FLORICULTURE AND NURSERY RESEARCH INNITIATIVE (FNRI)



FY2020 ANNUAL REPORTS

Floriculture and Nursery Research Initiative

FY 2020 Annual Reports

TABLE OF CONTENTS	Page
FNRI Summary	4
GERMPLASM	
Germplasm Resources for the Floriculture and Nursery Industry at the Ornamental Plant Germplasm Center (OPGC). [Pablo Jourdan]	9
Evaluation of Native and Underutilized Germplasm for Nursery and Landscape Use. [Nick Gawel and Lisa Alexander]	11
BIOTECHNOLOGY	
Improvement of Floricultural Plants through Gene Editing [Gan-Yuan Zhong]	14
Development of Transgene-free Approaches for Gene Editing in Landscape Plants. [Wusheng Liu, Thomas Ranney, Hamid Ashrafi, Darren Touchell, and Fred Gouker]	16
GREENHOUSE AND NURSERY PRODUCTION TECHNOLOGY	
Advancing Pest Management in Floriculture Crops Through Applications of Innovative Technologies. [Christian Nansen and Dan Kluepfel]	19
Improved Pest Control Application Technologies for Sustainable Crop Protection. [Heping Zhu]	22
ECONOMICS AND IMPACTS	
Strategies for Carbon Sequestration and Reducing Greenhouse Gas Emissions from Nursery Production Systems. [Stephen Prior, Brett Runion, Anna-Marie Murphy, Dave Williams, and Allen Torbert]	25
PESTS AND WEEDS	
Evolving and integration of management plans for major taxonomic groups and potential new invasive pests of ornamental and floriculture production. [Lance Osborne and Cindy McKenzie]	27
Identification of Methods for Quarantine Certification of Imported Fire Ant in Nursery Production Systems and Establishment of Biological Control Agents to Reduce Fire Ant Populations. [Jason Oliver, Karla Addesso, and Lisa Alexander]	31

TABLE OF CONTENTS continued	Page
DISEASE	
Improved Pathogen Detection and Management of Bulb Diseases. [Gary Chastagner and Dilip Laksham]	35
Discovery and Management of Diseases of Floriculture Crops. [Margery Daughtrey and Melanie Filiatrault]	38
New Technologies that Manage Profit-Limiting Diseases with a Focus on Botrytis. [Mary Hausbeck]	42

SUMMARY

The purpose of this report is to compile and update the yearly progress made by research directed by the Floral and Nursery Initiative funds located at USDA-ARS headquarters.

History of USDA-ARS Floriculture and Nursery Research Initiative (FNRI)

The ARS Floriculture and Nursery Research Initiative (FNRI) was presented to congress in 1998 by the American Nursery Landscape Association and Society of American Florists (SAF). The initiative was the culmination of five years of surveys and discussion among the green industry, academic researchers, and USDA Agricultural Research Service (ARS) to identify priority research goals and key projects in environmental horticulture. FNRI was first funded in FY 1999 with \$1 million in congressional appropriated funding to ARS. Since that time there have been six additional funding increases for more than \$6 million, although actual appropriated dollars have been adjusted for rescissions, sequestration, and other changes to federal base funding. FNRI uses a model of tripartite coordination between ARS Office of National Programs, commodity groups (AmericanHort and the American Floral Endowment), and university cooperators to set research priorities each year. Over the years, some FNRI funds have been moved to ARS base funds in order to hire permanent ARS scientists for FNRI objectives, mostly at the U.S. National Arboretum (USNA). The remaining funds are distributed to floral and nursery researchers on a yearly basis as determined by ARS Office of National Programs in consultation with AmericanHort and the American Floral Endowment. ARS leads floriculture and nursery research projects at 22 locations in the U.S. representing more than \$37 million in ongoing research efforts. FNRI funding represents a substantial portion of the overall ARS efforts and a unique public-private partnership funded through ARS. FNRI plays a critical role in generating scientific research on high-priority issues that affect all segments of the floral and nursery industries including post-harvest technology, water quality and pest and disease management. In FY2020, the total amount allocated for FNRI at ARS headquarters was \$2,862,590. This amount does not include FNRI funding that has been converted in ARS base funds.

Given the unprecedented events of the last year and the impacts of COVID19 on research, the following reports represent only a fraction of the FNRI research in FY2020.

FNRI-funded researchers presented their results in FY 2020 at:

- Scientific Conferences and Workshops 30 times (85 in FY 2019 and 78 in FY 2018)
- Grower Expos, Training, and Educational Workshops 2 times (48 in FY 2019 and 47 in FY 2018)
- Master Gardeners and Garden Clubs 11 times (4 in FY 2019 and 6 in FY 2018)
- Of these presentations, 1 was made outside the U.S. (9 in FY 2019 and 17 in FY 2018)

FNRI-funded researchers published their results in FY 2020 in:

- Peer-reviews journals 31 times (42 times in FY 2018, 89 in FY 2019)
- 4 peer-reviewed Proceedings and Abstracts (6 in FY 2019 and 7 in FY 2018)
- 9 Popular Press articles, Newsletters, Blogs, and Reference Guides (20 in FY 2019 and 15 in FY 2018)
- 3 Books (5 in FY 2019 and 4 in FY 2018)

- 2 Dissertations (2 in FY 2019 and 1 in FY 2018)
- FNRI funding was used to train 32 graduate students and postdocs (28 in FY 2019), as well as fund 9 undergraduate and technical help in FY 2020 (44 in FY 2019). One international visiting scientists was funded in part by FNRI. Three undergraduate students (16 in FY 2019) and 8 graduate students and postdocs (9 in FY 2019) participated in FNRI research without receiving funding.
- FNRI funding is currently being leveraged for \$490,910 in **competitive grants and unrestricted funding** (\$2.534 million in FY 2019 and \$2.375 million in FY 2018).

