from 0.73 to 15.61 μg/g (dw), facilitating translocation to roots. After feeding on 5 mg/L 2H-PSNPs-contaminated lettuce leaves for 14 days, snails accumulated NPs at 0.33 to 10.68 μg/kg (dw) with kinetic trophic transfer factors of 0.02–0.68, demonstrating trophic dilution in this food chain. The decreased ingestion rate from 6.43 to 3.18 mg g-1 d-1 can be attributed to elevated chemical defense metabolites caused by consumption of leaves with high levels of 2H-PSNPs, which affected palatability and disrupted snail digestive function and bile secretion. This study provides critical quantitative information on the characteristics of NPs bioaccumulation and the associated risks to terrestrial food chains exposed to atmospheric NPs pollution.

4. Chen, L., Qiu, T., Zeng, Y., Huang, F., **White, J. C**., Fang, L. (2024). Micro-nanoplastics pollution poses a potential threat to soil health: Evidence from a hierarchical meta-analysis. *Global Change Bio*. https://doi.org/10.1111/gcb.17470.

Abstract: A comprehensive understanding the impacts of microplastics (MPs) pollution in soil ecosystems is urgently needed to accurately assess risk. However, the impacts of MPs on soil health have not yet been explored. We conducted a hierarchical meta-analysis of over 5000 observations from 228 articles to assess the broad impacts of MPs on soil health parameters and whether the impacts depended on MPs properties. Our results found that MPs exposure significantly inhibited crop biomass and germination, and reduced earthworm growth and survival rate. Under MPs exposure, the emissions of soil greenhouse gases (CO2, N2O, and CH4) were significantly increased. MPs significantly decreased the Shannon index and increased the Simpson index of bacteria in soil, implying a decrease in soil bacteria diversity. Importantly, the magnitude of impact of the soil-based parameters was dependent on MPs dose and size; however, there is no significant difference in MPs type (biodegradable and conventional MPs). Moreover, MPs significantly promoted plant Cd accumulation and reduced As uptake by plants. Using an analytical hierarchy process, we quantified the negative impacts of MPs addition on soil health as a mean value of -10.2% (-17.5 to -2.57%). Overall, this analysis provides new insights for assessing potential risks of MPs pollution to soil ecosystem functions.

5. Chen, Li, Fang, L., Liu, J., Bing, H., Zeng, Y., Chen, X. Li, Z., Hu, W., Huang, F., Yang, X., **White, J. C.,** Shaheem S. M., Xing, B. (2024). Nano-enabled strategies to promote safe crop production in heavy metal(loid)-contaminated soil. *Sci. Total Environ.* 947 (2024) 174505.

Abstract: Nanobiotechnology is a potentially safe and sustainable strategy for both agricultural production and soil remediation, yet our current knowledge on the comprehensive effects of nanomaterials application in complex soil sols is fragmented. A global meta-analysis was conducted with over 5,000 observations to quantify the effects of nanomaterials on safe crop production in soils contaminated with heavy metal(loid) (HM), and a machine learning approach identified major contributing features. Nanomaterials elevated crop shoot (15.2%) and grain biomass (27.2%), and decreased shoot and grain HM concentration by 28.1 and 45.3%, respectively. Iron-nanomaterials showed a greater potential to inhibit crop HM uptake compared to other types of nanomaterials. A new model based on human health risk assessment models coupled with Monte Carlo simulation was developed and further demonstrates that nanomaterials application substantially reduces the probabilistic

health risk of HMs in crop grains. The nanomaterials-induced reduction in HM accumulation was associated with decreasing HM bioavailability, as well as increased soil pH and organic matter. A random forest model demonstrates that soil pH and total HM concentration are the two significant features affecting shoot HM accumulation. This analysis of the literature highlights the significant potential of nanomaterials application in promoting safe crop production in HM-contaminated agricultural soils.

6. Zhou, J., Tang, C., Kuzyakov, Y., Zhou, X., Gef, T., Xiao, M., Cai, Y., Yu, B., **White, J. C**., Jiang, Z., Li, Y. (2024). Biochar-based urea increases soil methane uptake in a subtropical forest. *Geoderma* 449, 116994.

Abstract: Novel biochar-based fertilizers, produced by combining biochar particles with chemical fertilizers, have strong potential to enrich soil with carbon (C) and nitrogen (N), and to increase plant productivity. Biochar-based fertilizer application modifies the soil microbial community composition, thereby influencing greenhouse gases emissions, including methane (CH4) fluxes. Due to the improved aeration in soils amended with biochar particles, we hypothesized that biochar-based urea will decrease CH4 production and increase CH4 oxidation, leading to increase of total CH4 uptake by soil. A three-year field trial was conducted in a subtropical Moso bamboo forest to compare the impacts of urea and biochar-based urea on seasonal CH4 uptake by soil, as well as on soil physico-chemical and microbial properties. Urea application reduced the annual soil CH4 uptake by 5.7% and 16% at 100 and 300 kg N ha−1, respectively, within the first year. The biochar-based urea application at 300 kg N ha−1 increased the annual CH4 uptake by 12% in both the first and second years, although the effects lessened over time. Soil CH4 uptake increased with CH4 oxidation rate but decreased with CH4 production rate. The urea-induced decrease in CH4 uptake was attributed to the increased NH4+ and NO3− content and mcrA gene abundance, as well as decreased pmoA/mcrA ratio, thereby increasing CH4 production rate. The biochar-based urea increased CH4 uptake was mediated by better soil aeration and labile C supply, creating favorable environments for methanotrophs, as well as increased pmoA gene abundance and pmoA/mcrA ratio, which accelerated CH4 oxidation. Overall, these results highlight that biochar-based fertilizers can increase CH4 uptake by increasing the CH4 oxidation rate and microbial activity in soils. Extrapolating these results, we deduce that the replacement of urea with biochar-based urea in Moso bamboo forests across China will increase the national annual soil CH4 uptake by around 4450 t CH4.

7. Hou, J., Lu, Y., Chen, Q., Lian, X., Wu, X., Sang, K., Xu, J., Zhang, J., Yang, K., Zhu, L., **White, J. C**., Gardea-Torresdey, J. L., Lin, D. (2024). Multifunctional biomolecular corona-inspired nanoremediation of antibiotic residues. *PNAS* (36) e2409955121.

Abstract: Biomolecular coronas derived from highly tolerant organisms under significant contamination scenarios can used in conjunction with nano-zero valent iron (nZVI) to remediate emerging contaminants of concern. Here, super worms (Tubifex tubifex) with high pollutant-tolerance were integrated with nZVI to effectively reduce the content of 17 antibiotics in wastewater within 7 d. Inspired by the synergistic remediation, nZVI-augmented worms were constructed as biological nanocomposite. Neither nZVI (0.3-3 g/L) nor worms (104-105 per liter) alone efficiently degraded florfenicol (FF, as a representative antibiotic), while their composite removed 87% of FF (3 μmol/L). Under antibiotic exposure, biomolecules secreted by worms formed a corona on and modified the nZVI particle surface, enabling the nano-bio interface greater functionality, including responsiveness, enrichment, and reduction. Mechanistically, FF exposure activated glucose-alanine cycle pathways that synthesize organic acids and amines as major metabolites, which were assembled into vesicles and secreted, thereby interacting with nZVI in a biologically response design strategy. Lactic acid and urea formed hydrogen bonds with FF, enriched analyte presence at the hetero-

geneous interface. Succinic and lactic acids corroded the nZVI passivation layer and promoted electron transfer through surface conjugation. This novel strategy highlights biomolecular coronas as a complex resource to augment nano-enabled technologies and demonstrate the potentially for rational manipulation of nanomaterial surfaces with coordinated multifunctionalities.