FY 2020 FNRI Funding went to ARS and university scientists for research on the projects summarized below.

Germplasm Collections and Evaluations, \$375,000

- 1. Pablo Jourdan, Department of Horticulture and Crop Science, at The Ohio State University, Columbus, OH and James Altland, ARS at Wooster, OH. The project is titled "Germplasm resources for the floriculture and nursery industry at the Ornamental Plant Germplasm Center (OPGC)" and the goals are to acquire, document, maintain, characterize, and distribute herbaceous ornamental genetic resources and associated information for conservation and scientific research.
- 2. Nick Gawel, Tennessee State University, McMinnville, TN, and Lisa Alexander, ARS, McMinnville, TN. The project is titled "Evaluation of native and underutilized germplasm for nursery and landscape use". Research includes replicated trials to develop, and evaluate improved germplasm of species native to the Southeastern US to expand market opportunities and decrease the environmental footprint of nursery production. Evaluations will emphasize disease incidence and tolerance, production value, ornamental merit, and potential for breeding improvement.

Biotechnology, \$367,798

- David Clark and Thomas Colquhoun from the University of Florida and Joe Patt, ARS, Ft. Pierce, FL. The project is titled "Basic Mechanisms of Plant Metabolism: Biotechnology Approaches for Manipulating Plant Biochemistry". The project focuses on Lilium and Caladium to understand plant metabolism and the production of fragrance, and to manipulate plant metabolism through environmental effects or gene editing.
- 2. Gan-Yuan Zhong, ARS, Geneva, NY. The project is titled "Improvement of Floricultural Plants through Gene Editing" and focuses on developing and applying CRISPR-based gene editing technologies for the improvement of floricultural plants. *New in FY 2020*
- 3. Wusheng Liu, Thomas Ranney, Hamid Ashrafi, and Darren Touchell at North Carolina State University, and Fred Gouker, ARS, Beltsville, MD. The project is titled "Development of transgene-free approaches for gene editing in landscape plants". The projects focuses on developing DNA- free genome editing techniques to avoid the presence of transgenes.
- 4. Michelle Jones, The Ohio State University and James Altland, ARS, Wooster, OH. The project it titled "Evaluating the Influence of Beneficial Microbes on Crop Quality in the

Nursery and Greenhouse Industry". Research focuses on screening cooperator's microbe collection for bacteria that can increase nutrient and water use efficiency and increase plant stress tolerance in cut flowers. *New in FY 2020*

5. Anna Whitfield, North Carolina State University and Scott Adkins, ARS, Fort Pierce, FL. The project is titled "Development of a Novel Control Strategy for Thrips and Tospoviruses in Floral Crops". The goal of this project is to develop new and effective technologies for management of tomato spotted wilt virus, including altered Bt toxins that are effective against the western flower thrips insect, which moves the virus around, and dsRNA technologies.

Greenhouse and Nursery Production Technology, \$623,000

- 1. Christian Nansen, University of California, Davis and Dan Kluepfel, ARS, Davis, CA. The project is titled "Advancing Pest Management in Floriculture Crops Through Applications of Innovative Technologies". The project improves monitoring for detection and diagnosis of emerging arthropod pest outbreaks using images, machine learning, and robotics. It also tests novel chemistries and best practices in floral and nursery production.
- 2. Heping Zhu, ARS, Wooster, OH. The project is titled "Improved Pest Control Application Technologies for Sustainable Crop Protection". The goal of this project is optimize an intelligent-decision air-assisted spraying system to increase pesticide application efficiency and minimize environmental impact for ornamental nursery crops.
- 3. Erik Runkle and Roberto Lopez, Michigan State University, and James Altland, ARS, Wooster, OH. The project is titled "Using Lighting Technologies and Genetics to Improve Growth and Flowering of Floriculture Crops". The goal of this project is to understand the impact of blue light and far-red radiation on growth attributes and subsequent flowering of common annual bedding plant seedlings grown indoors and determine LED supplemental lighting.
- 4. James Faust, Clemson University and James Altland, ARS, Wooster, OH. The project is titled "Optimizing Water Use in Floriculture Crops". The goal of this project is to reduce water use in propagation of vegetative annuals for the purpose of improved environmental stewardship and improved production efficiency.
- 5. Paul Fisher, University of Florida and James Altland, ARS, Wooster, OH. The project is titled "Resource Efficiency in the Floriculture Production and Value Chain". The goal of this project is produce grower decision support tools that improve resource use efficiency at each step of production and distribution of floriculture crops from propagation of the seed or unrooted cutting to a finished plant at retail.
- 6. John Erwin, University of Minnesota and James Altland, ARS, Wooster, OH. The applied focus of the work is to reduce energy inputs, reduce labor inputs, improve crop yield via environmental manipulation, and reduce chemical inputs by combining chemicals to produce synergistic responses.
- 7. Jim Owen and James Altland, ARS, Wooster, OH. The project is titled "Development of Technologies and Strategies for Sustainable Crop Production in Containerized and Protected Horticulture Systems". Research supports new pine-based substrates for container nursery production that are engineered to reduce water use, lower agrochemical inputs, and minimize crops stress and time to market.

- 8. Ariana Torres, Purdue University; Rosa Raudales, University of Connecticut; Dewayne Ingram, University of Kentucky; and Jennifer Boldt, ARS Toledo, OH. The project is titled "Strategies and technologies to optimize resources in controlled environment agriculture". This project proposes to measure energy, water, carbon, and nutrient benchmarks for growers to compare resource efficiency at their operations with that of the industry and competitors using data collected from commercial greenhouses. Benchmarks will help growers evaluate their internal use of resources and environmental impact (using the Water Footprint model and Life Cycle Assessment).
- 9. Neil Mattson, Cornell University, and Jennifer Boldt, ARS, Toledo, OH. Project is titled "Optimizing Crop Production while Reducing Energy Use through Improved Lighting and Greenhouse Control Systems". This research focuses on reducing energy use through improved lighting and greenhouse control systems while improving growth and flowering of floriculture crops. *New in FY 2020*

Economics and Impacts, \$250,792

- 1. Charlie Hall, Texas A&M University, and James Altland, ARS, Wooster, OH. The project is titled "Measuring Economic Efficiency in the Floriculture Production Value Chain". Research goals are to determine the morphological, physiological, quality, and economic responses of four bedding plant species to reduced substrate moisture content.
- 2. Ariana Torres, Purdue University, and Jennifer Boldt, ARS, Toledo, OH. The project is titled "Strategies and Technologies to Optimize Resources in Controlled Environment Agriculture". Research focuses on the economic feasibility of adopting water, energy, carbon, substrate, and nutrient-efficient growing practices and consumer preferences for those practices using consumer surveys to estimate the costs, benefits, and financial risks associated with adopting input-efficient strategies. This work will also assess consumers' purchasing behavior, attitudes, and preferences for "greener" plants. *New in FY 2020*
- 3. Stephen Prior, Brett Runion, and Allen Torbert, ARS, Auburn, AL; and Glenn Fain, Auburn University, Auburn, AL. The project is titled "Strategies for Carbon Sequestration and Reducing Greenhouse Gas Emissions from Nursery Production Systems". The goal of this project is to determine baseline greenhouse gas emissions for nursery systems, develop strategies to reduce emissions, and develop strategies to increase soil carbon storage.

Pests and Weeds, \$427,000

- 1. Peter Schultz, Virginia Tech, Jason Oliver and Karla Addesso, Tennessee State University with Chris Ranger and Mike Redding, ARS, Wooster, OH. The project is titled "New Strategies for Management of Invasive Ambrosia Beetles in Horticultural and Nursery Crops". The goal of this project is to adjust production practices to maximize tree health and minimize the risk of ambrosia beetle attacks by using repellents to push beetles away from vulnerable trees and attractants to pull beetles into annihilative traps.
- 2. Lance Osborne, University of Florida and Cindy McKenzie, ARS, Fort Pierce, FL. The project is titled "Evolving and Integration of Management Plans for Major Taxonomic Groups and Potential New Invasive Pests of Ornamental and Floriculture Production". The goal of this project to evaluate alternatives to neonicotinoid class of insecticides by working closely with the chemical industry to identify registered pesticides and those

near registration that could fill the void created if the neonicotinoid class is banned.

- 3. Jason Oliver and Karla Addesso, Tennessee State University, and Lisa Alexander, ARS, National Arboretum, McMinnville, TN. The project is titled "Identification of Methods for Quarantine Certification of Imported Fire Ant in Nursery Production Systems and Establishment of Biological Control Agents to Reduce Fire Ant Populations". The goal of this project is to develop new treatment options for the control of red imported fire ants that have lower cost, longer certification periods, rapid application potential, and less worker exposure.
- 4. James Altland, ARS, Wooster, OH and Chris Marble, University of Florida. The project is titled "Weed Management Strategies to Reduce Costs, Improve Control, and Mitigate Injury to Sensitive Crops". The goal of this project is to develop new strategies for growers to deal with the declining efficacy of traditional herbicides for weed control.

Disease, \$819,000

- Gary Chastagner, Washington State University, and Dilip Laksham, ARS, Beltsville, MD. The project is titled "Improved Pathogen Detection and Management of Bulb Diseases". The goal of this project is to provide growers with the necessary tools to manage gray mold. Research is focused on a better understanding of the diversity of Botrytis species, their pathogenicity and biology, source of inoculum, and sensitivity to fungicides to improve Botrytis disease management programs.
- 2. Margery Daughtrey, Cornell University, Riverhead, NY; and Melanie Filiatrault, ARS, Ithaca, NY. The project is titled "Monitoring and Molecular Characterization of Pythium species in Floricultural Greenhouses of NY and OK". This project is focused on molecular population analyses to identify, monitor, and describe new, emerging bacterial pathogens in commercial greenhouses.
- 3. Nik Grunwald, Jerry Weiland, and Carolyn Scagel, ARS, Corvallis, OR. The project is titled "Improving plant health for nursery production in the Pacific Northwest". The Pacific Northwest nursery industry produces stock in both containers, and field operations. The goal of this project is to prevent Phytophthora and Pythium infection through an integrated approach to plant pathology, genomics and marker development, and soil health and nutrient management.
- 4. Steven Jeffers, Clemson University; Carla Garzon, Oklahoma State University, and Nik Grunwald, ARS Corvallis, OR. The project is titled "Tracking and Managing Diseases of Floriculture Crops Caused by Oomycetes and Fungi". In collaboration with the US Lavender Growers Association, the project focuses on phytophthora root and crown rot on lavender and root knot nematodes impacting field-grown lavender, and Pythium root rot in chrysanthemums and poinsettias.
- 5. Carla D. Garzon, Oklahoma State University, OK and Jerry Weiland, ARS Corvallis, OR. The goal of this research is to assess the incidence and diversity of pathogenic Pythium species in floricultural crops grown outdoor (chrysanthemum) and indoor (poinsettia, dahlia) on the same season; and monitor the movement of inoculum between outdoor and indoor crops grown simultaneously in the same floricultural operations.
- 6. Mary Hausbeck, Michigan State University and Nik Grunwald, ARS Corvallis, OR. The project is titled "Advancing Novel Tactics and Triaging Fungicide Resistance for Botrytis cinerea and other Profit-limiting Pathogens". The goal of this project is to determine disease susceptibility among cultivars of seed-geraniums and snapdragons to Pythium,