Jason C. White, Ph.D. lecturing at the University of Galway.

ANALYTICAL CHEMISTRY

Anuja Bharadwaj, Ph.D. participated in the "USP Cannabis Regulator Forum: Workshop on Heavy Metals in Cannabis" on August 20 in Rockville, MD. Following the event, she held a Zoom meeting with Jennifer Mandzuk from the Drug Control Division of the Department of Consumer Protection to review and update key takeaways from the forum.

Anuja Bharadwaj, Ph.D., along with **Drs. Christian Dimkpa** and **Jason White,** presented their work on the Adult-Use Marijuana Program at two events: during the visit of DCP Commissioner Bryan T. Cafferelli and his team from the Drug Control Division of the Department of Consumer Protection, and the Station Associates Tour. At both events, attendees were briefed on the program's purpose and importance, followed by a demonstration of analytical instrument runs to provide insight into the laboratory processes.

DCP Officials or DCP Commissioner Bryan T. Cafferelli, Deputy Commissioner Shirley Skyers-Thomas and their team being addressed by **Anuja Bharadwaj, Ph.D.**

Station Associates being addressed by **Anuja Bharadwaj, Ph.D.**

NEW STUDENTS, STAFF, AND VOLUNTEERS:

Jiashu Zhou is a student at the Zhejiang A&F University, China. He joined the Analytical Chemistry Department in August as a research visitor through the Zhejiang A&F University Conservation Scholars program. At CAES, Jiashu is working with Dr. Yi Wang and Dr. Chaoyi Deng to investigate the synergistic effects of sulfur nanoparticles on soil and plants. He has a personal interest in the fabrication of nanoparticle-based nutrient element and aims to deepen his understanding of the ecological processes of nanoparticles in soil. Additionally, Jiashu is eager to gain experience in analyzing nanoparticle-related experimental samples using LC-ICP-MS and GC-MS techniques at the station. His research interests include the application of nanoparticle-biomass coupling strategies in agricultural and forestry ecosystems and their implications for environmental and human health. Jiashu's tenure at CAES ends in late November.

PUBLICATIONS:

1. Johnson, K.I., Ilacas, G., Das, R., Chang, H-Y., Sharma, P.R., **Dimkpa, C.O.**, Hsiao, B.S. (2024). A circular solution to enhance the food-water nexus by nanocellulose technologies for ammonium recovery and reuse. Sustainability Science and Technology 1: 014001 https://doi.org/10.1088/2977-3504/ad6cdf

Abstract: In this study, a circular solution to enhance the food and water nexus by using a zero-waste process to produce carboxylated nanocellulose adsorbents from a model lignocellulose feedstock (jute) for ammonium (NH_4^+) nutrient recovery and reuse was demonstrated. The study represents a new pathway to close the nitrogen loop that will be suitable for some

agricultural practices. In specific, anionic nanocellulose containing COO^- groups, produced by the nitro-oxidization process, are efficient for removing NH_4^+ from contaminated water, where the spent adsorbent can be repurposed as an effective plant fertilizer. The nitro-oxidized cellulose nanofiber (NO-CNF) scaffold prepared from raw jute exhibited a maximum adsorption capacity of 22.7 mg g^{-1} for NH_4^+ removal, which is significantly higher than any natural sorptive materials reported thus far, including biochar and activated carbons. The effect of pH (from 2 to 10) on the ammonium adsorption efficiency and the corresponding zeta potential of NO-CNF was investigated, where the optimal adsorption capacity was near neutral pH conditions. The zeta potential of ammonium-loaded NO-CNF was found to covert from a negative value (−50 mV) to a positive value (100 mV) with the increasing ammonium content at a critical NH_4^+:COO^- molar ratio around 0.85). The NH_4^+-loaded NO-CNF was repurposed as a fertilizer for soybean growth with efficacy similar to a typical urea fertilizer. This study illustrates an exemplary advance in the development of zero-waste process to upcycle natural waste feedstocks into valued products that can simultaneously reduce nitrogen pollution problems and close the nitrogen loop for food production.

2. Deng, C., Wang, Y., Castillo, C., Zhao, Y., Xu, W., Lian, J., Rodriguez-Otero, K., Brown, H.J., Cota-Ruiz, K., Elmer, W.H., **Dimkpa, C.O.,** Giraldo, J.P., Hernandez, R., Wang, Y., **White, J.C.** (2024). Nanoscale iron (Fe_3O_4) surface charge controls Fusarium suppression and nutrient accumulation in tomato (*Solanum lycopersicum* L.). ACS Sustainable Chemistry and Engineering. 12, 35, 13285–13296.

Abstract: With the growing recognition that conventional agriculture will not be able to meet food production demands, innovative strategies to reach food security are imperative. Although nanoscale fertilizers are attracting increased attention as a sustainable platform for agricultural applications, limited data exist on how surface charge influences overall efficacy relative to disease suppression and nutrient accumulation. This study investigated the effect of positively and negatively charged iron oxide nanoparticles (Fe_3O_4 NPs) on the growth of tomato (*Solanum lycopersicum* L.) plants and their disease resistance against the pathogen *Fusarium oxysporum* f. sp. *lycopersici* at both the greenhouse and field scale. In addition, a theoretical model of the biointerface was employed for a mechanistic understanding of the interaction and attachment efficiency between NPs and tomato leaves after foliar exposure. In the greenhouse, both positively and negatively charged Fe_3O_4 NPs significantly suppressed Fusarium wilt by 41.4 and 44.6% and increased plant shoot biomass by 327.6 and 455.0%, respectively, compared to the diseased control. The impact of the NP surface charge was apparent; positively charged Fe_3O_4 NPs demonstrated superior efficacy compared to their negatively charged counterparts in mitigating disease damage and regulating nutrient (Na, Si, and Cu) accumulation. Computationally, positively charged Fe_3O_4 NPs consistently migrate toward lipid layers, indicative of a pronounced affinity between these entities compared with the negatively charged particles, which aligns with the experimental data. The findings highlight the importance and tunability of nanomaterials properties, especially the surface charge, in optimizing the use for disease suppression and nutrient modulation, which offers a great potential for sustainable agriculture.