determine the host range and virulence of Pythium species that may infect greenhouse floral crops, and characterize the efficacy of new active ingredients, including reducedrisk fungicides, biocontrol agents or biopesticides.

7. Jo Anne Crouch, ARS, Beltsville, MD; Nina Shishkoff, ARS, Frederick, MD; Jerry Weiland, ARS, Corvallis, OR; and Marc Cubeta, North Carolina State University. The project is titled "Minimizing the Impact of Boxwood Blight Disease". The goal of this project is to understand the causal agent for boxwood blight and develop control strategies.

Germplasm resources for the floriculture and nursery industry at the Ornamental Plant Germplasm Center (OPGC)

Pablo Jourdan, The Ohio State University, Columbus OH

Background: Genetic resources are fundamental for continued development of ornamental crops. Availability of such resources is critical for the success of the industry. The project is designed to provide genetic resources for the Floriculture and Nursery industry through support of the Ornamental Plant Germplasm Center, whose mission is to acquire, document, maintain, characterize, and distribute herbaceous ornamental genetic resources and associated information for conservation, enhancing scientific research and the industry.

Progress and Accomplishments in FY2020:

Clonal collection: Tissue culture backup and maintenance of clonal accessions including *Begonia, Phlox, Pelargonium* and other genera is steadily being maintained and refined. The base collection is stored at 20C; wherever possible, accessions are being replicated at 4C and 10C in an attempt to slow growth and extend time between subcultures. We have established that some accessions can remain in the same container for up to 2 years at 4C, whereas the same accession would require subculture every 6 months at 20C.

Distribution of germplasm continues to be an important activity; 33 germplasm requests containing 247 items were distributed the past year; COVID-19 has had a significant negative impact on distributions since March, 2020. Since the OPGC began distributing germplasm in 2002, 1,454 orders and 9,945 germplasm items have been distributed.

Acquisitions: 588 accessions were acquired in the past year, including 577 accessions of native U.S. plants collected through the Seeds of Success program received in August 2020. The bulk of the OPGC collection now consists of native North American species.

Accessions: Currently 7,324 accessions are maintained at the OPGC; this encompasses a diverse collection of 389 genera, and 2092 taxa of 1845 species. Approximately 42% of the collection is currently available for distribution. 5,154 accessions are backed up off-site (70% of the collection). PI numbers were assigned to 176 *Pelargonium* accessions. *Begonia* accession grow-out of seeds for taxonomic confirmation continues; 15 accessions were sown, taxonomy of 5 accessions was confirmed. Other activities continue, including seed regeneration (e.g. for *Antirrhinum, Bidens* and *Verbena*) and viability testing of seeds; the 2020 growing season has been significantly affected by COVID-19 because of inability to access production fields during the pandemic.

FY 2020 Publications

Farinas, C., Jourdan, P., Paul, P.A., Peduto Hand, F. 2019. Development and evaluation of laboratory bioassays to study powdery mildew pathogens of Phlox in vitro. Plant Dis. 103(7):1536-1543. Farinas, C., Jourdan, P., Paul, P., Slot, J., Daughtrey, M., Devi Ganeshan, V., Baysal- Gurel, F., Peduto Hand, F. (2020) Phlox species show quantitative and qualitative resistance to a population of powdery mildew isolates from the eastern United States. Phytopathology -Published Online:10 Jun 2020 https://doi.org/10.1094/PHYTO-12-19-0473-R

Evaluation of native and underutilized germplasm for nursery and landscape use

Lisa Alexander, ARS, Floral and Nursery Plants Research Unit, McMinnville, TN

Nick Gawel, Tennessee State University, McMinnville, TN

Background: Maintaining the growth and profitability of the U.S. nursery industry is dependent on developing new and novel woody ornamental cultivars while protecting natural resources and worker health. Establishment of long-term experimental populations, coupled with the development of database-centered information management systems are essential to full utilization of existing genetic and genomic information for nursery crop improvement. Genomic information and new phenotyping methods applied to current breeding lines and experimental populations are needed to accelerate the production of high-value nursery crop cultivars. Native and underrepresented woody landscape species improved for product quality and disease resistance will stimulate consumer interest in landscape plants, provide producers with expanded marketing opportunities, and reduce the environmental footprint of nursery production by reducing the need for pesticides.