3. Irewale, A.T., **Dimkpa, C.O,** Elemike, E.E., Oguzie, E.E. (2024). Water Hyacinth: Prospects for Nanobiochar and Biofertilizer Development. Heliyon 10 (2024) e36966; https://doi.org/10.1016/j.heliyon.2024.e36966

Abstract: The widespread proliferation of water hyacinth (*Eichhornia crassipes*) in aquatic ecosystems has raised significant ecological, environmental, and socioeconomic concerns globally. These concerns include reduced biodiversity, impeded water transportation and recreational activities, damage to marine infrastructure, and obstructions in power generation dams and irrigation systems. This review critically evaluates the challenges posed by water hyacinth (WH) and investigates potential strategies for converting its biomass into value-added agricultural products, specifically nanonutrients-fortified, biochar-based, green fertilizer. The review examines various methods for producing functional nanobiochar and green fertilizer to enhance plant nutrient uptake and improve soil nutrient retention. These methods include slow or fast pyrolysis, gasification, laser ablation, arc discharge, or chemical precipitation used for producing biochar which can then be further reduced to nano-sized biochar through ball milling, a top-down approach. Through these means, utilization of WH-derived biomass in economically viable, eco-friendly, sustainable, precision-driven, and smart agricultural practices can be achieved. The positive socioeconomic impacts of repurposing this invasive aquatic plant are also discussed, including the prospects of a circular economy, job creation, reduced agricultural input costs, increased agricultural productivity, and sustainable environmental management. Utilizing WH for nanobiochar (or nano-enabled biochar) for green fertilizer production offers a promising strategy for waste management, environmental remediation, improvement of waterway transportation infrastructure, and agricultural sustainability. To underscore the importance of this work, a metadata analysis of literature carried out reveals that an insignificant section of the body of research on WH and biochar have focused on the nano-fortification of WH biochar for fertilizer development. Therefore, this review aims to expand knowledge on the upcycling of non-food crop biomass, particularly using WH as feedstock, and provides crucial insights into a viable solution for mitigating the ecological impacts of this invasive species while enhancing agricultural productivity.

ENTOMOLOGY

PHILIP ARMSTRONG, SC.D. spoke with reporters from NBC Connecticut (August 2), Hearst Media (August 5), and Fox 51 (August 5) about the detection and risk of EEE and West Nile viruses this season in Connecticut, met with Yale Microbiology Fellow Donna Hill to discuss the Connecticut Mosquito Surveillance Program (August 13), gave an online talk titled "Sychrony of mosquito and arbovirus collections in Connecticut" to the CDC Vector-Borne Disease Forcasting Workgroup (August 26; 40 attendees), interviewed by: news channel 12 about West Nile virus risk and disease prevention (August 27), Hearst Media about EEE virus (August 27), NBC Connecticut about EEE and West Nile virus in Connecticut (August 28) WTIC about an insecticide spraying event in Connecticut (August 28), DW news about the validity of a EEE virus conspiracy theory (August 29), Harford Courant (August 29), Univision News (August 29), and NPR CT (August 30) about the EEE virus activity in Connecticut and the surrounding region.

ANGELA BRANSFIELD met with Yale Microbiology Fellow Donna Hill to discuss the Biosafety Level 3 (BSL-3) laboratory and viral diagnostics in relation to the CT Mosquito Surveillance Program (August 13), participated via Zoom in Yale University's Biosafety Committee meeting (August 15), and participated in Yale University's emergency response drill (August 27).

KELSEY E. FISHER, PH.D. held a bumblebee outreach event for 28 K-3 children at Gather New Haven's Schooner Environmental Summer Camp (Aug 2); presented a barn exhibit at Plant Science Day "Mighty Monarchs and Busy Bumblebees" (Aug 7); participated in the International Congress of Entomology 2024, hosted in Kyoto Japan, and presented the talk "Influence of habitat configuration on breeding-season female monarch butterfly movement and space use in north central USA agroecosystems" (Aug 25-30; 120 attendees).

ANDREA GLORIA-SORIA, PH.D. Presented the research short talk "New challenges in vector control and how population genetics can help" (~70 attendees) and hosted the "Kids Corner" activity booth during the 114th Plant Science Day of the Connecticut Agricultural Experiment Station (August 7, 2024); participated in the International Congress of Entomology 2024, hosted in Kyoto Japan,and presented the talk "Population genetics of insecticide resistance in the *Culex* pipiens complex within the USA" (August 25-30, 2024; 80 attendees).

MEGAN LINSKE, PH.D. co-hosted the Wildlife Society Leadership Institute Committee meeting as Co-Chairperson (Aug 8); participated in the Northeast Center for Excellence in Vector Borne Diseases and Training and Evaluation Center's Leadership Committee meeting (Aug 13); hosted Yale Medical Microbiology Fellow Dr. Donna Hill for a meeting to discuss the integrated tick management research programs (Aug 13) and a field day at our systemic treatment study sites to partake in white-footed mouse processing and surveillance (Aug 14); participated in a meeting with collaborators from BanfieldBio, Inc. and North Carolina State University to discuss blacklegged tick repellency trials and developments for the upcoming field season (Aug 20); participated in a call with staff from the Centers for Disease Control and Prevention's Division of Vector-Borne Diseases on progress made on a funded integrated tick management and seasonal spray projects (Aug 29).

GOUDARZ MOLAEI, PH.D. attended the monthly meeting of longhorned tick, *Haema-*

physalis longicornis, and discussed this tick activity in CT (August 5); attended the CAES Plant Science Day 2024 and had a display "Passive Tick and Tick-borne Pathogen Surveillance Program in Connecticut" under the public health tent (August 7); was interviewed by WTNH Channel 8 about the CAES Tick Testing Laboratory and challenges with ticks and tick-borne diseases (August 7); discussed collaborative research projects with Suffolk Mosquito Control District in Virginia (August 8); attended NEVBD TEC leadership meeting to discuss projects and progress (August 13); hosted Dr. Donna Hill, a Yale University Microbiology Fellow and discussed diagnostic services at the CAES Tick Testing Laboratory and research projects on ticks and mosquitoes (August 13); presented a lecture on vector-borne diseases at the Southern Connecticut State University (August 28); and was interviewed by Hearst Connecticut Media Group on increases in tick and tick-borne disease activity (August 29).

JACOB RICKER participated in UConn Extension's Biological Control Conference (August 15), which concerned topics related to ornamental growers like beneficial insects, *Thrips parvispinus*, and biopesticides.

GALE E. RIDGE, PH.D. met with the director of health, Jennifer Muggeo from the Ledge Light Health District to inspect a neighborhood fly infestation that was coming from a farm (August 9); and presented a talk on bed bugs to the Western Connecticut Mental Health Network (20 online attendees) (August 23).

CLAIRE RUTLEDGE, PH.D. prepared a poster 'Potential for biological control of Spotted Wing Drosophila in Connecticut' along with childrens' activities for the 114th Plant Scence Day of the Agricultural Experiment Station (August 7).

KIRBY C. STAFFORD, PH.D. participated in a NEVBD-TEC leadership call on Zoom (August 13).

PAULA WOLF Participated in the Eastern Apicultural Society Conference involving several workshops surrounding honey bee health and disease management (August 5-6 & 8-9) and displayed an observation hive, speaking with attendees at the 114th Plant Science Day of the Connecticut Agricultural Experiment Station (August 7th).