Funding for this project supports research on replicated trials to develop, evaluate, and release improved germplasm of species native to the Southeastern US to expand market opportunities and decrease the environmental footprint of nursery production. Trials will include but are not limited to Callicarpa, Chionanthus, Cornus, Fothergilla, Hamamelis, Hydrangea, and Viburnum. Evaluations will emphasize disease incidence and tolerance, production value, ornamental merit, and potential for breeding improvement. Disease incidence and tolerance will be evaluated both in situ and in replicated laboratory assays designed to identify and screen for diseases in juvenile stages and propagation. Methods of controlling fungal and bacterial diseases including biocontrol methods and biorational pesticides will be investigated. Evaluation for production merit will include investigations into propagation methods and substrates, insect and weed pests, and correct siting. Ornamental merit and potential for breeding improvement will be evaluated based on comparisons to industry standard cultivars, ability of plants to mate successfully within and between species, and genetic inheritance of ornamental traits.

The potential benefits expected from this project from the new and improved nursery crop cultivars will stimulate consumer interest in landscape plants, provide producers with expanded marketing opportunities, and improve the environment by reducing the need for pesticides. This research will impact other scientists, the agricultural community, the general public, nursery producers, retail garden center operators, landscapers, and public and private gardeners

Progress and Accomplishments in FY 2020

- We provided recommendations to growers for biorational products to treat botrytis blight on cut bigleaf hydrangeas. These recommendations will provide ornamental growers with a new, sustainable alternative to protect the quality and value of cut flowers and greenery.
- We identified the gene responsible for the mophead/lacecap inflorescence trait in bigleaf hydrangea and developed a molecular marker to characterize this trait quickly and efficiently in hydrangeas. This is the first marker developed for hydrangea breeding

improvement and will be used by plant scientists and plant breeders for ecological research, functional genomics, and cultivar improvement.

- We developed and used molecular markers to verify parentage of novel *Osmanthus* hybrids between *Osmanthus armatus* and other *Osmanthus* species. Hybrids are being evaluated for growth, flowering, ornamental merit, and cold-hardiness. Results of this research will provide new, cold-hardy *Osmanthus* varieties for growers and consumers.
- We completed the second year of evaluation of oakleaf hydrangea seedlings from 17 populations across the six-state native range of the species. Seedlings were evaluated for growth, disease incidence, and ornamental traits in May and October of 2020.
- We established a replicated field trial of *Osmanthus fragrans* cultivars and *Osmanthus* hybrids developed in our program to improve cold-hardiness of the species. Accessions will be evaluated for growth, flowering, and winter injury.
- We established a replicated field trial of sourwood representing ten parental populations collected across the southeast. Accessions will be evaluated for growth, flowering, foliage health, and fall color.
- We collected and propagated germplasm for a comprehensive trial of reblooming in commercially available bigleaf hydrangea cultivars.

FY 2020 Presentations

- 1. Baysal-Gurel, F. and L. W. Alexander. Susceptibility of bigleaf hydrangea cultivars and hybrids to powdery mildew disease. Plant Sciences Symposium, Tennessee State University, Nashville, TN, November 5, 2019.
- 2. Alexander, L. W. Developing superior floral and woody nursery plants at the U.S. National Arboretum. Warren County Garden Club, McMinnville, TN, March 3, 2020.
- Alexander, L. W. and F. Baysal-Gurel. Ploidy level influences powdery mildew disease severity in *Hydrangea macrophylla*. 42nd Annual Tennessee State University Research Symposium, Tennessee State University, Nashville, TN, March 23 – 27, 2020.
- Oliver, J., Addesso, K., Witcher, A., Dant, L., Youssef, N., O'Neal, P., Ojha, V., Dismukes, A., Weeks, R., and L. W. Alexander. Continuing efforts to develop chlorpyrifos replacements for fire ant quarantine certification. 42nd Annual Tennessee State University Research Symposium, Tennessee State University, Nashville, TN, March 23 – 27, 2020.
- 5. Bika, R., Simmons, T., Alexander, L. W., and F. Baysal-Gurel. *Fusarium oxysporum* causing post-harvest meltdown of Zinnia flowers. Annual Meeting of the American Phytopathological Society, Virtual, August 4, 2020.
- 6. Bika, R., Alexander, L., Palmer, C., and F. Baysal-Gurel. Efficacy of fungicides and biorational product in management of *Botrytis cinerea* in cut flower of *Hydrangea macrophylla*. Annual Meeting of the American Society for Horticultural Sciences, Virtual, August 10, 2020.
- 7. L. W. Alexander and F. Baysal-Gurel. Using AUDPC scores for disease resistance phenotyping of hydrangea. In Workshop, "Focusing on Phenotyping: Tools and Techniques for Ornamental Breeders". Annual Meeting of the American Society for Horticultural Sciences, Virtual, August 11, 2020.
- 8. L. W. Alexander and X. Wu. High-throughput phenotyping of inflorescence type in hydrangea. In Workshop, "High Throughput Phenotyping for Germplasm Selection and

Development in Vegetable Crops". Annual Meeting of the American Society for Horticultural Sciences, Virtual, August 14, 2020.