GRANTS AWARDED:

'Biological control of spotted wing drosophila' USDA Biological Control Cross-Agency Functional Working Group. PI's **Claire Rutledge**[1]**, Richard Cowles**[1]**,** Jaime Pinero[2], Matther Buffington[3] $14,500

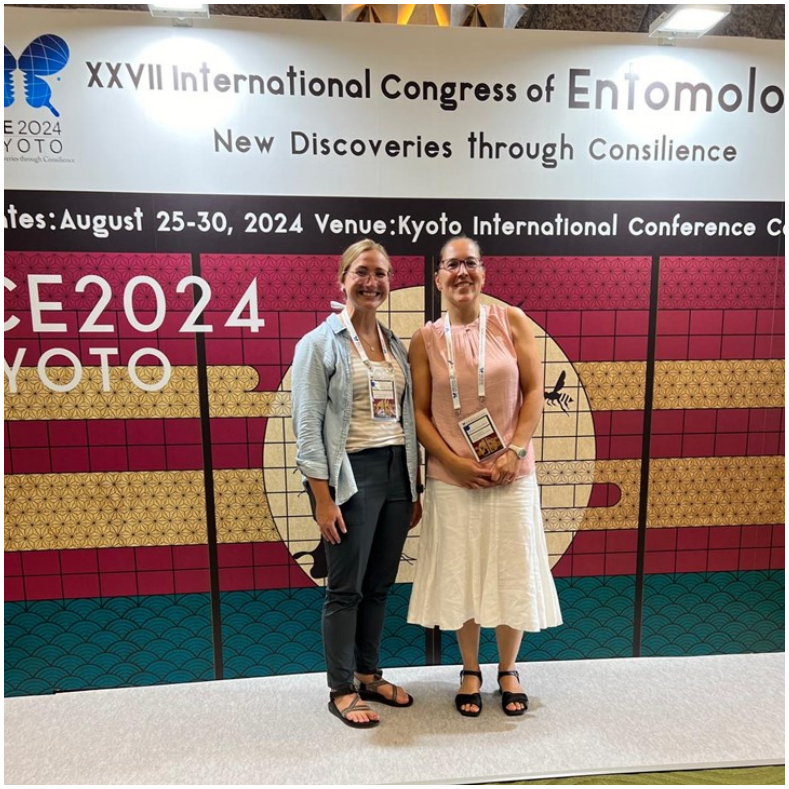
[1] Connecticut Agricultural Experiment Station, [2] University of Massachusetts, Amherst, [3] USDA Systematic Entomological Laboratory.

Spotted wing drosophila (hereafter referred to as SWD) is a serious invasive pest of soft fruits that has been present in Connecticut since 2011. Its arrival seriously disrupted pest management strategies in these crops and increased the usage of pesticides manyfold. A specialist Asian parasitoid of SWD has recently been approved by USDA for release in the USA. However, both the approved parasitoid, *Gnapsis kimorum* and an adventitious Asian parasitoid, *Leptopilina japonica*, with a broader host range of many drosophilid species, have been spreading unaided. This project is focused on surveying Connecticut and Massachusetts for the presence of one, or both parasitoids to assess the need for active releases of *G. kimorum* in these states next year.

PUBLICATIONS:

1. Galletti, M. F. B. M., Hecht, J. A., McQuiston, J. R., Gartin, J., Cochran, J,. Blocher, B. H., Ayres, B. N., Allerdice, M. E. J., Beati, L., Nicholson, W. L., Snellgrove, A. N., Paddock, C. D., Kennedy, A., **Molaei, G.**, Lado, P., Foley, J., Goddard, J., Occi, J.L., Padgett, K., Dykstra, E., Nolan, M., Cortinas, R., Sambado, S., Fink, S., Campbell, S. R., and Romer, Y. (2024). Applying MALDI-TOF MS to resolve morphologic and genetic similarities between two Dermacentor tick species of public health importance. *Sci Rep.* 14 (1):19834. DOI: 10.1038/s41598-024-69768-8.

Abstract: Hard ticks (Acari: Ixodidae) have been historically identified by morphological methods which require highly specialized expertise and more recently by DNA-based molecular assays that involve high costs. Although both approaches provide complementary data for tick identification, each method has limitations which restrict their use on large-scale settings such as regional or national tick surveillance programs. To overcome those obstacles, the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been introduced as a cost-efficient method for the identification of various organisms, as it balances performance, speed, and high data output. Here we describe the use of this technology to validate the distinction of two closely related Dermacentor tick species based on the development of the first nationwide MALDI-TOF MS reference database described to date. The dataset obtained from this protein-based approach confirms that tick specimens collected from United States regions west of the Rocky Mountains and identified previously as *Dermacentor variabilis* are the recently described species, *Dermacentor similis*. Therefore, we propose that this integrative taxonomic tool can facilitate vector and vector-borne pathogen surveillance programs in the United States and elsewhere.



Dr. Gloria-Soria and **Dr. Fisher** representing CAES at the XXVII International Congress of Entomology. August 25-30, Kyoto, Japan.

ENVIRONMENTAL SCIENCE & FORESTRY

SCOTT WILLIAMS, PH.D. participated in a Zoom call with staff from MaineHealth and White Buffalo, Inc. regarding an ongoing collaborative host-targeted integrated tick management project (August 8); participated in the leadership meeting of the Northeast Center for Excellence in Vector Borne Disease's Teaching & Evaluation Center (August 13); met with Dr. Donna Hill, Yale University School of Medicine Microbiology Fellow Rotation and spoke of ticks and tick-borne pathogen abatement (August 13); hosted a field visit to demonstrated rodent trapping and processing in residential settings with Dr. Donna Hill, Yale University School of Medicine Microbiology Fellow (August 14); hosted Dr. Michael Zarfos, Executive Director of Great Mountain Forest (Norfolk, CT) to introduce him to CAES, our research, and forest scientists (August 16); participated in a collaborative Zoom call with members of the Banfield Biologic NIH SBIR-funded tick repellent fabric team (August 20); participated in a Zoom call with staff from the CDC Division of Vector-Borne Diseases on progress made on a funded integrated tick management project (August 21).

JOSEPH P. BARSKY met with Michael Zarfos, Ph. D. of Great Mountain Forest Corporation to discuss collaborative research (August 16); interviewed by Jennifer Aherns of Connecticut Public Radio about the Connecticut Oak Mast Surveillance Program (August 20); met with Gail Cameron of the Sleeping Giant Park Association to discuss the Connecticut Oak Mast Surveillance Program (August 20); participated, as chair-elect, at the New England Society of American Foresters Board of Directors Meeting (August 29).

JESSICA BROWN, PH.D. participated in a Zoom call with members of The Wildlife Society's Conservation Affairs Network (August 29).

GREGORY BUGBEE gave an update on "Hydrilla in the Connecticut River and its Spread to Lakes" at a virtual meeting of the Connecticut River Conservancy (25 attendees) (August 13); presented an invited talk on "Hydrilla in Gardner Lake" to the Gardner Lake Association at the Gardner Lake Volunteer Fire Department in Salem (75 attendees) (August 15); gave an invited talk on "Container Gardening Indoors and Out" to the Oxford Garden Club at the Oxford Public Library (40 attendees) (August 27); participated in the quarterly meeting of the Northeast Aquatic Nuisance Species Panel (August 26); interviewed by Brian Murphy of the United States Army Corps of Engineers Press Corps on hydrilla in the Connecticut River (August 13); met via video conference with the United States Army Corps of Engineers Hydrilla Research and Demonstration Education & Outreach Working Group to discuss the CT River Hydrilla Project (August 19, 22).

JEREMIAH FOLEY, IV, PH.D. was interviewed by The Lakeville Journal for the article "Hydrilla's 'Alarmingly Rapid' Spread Prompts State Survey of Boat Launches" (August 3); presented a invited lecture on the spread and impact of aquatic plants in CT (100 attendees) (August 3); interviewed by Ben James for an NPR segment on the CT River (Aug 6).

SUSANNA KERIÖ, D.SC. attended a zoom meeting to plan the Annual Symposium of The American Chestnut Foundation (August 14); met with Mathew Verry, Eveleen Jiang (DOT intern 2024, CAES intern 2023 in Keriö lab), and Madeline Schad at the CT Department of Transportation to discuss collaboration on urban tree research (August 15); recorded an invited

presentation on "Increased Risks to Urban Forests from Climate Change" for Yale online certificate course on Urban Climate Leadership (August 20); met **RICHARD JAYNES, PH.D.** (former CAES scientist) at Lockwood Farm to plan tours in the CAES chestnut plots for the Annual Symposium of The American Chestnut Foundation (August 29).

SARA NASON, PH.D. participated in meetings for the Best Practices for Non-Targeted Analysis working group (August 1, 5, 20, virtual); presented a talk titled "PFAS Research at CAES" to a group of visiting student researchers from the University of Massachusetts, Amherst (August 26, New Haven, CT).

SUMMER STEBBINS attended the NASA Invasive Species Monitoring with Remote Sensing Training (August 14, 21, 28); recorded educational videos for the UConn Invasive Plant Certificate training course (August 21); met with UConn Extension 4H Educators to restart the Students in STEM program (August 28); gave a training on hand-harvesting hydrilla at Amos Lake in Preston (7 attendees) (August 29).

BLAIRE STEVEN PH.D. attended the International Society of Microbial Ecology Microbes meeting in Cape Town, South Africa and presented a research poster titled "Rewilding the Microbiome: Introducing Environmental Bacteria to Axenic Mosquitoes alter Microbiome Assembly and Host Phenotypes" (August 18-23).

ELISABETH WARD, PH.D. participated in the monthly Master Woodland Managers partner meeting (August 6); participated in the monthly State Coordinators meeting for the Forest Ecosystem Monitoring Cooperative (August 12); met with Jonathan Zeiner (Forester, Aquarian Water Company) to tour and discuss ash salvage harvests from emerald ash borer invasion on Torrington Water Company land along with **Joseph P. Barsky** (August 14); met with Mike Zarfos, Ph.D. (Executive Director, Great Mountain Forest) to discuss collaborative research between Great Mountain Forest and CAES along with **SCOTT C. WILLIAMS, PH.D.**, **Megan Linske, Ph.D.** and **Joseph P. Barsky** (August 16); was featured in an article published by the Yale School of the Environment on mentoring New Haven Promise interns in the field (August 20); was interviewed by the Northeast Wilderness Trust on current forest ecology research and priorities(Aug 26); met with Helen Poulos, Ph.D. (Associate Professor, Wesleyan University) and her student at Meshomasic State Forest to sample non-structural carbohydrates from trees affected by Beech Leaf Disease along with **Susanna Keriö, Ph.D.** (August 27); met with forestry staff from the Rhode Island Department of Environmental Management to plan collaborative grant proposal for Beech Leaf Disease management (August 28); met with Forestry Division staff from the Connecticut Department of Energy and Environmental Protection to plan collaborative grant proposal for Beech Leaf Disease management (August 28).

JEFFREY S. WARD, PH.D. spoke on "Tree identification and silvics" at the Woodland Academy at Great Mountain Forest in Norfolk (29 attendees) (August 3); participated in a meeting of the Great Mountain Forest Trustees in Norfolk (August 10); meet with Connor Hogan (McLean Game Refuge Director) to discuss forest regeneration (August 13); spoke on "Natural Disturbance and Silvicultural Systems" at the Woodland Academy at Great Mountain Forest in Norfolk (31 attendees) (August 17); meet with representatives of Norcross Wildlife Sanctuary and Great Mountain Forest to discuss deer pressure and forest regeneration (9 attendees) (August 27); participated in a Zoom call with New England Forestry Foundation to

discuss options for forest management on private lands (6 attendees) (August 28).

LEIGH WHITTINGHILL, PH.D. produced materials for two field exhibits titled "Baby Pools: Low-Cost Containers for Vegetable Production in Urban Agriculture" and "The Effect of Different Fertilizer Applications on Cut-and-come-again Kale Production" presented by the Plant Health Fellows and Sofia Shubin at the 2024 annual Plant Science Day (Aug 7).

YINGUE (CHARLIE) YU, PH.D. attended the online workshop from EMSL "Integrated chemical and physical imaging of biogeochemical processes and interfaces: molecular to regional scale" (August 12–13); attended the EPA Funding Opportunity Webinar "Developing and Demonstrating Nanosensor Technology to Detect, Monitor, and Degrade Pollutants" (August 29).

GRANTS AWARDED:

GREGORY J. BUGBEE Received a grant for **$43,500** from the CT DEEP Aquatic Invasive Species Fund to survey areas around boat launch ramps for hydrilla.

SUMMER STEBBINS received grant funding from the United States Fish and Wildlife Service to perform a "Comparative Analysis of AIS Mapping Techniques on Seasonal Abundance and Distribution of *Hydrilla verticillata* ssp. *lithuanica* at Connecticut River Boat Ramps" **$44,876.42**

PUBLICATIONS:

1. Ruiz-Carrascal, D., Bastard, J., **Williams, S. C.,** and Diuk-Wasser, M. (2024). Modeling platform to assess the effectiveness of single and integrated *Ixodes scapularis* tick control methods. *Parasites & Vectors* 17, 339. DOI: 10.1186/s13071-024-06387-2

Abstract: Background - Lyme disease continues to expand in Canada and the USA and no single intervention is likely to curb the epidemic.
Methods- We propose a platform to quantitatively assess the effectiveness of a subset of *Ixodes scapularis* tick management approaches. The platform allows us to assess the impact of different control treatments, conducted either individually (single interventions) or in combination (combined efforts), with varying timings and durations. Interventions include three low environmental toxicity measures in differing combinations, namely reductions in white-tailed deer (*Odocoileus virginianus*) populations, broadcast area-application of the entomopathogenic fungus *Metarhizium anisopliae*, and fipronil-based rodent-targeted bait boxes. To assess the impact of these control efforts, we calibrated a process-based mathematical model to data collected from residential properties in the town of Redding, southwestern Connecticut, where an integrated tick management program to reduce *Ixodes scapularis* nymphs was conducted from 2013 through 2016. We estimated parameters mechanistically for each of the three treatments, simulated multiple combinations and timings of interventions, and computed the resulting percent reduction of the nymphal peak and of the area under the phenology curve.
Results- Simulation outputs suggest that the three-treatment combination and the bait boxes–deer reduction combination had the overall highest impacts on suppressing *I. scapularis* nymphs. All (single or combined) interventions were more efficacious when implemented for a higher number of years. When implemented for at least 4 years, most interventions (except

the single application of the entomopathogenic fungus) were predicted to strongly reduce the nymphal peak compared with the no intervention scenario. Finally, we determined the optimal period to apply the entomopathogenic fungus in residential yards, depending on the number of applications.
Conclusions- Computer simulation is a powerful tool to identify the optimal deployment of individual and combined tick management approaches, which can synergistically contribute to short-to-long-term, cost-effective, and sustainable control of tick-borne diseases in integrated tick management (ITM) interventions.