FY 2020 Publications

- Wu, X. and Alexander, L.W. 2019. Genetic diversity and population structure analysis of bigleaf hydrangea using genotyping-by-sequencing. Journal of the American Society for Horticultural Science. 63: 18-27.
- Alexander, L.W. 2020. Ploidy level influences pollen tube growth and seed viability in interploidy crosses of *Hydrangea macrophylla*. Frontiers in Plant Science. 11:100.
- Wu, X. and Alexander, L.W. 2020. Genome-wide association studies for inflorescence type and remontancy in *Hydrangea macrophylla*. Horticulture Research. 7:27.
- Alexander, L.W. and Hassler, S. 2020. Production and verification of *Osmanthus armatus* hybrids. Proceedings of the Southern Nursery Association 64:79-84.
- Bika, R., Palmer, C., Alexander, L. W., and F. Baysal-Gurel. 2020. Comparative performance of reduced-risk fungicides and biorational products in management of postharvest botrytis blight on bigleaf hydrangea cut flowers. HortTechnology. Published online 10/5/2020.

Improvement of Floricultural Plants through Gene Editing

Gan-Yuan Zhong, USDA-ARS, Grape Genetics Research Unit, Plant Genetic Resources Unit AgriTech, New York State Agricultural Experiment Station Cornell University, 630 W. North Street, Geneva, NY 14456

Background: CRISPR-based gene editing is a recently developed genomic technology which can be used to modify a gene in plants in a precise manner with no unintended genetic changes or footprint left in the genome modified. This technology offers tremendous opportunities in plant cultivar improvement, especially in modifying elite plant cultivars for which improvement of only one or few specific genes/traits is desired. Because of its potential in revolutionizing plant breeding, CRISPR-based gene editing has been actively pursued and successfully demonstrated in many model species, such as Arabidopsis and tobacco, as well as major field crops, such as wheat, rice, corn, and cotton. In contrast, exploration of the editing technology for the improvement of floricultural plants is still at the infancy stage. **The objective of this proposed study is to develop and apply CRISPR-based gene editing technologies for the improvement of floricultural plants**.

Like in many other plants, one significant challenge in developing and applying CRISPR editing technologies for the improvement of floricultural plants is to deliver various editing components into plant cells for realizing the editing. Most of the previous gene editing work was done through a transgenic approach. Such approach is acceptable for developing new plant cultivars involving some subsequent crosses in which the transgenic footprint can be segregated out. However, such transgenic approach is not desirable for improving a specific trait in an elite cultivar, especially in an elite floricultural cultivar due to its highly heterozygous nature. One subobjective in this study is to develop a transgene-free editing technology for the improvement of elite floricultural cultivars. The research field of method development for creating transgene-free edited plants continues evolving. Interesting progress has been reported in the exploration of protoplast transfection, Agrobacterium- or biolistic-mediated transient transformation, and nanoparticles to facilitate delivery of gene editing components into target cells for editing. Based on various considerations, we will focus on the Agrobacterium- and/or biolistic-mediated transient transformation approach in this study. To make the Agrobacteriumand/or biolistic-mediated transient transformation effective for generating transgene-free editing events, one must be able to select a small number of cells with the target gene edited among millions of non-edited callus cells without relying on an introduced selection marker gene. Clearly, this is very difficult. To solve this technical challenge, an idea has recently been demonstrated in wheat in which an endogenous gene for herbicide resistance was simultaneously edited along with a target gene to make the edited cells herbicide resistance. The herbicide resistance cells can easily be selected for by applying appropriate herbicides in the tissue culture medium. We have been testing this idea in grapevine and will extend the idea to floricultural plants in this study.

Another significant challenge in developing and applying CRISPR editing technologies for the improvement of floricultural plants is to optimize various editing components for

realizing efficient editing. The editing components mainly include CRISPR-associated proteins (Cas9 vs. Cas12a), gRNAs, and promoters. **One other subobjective in this study is to optimize gene editing components for editing TFL1, a flowering pathway gene, in woody peonies** (tree peonies) (*Paeonia suffruticosa*). Tree peonies are globally ornamentals with large, colorful, and various types of flowers. However, a relatively short and uniform flowering period limits the commercial opportunities of these tree peonies. TFL1 is a critical gene in plant flowering pathway and silencing the gene often results in early and continuous flowering in plants. If we could extend the flowering period of tree peonies by editing their TFL1-like genes, the economic benefits of ornamental tree peonies would be significantly increased. Because tree peonies are difficult to transform, we will first explore a suitable transformation method for the species.

Progress and Accomplishments in FY 2020

This project was initiated about 3 months ago. The following progress has been made.

• We have made significant progress in evaluating a dozen of mutant versions of the ALS gene for herbicide resistance in grapevine. This is an important step for exploring the co-editing strategy for selection of edited cells among millions of non-edited ones. The concept, once proven successful, will be extended to floricultural plants.

• We have identified and done initial analysis of TFL1- like genes in tree peonies. We have also identified a collaborator who will collaborate with us on tree peony transformation.

Development of transgene-free approaches for gene editing in landscape plants

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Darren Touchell, Horticultural Science Department, North Carolina State University, Mills River, NC

Fred Gouker, USDA-ARS, FNPRU, Bldg. 010A Beltsville, MD

Background: The nursery and floricultural industries are facing pressing problems such as disease resistance and management, invasiveness, and the need to develop advanced breeding methods to modify or edit gene expression, which are both threats and opportunities for future growth, profitability and sustainability of nursery/floriculture crops. As many of these crops are highly heterozygous and propagated asexually, in many cases it would be desirable to develop DNA- free genome editing techniques to avoid the presence of transgenes. To address these issues, we propose to develop bioengineering pipelines for specific landscape crops with high economic value to provide the most immediate impacts for crop improvement. To achieve that goal, our objective is to develop bioengineering pipelines for Rosa and Tripidium as model crops. We will focus on transgene-free approaches for gene editing using Agrobacterium-mediated plant transformation and subsequent breeding and/or other approaches. These systems will have broad utility and provide a foundation for developing improved crops with enhanced disease and insect resistance, non-invasiveness, unique phenotypes, and greater commercial potential. Transgene-free end products will be realized by segregating out transgenes with selective breeding or with the use of DNA-free delivery systems.

Progress and Accomplishments in FY 2020

- We are developing a regeneration/transformation system for rose cultivars. We have completed experiments to optimize regeneration systems on three cultivars. Davis Harmon has started his MS research on this project. Experiments to optimize *Agrobacterium*-mediated plant transformation of these rose cultivars are in beginning stages.
- We are completing the tissue culture and transformation methods for *Tripidium* hybrids. Nathan Maren has worked on this as part of his PhD dissertation.
- We have completed a developmental flowering transcriptome analysis for *T*. *ravennae*. A manuscript is in preparation. Nathan Maren has worked on this as part of his PhD dissertation.
- We have finished a complete genome sequence for *T. ravennae*. A manuscript is in

preparation. Nathan Maren has worked on this as part of his PhD dissertation.

FY 2020 Publications

Maren, Nathan. Characterization and Improvement of Novel Bioenergy Grasses (*Tripidium* spp.). PhD Dissertation, NC State University. In press.

Advancing Pest Management in Floriculture Crops Through Applications of Innovative Technologies.

Christian Nansen, Insect ecology and remote sensing, University of California Davis, Davis, CA

Background: The Nansen lab at University of California Davis (UC Davis) conducts applied and fundamental research, and also teaching and student mentoring, in a number of areas with strong relevance to nursery and floral production operations. These research and teaching/mentoring activities are presented on the team website (http://chrnansen.wix.com/nansen2).

Among more conventional pest management activities, the Nansen lab conducts phytotoxicity and pest management trials in collaboration with industry partners and IR4. A video has recently been developed, in which it is described how pesticide performance testing is executed, and also how it is integrated into teaching by Nansen (https://youtu.be/DtYFrvkguKQ). In addition, the Nansen lab undertakes studies to characterize and describe behavioral avoidance of insecticides (also referred to as behavioral resistance) by key pests. Behavioral avoidance of insecticides is closely linked to poor/incomplete spray coverages of pesticides, and that is a topic of major importance to the Nansen lab.

Regarding technology-driven and more cutting-edge pest management activities, the Nansen lab: 1) is developing, testing, and disseminating (freely) smartphone based decision support tools to optimize pesticide spray applications (https://chrnansen.wixsite.com/nansen2/smartspray), 2) performs fundamental studies into spectral calibration with the end goal of obtaining more consistent and therefore reliable optical data, so that crop stress symptoms can be detected with high degree of sensitivity and accuracy, 3) investigates fundamental aspects of hyperspectral sensing as a technology to detect and diagnose abiotic and biotic stress of crops, including ornamental plants – this research includes studies into relationships between crop physiology and leaf reflectance features, 4) is performing engineering optimization of: lighting, robotic rail movement of hyperspectral camera, and mounting of hyperspectral imaging onto an octocopter drone, and 5) collaborates with the Kong lab in the department of Mechanical Aerospace Engineering on both development and performance testing of a drone-based device, the "bugbot", to precision-release natural enemies, such as, predatory mites onto hotspots in crop fields with emerging spider mite outbreaks.

The overall goals with all abovementioned activities is to perform research at the highest possible standard, so that it can: 1) be published/disseminated in internationally recognized research journals, 2) lead to industry collaborations and development of commercially viable solutions, and 3) involve students and postdocs and therefore lead to capacity building and career development of aspiring scientists with an interest in solution-based research into integrated pest management.

Progress and Accomplishments in FY 2020

• A new technician, Mr. Anil Mantri, with unique engineering skills has joined the Nansen lab and he has been working on improving the robotic rail system, which runs and controls the hyperspectral imaging and active lighting. An issue has been that the original rail system was quite heavy and bulky, which made it challenging to move and potentially bring to commercial greenhouse facilities to show case to growers. In 2021 (if Covid-19 induced restrictions allow), we intend to organize field trips to key nursery and ornamental growers. The primary objectives with these visits will be to demo the robotic rail system under commercial operations and also discuss ways to move the existing prototype further towards commercialization.



ssentative image of marigold plants imaged (B), and the same arigold after leaf reflectance data from low, medium, and high elative treatment effect with the dotted horizontal line f control plants).

• The improved robotic rail system is used inside a dark room with an active light source to acquire high-precision leaf reflectance data from crop plants and quantify their direct responses to pesticides (i.e. phytotoxicity) and/or arthropod infestations.

• In 2021, the robotic rail system will also be configured, so that it can operate outside and/or inside commercial greenhouses.

• Multiple data sets from gerbera and chrysanthemum plants have been successfully acquired and integrated with photosynthesis data and data on plant element composition. The compiled data set has been processed and a manuscript was submitted for publication in the journal Pest management Science. Two hypotheses underpin accurate and reliable use of remote sensing data in detection and diagnosis of biotic stressors in crops, and they are examined in the study: 1) infestation by a pest is associated with unique changes in plant compositional traits, and 2) pest-induced changes in plant compositional traits are associated with detectable and unique features in leaf reflectance profiles.