2. Hillen, A. P., **Foley, J. R. IV**, Gross, A. D., Mayfield, A. E. III, Williams, J., Xia, K. et al. (2024). The impact of imidacloprid on the subterranean survivorship of *Laricobius* (Coleoptera: Derodontidae), a biological control agent of *Adelges tsugae* (Hemiptera: Adelgidae). *Agricultural and Forest Entomology*, 1–11. DOI: 10.1111/afe.12647

Abstract: 1. The invasive hemlock woolly adelgid (HWA), *Adelges tsugae* (Annand) (Hemiptera:Adelgidae), has spread throughout most of the range of eastern hemlock, *Tsuga canandensis* (L.), and the entire range of Carolina hemlock, *Tsuga caroliniana* (Engelman), in the United States.
2. Integrated pest management (IPM) of HWA combines chemical applications with the release of biological control agents on untreated trees within the same stand. *Laricobius* spp., Rosenhauer (Coleoptera: Derodontidae), have been used as biological control agents of HWA since 2003 and have subterranean and arboreal life phases that are synchronous with HWA's lifecycle. When utilizing IPM tactics, there is potential for *Laricobius* spp. to settle below an insecticide-treated tree for its subterranean phase.
3. Field investigations assessed the impact of historical (five years post treatment in 2017) and recent imidacloprid soil treatments (via soil injection, soil drench, and tablet in November 2020) on the subterranean survivorship of *Laricobius* spp. by quantifying concentrations of imidacloprid and its metabolites to determine its potential impact on percent adult emergence from the soil.
4. We observed a significant treatment effect on mean soil concentration among application methods at the recent treatment site, but not the historical treatment site. Additionally, at the more recently treated site, significantly lower mean percent emergence was observed from soil drench and tablet imidacloprid applications after one year (2021), but by the following year (2022), that effect was no longer present.
5. This study supports recent recommendations to delay releases of *Laricobius* spp. for one-year post-treatment with imidacloprid. Furthermore, these data suggest when applying imidacloprid to a stand established with *Laricobius* spp., soil injection techniques pose the least risk to their subterranean survivorship.

NEW STUDENTS, STAFF, AND VOLUNTEERS:

Alice Zhou, Ph.D. joined the Department of Environmental Science and Forestry as a Postdoctoral Scientist in August. She will be working under **Itamar Shabtai, Ph.D.**, and her research will focus on characterizing how calcium amendments affect soil organic carbon cycling and sequestration across a range of soil types. **Dr. Zhou** graduated from the University of Michigan in July with a Ph.D. in Earth and Environmental Sciences, where she studied microbial iron cycling and biomineralization in conditions simulating Earth's oceans before the

rise of atmospheric oxygen. In her free time, Alice enjoys running, camping, and playing Magic: The Gathering.

Drs. Elisabeth Ward and Helen Poulos (Wesleyan University) demonstrate how to collect tree cores to assess non-structural carbohydrates in beech trees affected by Beech Leaf Disease to a high school student.

New Haven Promise interns and Yale Masters student mentored by **Elisabeth Ward, Ph.D.** attend Plant Science Day.

Dr. Alice Zhou

PLANT PATHOLOGY AND ECOLOGY

LINDSAY TRIPLETT, PH.D. hosted representatives from Bayer, Ginko Bioworks, Eco-Tech, and Biosafe systems for a Zoom career panel for undergraduate interns on industry jobs in plant health (August 5th, 15 adults); coordinated and hosted the final research symposium for the 7th year of the Plant Health Fellows program (August 8th, 62 adults in person and virtually); hosted Lt. Governor Susan Bysiewicz and Hamden Mayor Lauren Garrett for a tour of Lockwood Farm on Plant Science Day (2 adults) (August 7th), and presented a talk and poster on the funded CAES project titled "Defining the taxonomy, function, and plant health impact of protist-associated bacteria in the rhizosphere" at the Project Director Meeting of the USDA-NIFA Agricultural Microbiomes program in St. Louis, MO (46 adults) (August 22); and presented at a mini-symposium for the UMass REEU internship program on research on agricultural microbiomes (August 26, 10 adults).

YONGHAO LI, PH.D. participated in the National Plant Diagnostic Network Northeast Reginal meeting via Zoom (August 8, 16 adults); participated in the National Plant Diagnostic Network Online Communication & Web Portal Committee meeting via Zoom (August 14, 8 adults).

ROBERT MARRA, PH.D., as Division Forum Representative for the Northeastern Division of American Phytopathological Society, participated in the Divisional Forum monthly meeting. (5 adults) (August 27)**.** With Adam Argraves, performed remeasurements of Long-term BLD Monitoring Plots at Centennial Watershed, Easton (August 15); Naugatuck State Forest, Portland (August 16); Great Mountain Forest, Norfolk, and Tunxis State Forest, Barkhamsted (August 21); MDC Reservoir #6, West Hartford, and Yale Myers Forest, Ashford (August 22); Nehantic State Forest, East Lyme, and Oswegatchie Hills Nature Preserve, Niantic (August 29).

FELICIA MILLETT presented "Get Crafting with Grafting: Tomato/Potato Plant" at the Technical Demonstration Tent at Plant Science Day (August 7); Presented "Identifying Native Trees and Shrubs in Connecticut" to the Garden Club of Brookfield (17 adults) (August 15); participated in the NPDN Proficiency Committee monthly meeting (9 adults) (August 20).

RAQUEL ROCHA, PH.D. presented at the CAES mini-symposium with the UMass REEU internship program in understanding nematode parasitism to design better management strategies. (August 26, 10 adults).

QUAN ZENG, PH.D. presented at a mini-symposium with the UMass REEU internship program in using nanotechnology in controlling plant bacterial diseases. (August 26).

NEW STUDENTS, STAFF, AND VOLUNTEERS:

Two PhD students, **Jewell Jung and Himanshi Jayasinghe** joined the Department of Plant Pathology on August 22, 2024. They will pursue graduate study at University of Connecticut while conducting research at CAES under the guidance of **Dr. Quan Zeng.** Jewell is originally from Washington and received her Bachelor of Science from Oregon State University;

Himanshi is from Shri Lanka and received her Master of Science from National Taiwan University. Their research topics are yeast-apple-fire blight interactions and interspecies bacterial communication in rhizosphere, respectively. **Photo: left: Jewell Jung; right: Himanshi Jayasinghe**

PUBLICATIONS:

1. Wang, N., Sundin, G. W., De La Fuente, L., Cubero, J., Tatineni, S., Brewer, M. T., **Zeng, Q.**, Bock, C. H., Cunniffe, N. J., Wang, C., Candresse, T., Chappell, T., Coleman, J. J., Munkvold, G. (2024) Key Challenges in Plant Pathology in the Next Decade. *Phytopathology* 114:837-842 https://doi.org/10.1094/PHYTO-04-24-0137-KC

Abstract: Plant diseases significantly impact food security and food safety. It was estimated that food production needs to increase by 50% to feed the projected 9.3 billion people by 2050. Yet, plant pathogens and pests are documented to cause up to 40% yield losses in major crops, including maize, rice, and wheat, resulting in annual worldwide economic losses of approximately US$220 billion. Yield losses due to plant diseases and pests are estimated to be 21.5% (10.1 to 28.1%) in wheat, 30.3% (24.6 to 40.9%) in rice, and 22.6% (19.5 to 41.4%) in maize. In March 2023, The American Phytopathological Society (APS) conducted a survey to identify and rank key challenges in plant pathology in the next decade. Phytopathology subsequently invited papers that address those key challenges in plant pathology, and these were published as a special issue. The key challenges identified include climate change effect on the disease triangle and outbreaks, plant disease resistance mechanisms and its applications, and specific diseases including those caused by Candidatus Liberibacter spp. and Xylella fastidiosa. Additionally, disease detection, natural and man-made disasters, and plant disease control strategies were explored in issue articles. Finally, aspects of open access and how to publish articles to maximize the Findability, Accessibility, Interoperability, and Reuse of digital assets in plant pathology were described. Only by identifying the challenges and tracking progress in developing solutions for them will we be able to resolve the issues in plant pathology and ultimately ensure plant health, food security, and food safety.

2. Taerum, S. J., Patel, R. R., Micciulla, J., **Steven**, **B.,** Gage, D., and **Triplett, L. R.** Establishment and effect of a protist consortium in the maize rhizosphere. *Phytobiomes early access*; https://doi.org/10.1094/PBIOMES-04-24-0035-R

Abstract: Protists are abundant in the rhizosphere, and protist inoculation onto plants has revealed protist roles in plant growth and development, disease suppression, and relationships with plant beneficial bacteria. In this study, we evaluated the establishment of an 18-member defined protist consortium after inoculation onto maize seedlings, along with the effects on the bacterial populations in the rhizosphere. Inoculated protists established a community that could be detected in the rhizosphere three weeks after inoculation. Plants inoculated with the

protist consortium developed a bacterial community structure similar to that of plants inoculated with protist-free rhizosphere bacteria, but different from that of uninoculated plants. This indicates that the protist cultures introduced rhizosphere-competent bacteria to the seedling. We also observed a plant growth phenotype, as inoculation with rhizosphere bacteria suppressed plant growth in the absence, but not the presence, of protists. Maize inoculated with an antibiotic-treated protist had lower biomass than plants inoculated with an untreated protist, indicating that growth promotion by protists depended on bacteria in the protist culture microbiome. This study shows that inoculation of germinated seeds establishes protist and bacterial communities on maize roots, and that plant detrimental effects of rhizosphere bacteria can be mitigated by the presence of protists.

3. Schultes, N. P., Sinn, J. P., Swenson, E. S., McNellis, T. W. Synthesis of aspartic acid and tyrosine by the fire blight pathogen *Erwinia amylovora* is not required for proliferation on apple flower stigmas or virulence in fruitlets. *J. Appl. Microbiol*. 135(8); https://doi.org/10.1093/jambio/lxae185

Abstract: Aims- The gram-negative bacterium *Erwinia amylovora* (*Ea*) is the causal agent of fire blight, a devastating disease of apples and pears. In the fire blight disease cycle, *Ea* grows in different plant tissues, each presenting a distinct nutrient environment. Here, we investigate the ability of aspartate and tyrosine double auxotroph *Ea* lines to proliferate on apple flower stigma surfaces representing the epiphytic growth stage of *Ea* and in developing fruitlets representing one endophytic growth stage of *Ea*.
Methods and Results- Heterologous complementation studies in an *Escherichia coli* aspartate and tyrosine auxotroph verify that *Ea*AspC and *Ea*TyrB act as aspartate and tyrosine amino transferases. Growth analysis reveals that *Ea aspC tyrB* mutants multiply to near wild type levels on apple flower stigmas and immature fruitlets.
Conclusions- *Ea aspC* and *tyrB* are reciprocally complementing for aspartate and tyrosine synthesis in *E. coli* and in *Ea*. *Ea aspC* ~~and~~ *tyrB* mutants obtain sufficient aspartate and tyrosine to support multiplication on stigma surfaces and virulence in immature fruitlets.
Impact Statement: *Ea* auxotrophic mutants are used to define nutritional landscapes in and on host tissues, typically as single mutations affecting specific metabolites. Due to the complex, interconnected genetics of aspartate and tyrosine biosynthesis, these metabolites cannot be probed using single mutants. We used double mutants to determine the sufficiency of aspartate and tyrosine on host stigmatic surfaces and fruitlets.

VALLEY LABORATORY

DEWEI LI, PH.D. participated in the 12th International Mycological Congress in Maastricht, the Netherland, August 10-16 and made a presentation 'A *Scytalidium*-like indoor fungus revealing polyphyletic relationships in *Scytalidium'* (86 attendees).

JATINDER S AULAKH, PH.D. attended the Connecticut Christmas Tree Growers' annual fall field day and gave a talk on identification and postemergence control of black swallow-wort, Japanese hops, porcelain berry, and mile-a-minute in Christmas trees at Castle Hill Christmas Tree Farm – 5 Hattertown Road, Newtown, CT (August 3, 2024, ~ 85 attendees); and presented a poster on "Nonchemical Control of Mugwort" at the annual plant science day at Lockwood research farm in Hamden, CT (August 7, 2024, ~70 visitors); and attended the international Christmas Tree Research and Extension meeting in Kerteminde, Denmark and gave a talk on "Weed control efficacy and Fraser x balsam Hybrid Tolerance to Topramezone Herbicide" (August 10 - 18, 2024, ~70 attendees).

CAROLE CHEAH, PH.D. toured a Simsbury Land Trust property to assess HWA and biological control needs with 3 volunteers June 7; gave a demonstration of biological control releases for New Hartford Land Trust volunteers (9), assessed a number of properties and released *S. tsugae* for HWA management July 14; met with CT DEEP staff (6) and assessed hemlocks for HWA at Dinosaur State Park in Rocky Hill July 11, returning to guide releases of donated ladybeetles on July 26 (4); gave the annual Ted Byers Lecture on hemlock woolly adelgid at the Doolittle Lake Clubhouse, Norfolk, August 3 (35); through a July donation of *S. tsugae* from Tree Savers, PA, released hundreds of *S. tsugae* at Dean's Ravine, Housatonic State Forest; Black Spruce Bog Natural Area, Mohawk Mountain State Park and Lake Winchester, August 4; Sunnybrook State Park, August 5; Salmon River State Forest, August 13; Lost Pond, Algonquin State Forest August 14; and along Falls Brook, Tunxis State Forest with a CT DEEP state forester August 20.
Gave an interview August 9, to Jennifer Ahrens, Connecticut Public Radio, on Connecticut's HWA biological control program:
https://www.ctpublic.org/news/2024-09-04/connecticut-hemlock-woolly-adelgid

RICHARD COWLES, PH.D. presented "How to interpret pesticide labels, and usefulness of controlled flow valves," to the CT Christmas Tree Growers' Association fall meeting, Aug. 3, Newtown, CT (63 attendees). He spoke about "Needle retention" and "Phytophthora root rot" at the MA Christmas Tree Growers' Association, Aug. 4, Fitchburg, MA (80 attendees). He discussed "A bioassay method for treatments to prevent needle loss due to ethylene response," to the 16th International Christmas Tree Research and Extension Conference, Kerteminde, Denmark, Aug. 12 (60 attendees). He was the keynote speaker for the Exotic Conifer Association Meeting, East Burke, VT (25 attendees), presenting "Climate change and its impacts on conifers: 2025 – 2050" on Aug. 23 and "Insects and diseases as our climate changes: threats to our success" on Aug. 24. He is receiving a grant of $24,653 from the Christmas Tree Promotion Board to support his project "Bioassays to test pre-and post-harvest chemistry to prevent needle loss".

NATE WESTRICK, PH.D. presented a poster at entitled "Protecting Connecticut Strawberries from Invasive Fungal Pathogens" at the Connecticut Agricultural Experiment Station (CAES) Plant Science Day (743 Attendees)(August 7); presented an update on white mold and strawberry disease research to the CAES Board of Control (9 attendees)(August 7); participated in the quarterly project-wide meeting of the Boxwood Blight Insight Group (BBIG) to discuss updates in research on boxwood blight (18 Attendees)(August 21)

PUBLICATIONS:

1. Xie, J. – Y., Li, H., Wan, Y., **Li, D.- W.**, and Zhu, L. – H. (2024). Shoot blight of *Pinus bungeana* caused by *Diplodia sapinea* newly reported in China. *Crop Protection* 181, 106711. https://doi.org/10.1016/j.cropro.2024.106711

Abstract: *Pinus bungeana* is a rare and endangered plant in China with high ornamental value. In September 2022, *P. bungeana* shoot blight was discovered in Changzhou, Jiangsu, China. This disease led to the chlorosis, withering and death of the stems and needles of shoot tips of the host. It seriously affected the growth of plants. The fungus was identified to be *Diplodia sapinea*, based on morphological characteristics and multilocus phylogenetic analyses of ITS region (the internal transcribed spacer) and EF gene (elongation factor 1-alpha). The pathogenicity test was carried out by inoculating the tips of the shoots using conidial suspension, and the Koch's postulates were confirmed. This is the first report of *P. bungeana* blight caused by *D. sapinea* in the world.

2. Castañeda-Ruiz, R. F., Mardones, M.,. Primo, M. G. A., **Li, D.- W.**, Kirk, P. M., Gusmão, L. F. P. (2024). New and validated hyphomycetes taxa described by A. Rambelli from Costa Rica. *Phytotaxa* 664 (2), 151-154. https://doi.org/10.11646/phytotaxa.664.2.8

Abstract: *Rambellia nodosa* gen. & sp. nov. and *Anabeltraniomyces maximus* gen. & sp. nov. are described as replacements of the two invalid species described in *Dendryphion* and *Beltrania*. The validation and orthographical corrections of the invalid genus *Parabeltrania* and species of the genera *Gyrothrix*, *Hansfordiellopsis*, *Hemibeltrania* and *Subulispora* are proposed.

3. Westrick, N. and **Salvas, M. R.** (2024). First Report of strawberry anthracnose crown rot caused by *Colletotrichum siamense* in New England. *Plant Dis.* 2024 Aug 15. doi:

10.1094/PDIS-04-24-0935-PDN. Epub ahead of print.

Abstract: In the summer of 2023, the Connecticut Agricultural Experiment Station was contacted by a farm in southern Connecticut due to reports of strawberry (*Fragaria* × *ananassa*) plants showing signs of severe wilting and crown rot across multiple fields, covering ~20 hectares. Cut crowns from diseased plants had marbled red and white lesions typically associated with anthracnose crown rot (ACR). Symptomatic plants were collected from five June-bearing cultivars (cvs. AC Valley Sunset, Lyla, Dickens, and Allstar) spanning four non-adjacent fields with incidence ranging from 5-90% and severity ranging mild wilting in low incidence fields to severe wilting/mortality in high incidence fields. Internal tissue from diseased crowns was surface sterilized in 0.6% NaOCL for 3 minutes, rinsed with sterile water, and plated on potato dextrose agar. After one-week, hyphal tips of fungi were transferred to fresh plates which formed dense mycelial mats of fluffy, greyish-white hyphae. Orange spore masses formed near the center of the colonies, each of which contained numerous cylindrical and fusiform straight conidia, matching spores within the genus *Colletotrichum* (De Silva et al. 2019). Average conidia (n=192) length was 15.7 ± 1.6 µm and width was 5.4 ± 0.7 µm. Fungi matching this morphology were isolated from 83% of the collected symptomatic crowns and hyphae were collected from two isolates, CT5-1 and CT23-1, for DNA extraction using the GeneJET Plant Genomic DNA Purification Kit. PCR was performed using primers targeting actin (*ACT*), calmodulin (*CAL*), β-tubulin (*TUB2*), GAPDH (*gpdA*), and ITS, followed by Sanger sequencing, which yielded identical sequences for both isolates (CT5-1 Accessions numbers: PP002078-81, OR999066)(Carbone and Kohn 1999; Hassan et al. 2018; Templeton et al. 1992). These were combined with sequences from fourteen *Colletotrichum* genomes, all of which were aligned, trimmed, and concatenated using Mega11 (Tamura, Stecher, and Kumar 2021). Model selection was conducted using IQ-TREE and selected parameters were used to generate maximum-likelihood trees from all five loci individually and the concatenated sequence, all of which placed the isolates in a high confidence cluster with *Colletotrichum siamense* (Nguyen et al. 2015). To confirm the pathogenicity of the pathogen, strawberry plants (cv. Jewel) (n=5) five weeks after bare root transplant were infected. The base of each crown was penetrated 5 mm deep with a sterile 20 µL pipette tip and then inoculated with 10 µL of spores at a concentration of 106 spores/mL. Control plants (n=5) were inoculated with 10 µL of sterile water. Plants were maintained at 30°C day (16-hour)/20°C night (8-hour) in a growth chamber and assessed after 14-days. Four of the five inoculated plants had visible wilt symptoms and bisected crowns revealed the marbled red and white lesions typifying ACR. Control plants had no clear wilting and bisected crowns were visibly healthy. *C. siamense* re-isolated from infected tissue presented with identical hyphal /spore morphology and ITS/*Tub2* were re-amplified and sequenced, yielding identical sequences to CT5-1. Plant inoculations with the same variety were repeated, yielding identical symptom development and crown lesions. *C. siamense* has been a dominant source of ACR throughout the southeastern US but has not previously been a major problem in the Northeast. Given the extent of the field infection, it is likely that these isolates can survive the colder winter temperatures of New England, but further experimentation is necessary to determine the extent of the pathogen's winter hardiness.

4. Huo, D.*, **Westrick, N. M.***, Nelson, A., Kabbage, M., and Koch, P. (2024). The role of oxalic acid in *Clarireedia jacksonii* virulence and development on creeping bentgrass. *Phytopathology* 2024 Aug 15. doi: 10.1094/PHYTO-03-24-0094-R. Epub ahead of print.

Abstract: Dollar spot is a destructive foliar disease of amenity turfgrass caused by the fun-

gus *Clarireedia* spp., and mainly *Clarireedia jacksonii* on the northern US region's cool-season grass. Oxalic acid (OA) is an important pathogenicity factor in related fungal plant pathogens such as *Sclerotinia sclerotiorum*, however, the role of OA in the pathogenic development of *C. jacksonii* remains unclear due to its recalcitrance to genetic manipulation. To overcome these challenges, a CRISPR/Cas9-mediated homologous recombination approach was developed. Using this novel approach, the oxaloacetate acetylhydrolase (*Oah*) gene that is required for the biosynthesis of OA was deleted from *C. jacksonii* wild-type stain. Two independent knockout mutants, Δ*Cjoah-1* and Δ*Cjoah-2*, were generated and inoculated on potted creeping bentgrass along with a wild-type isolate (WT) and a genome sequenced isolate LWC-10. After 12 days, bentgrass inoculated with the mutants Δ*Cjoah-1* and Δ*Cjoah-2* exhibited 59.41% lower dollar spot severity compared to the WT and LWC-10 isolates. Oxalic acid production and environmental acidification were significantly reduced in both mutants when compared to the WT and LWC-10. Surprisingly, stromal formation was also severely undermined in the mutants in vitro, suggesting a critical developmental role of OA independent of plant infection. These results demonstrate that OA plays a significant role in *C. jacksonii* virulence and provide novel directions for future management of dollar spot.

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