FY 2020 Presentations

- 1. Blog: Oct. 6, 2020 (https://ucanr.edu/blogs/blogcore/postdetail.cfm?postnum=43856): Technology-driven frontiers in entomology.
- 2. Nansen C. 2020. Three separate technology-driven frontiers in Entomology. Keynote presentation at the virtual conference: "Frontiers in Entomology" 47th Congress of the

Colombian Entomological Society (https://socolen47.agroclick.org/dr-christian-nansen/), October 8-9, 2020.

- Nansen C. 2020. Leaf Reflectance Responses by Strawberry Plants to Fertilizer Regimes and Arthropod Stressors. Ontario Berry Growers Meeting (https://www.amahort.com/berry-growers-of-ontario/). Niagara Falls, Ontario, Canada, Feb 18-19, 2020
- Nansen C, Teske A L, Ma N, Chen G, Kong Z. 2020. Optimization of drone-based releases of predatory mites to control spider mites in strawberry fields – cutting-edge research. Ontario Fruit and Ontario Fruit and Vegetable Convention (http://www.ofvc.ca/sessions_day1.html#berries). Niagara Falls, Ontario, Canada, Feb 19-20, 2020

FY 2020 Publications

- Nguyen HDD, Pan V, Pham C, Valdez R, Doan K, Nansen C. 2020. Night-based hyperspectral imaging to study association of horticultural crop leaf reflectance and nutrient status. Computers and Electronics in Agriculture. DOI: https://doi.org/10.1016/j.compag.2020.105458.
- Nguyen HDD, Nansen C. 2020. Hyperspectral remote sensing to detect leafminer-induced stress in bok choy and spinach according to fertilizer regime and timing. Pest Management Science. DOI: 10.1002/ps.5758

Nansen C, 2020. Robotics, machine vision, and automation – The Future Reality of Flower Production. American Floral Endowment Newsletter – June 2020 (https://endowment.org/robotics-machine-vision-and-automation-the-future-reality-of-flower-production/)

Improved Pest Control Application Technologies for Sustainable Crop Protection

Heping Zhu, ARS, Application Technology Research, Wooster, OH

Background: This project envisions that research on intelligent spray technologies, efficient applications of bio-products as alternative pesticides, and coordinated strategies can enhance pesticide application efficiency for efficacious and affordable control of insects, diseases and weeds. Selective approaches to achieve the objectives will be to: (1) innovate advanced intelligent-decision spraying systems to increase spray application efficiency; investigate spray deposition uniformity, spray drift, off-target losses and pesticide savings for ornamental nurseries, orchards, greenhouses and other specialty crops with intelligent-decision controlled sprayers; (2) develop drift reduction technologies with intelligent decision systems to aid in enabling development of sustainable production programs.

Progress and Accomplishments in FY 2020

A commercial version of an intelligent spray control system as a retrofit kit to existing sprayers was developed with joint efforts between ARS researchers at Wooster Ohio and engineers at a commercial partner. The commercial product was released to the marketplace. The commercial intelligent spray control product received 2020 American Society of Agricultural and Biological Engineers (ASABE) Davidson Prize, 2020 ASABE AE50 Award Winners, and 2020 World Ag Expo Top-10 New Product Winners. The availability of the commercial product enables growers and sprayer manufacturers to upgrade their existing sprayers without purchasing or redesigning new sprayers to achieve intelligent spray benefits of significant chemical savings and environmental protection.

To provide evidences for growers to reliably use the intelligent spray technology, comparative tests of the ability to control insects and diseases with intelligent sprayers and the same sprayers with conventional constant-rate mode were conducted at one fruit farm and two ornamental nurseries in Ohio for three consecutive years. Test crops were apple, peach, blueberry, black raspberry, crabapple, maple, birch, and dogwood. Pest insects and plant diseases included codling moth, oriental fruit moth, spotted wing drosophila, scab, powdery mildew, brown rot, mummy berry, phomopsis, and anthracnose in the fruit farm, and scab, leafhopper, aphid, and powdery mildew in the two nurseries. The field tests illustrated that the retrofitted sprayers were equal or more effective for control of the insects and diseases while reducing average 30%-65% pesticide and foliar fertilizer uses. This new spray technology avoids a significant portion of pesticides reaching non-target areas, thus, provides a highly efficient, low cost, and environmentally- and worker-friendly pesticide and foliar fertilizer applications for fruit and ornamental nursery industries.

A laser-guided variable-rate intelligent spray system was developed and tested in a laboratory before its integration with watering boom systems commonly used in greenhouses. Parameters in the spray system were configured according to the physical positions of the laser sensor and nozzles, and the sensor travel speed. The system accuracies were validated for synchronization of nozzle activation and laser sensor detection of objects and for desired spray volume discharged to the objects. Based on the laboratory evaluations, an experimental automatic laser-

guided variable-rate spray boom system was developed and attached to a mobile watering boom in a commercial greenhouse. The article reporting this research received the 2020 ASABE Superior Paper Award.

An electronic nose (E-nose) system equipped with a set of sensitive sensor array was developed for a fast diagnosis of aphid infestation at early stages in tomato plants growing in greenhouse. Volatile Organic Compounds (VOCs) of tomato plants with and without aphids were detected by the E-nose system and gas chromatography mass spectrometry (GC-MS), respectively. Sensor performances with fast sensor responses and high sensitivity were observed using the developed E-nose system. An accurate diagnosis of aphids-stressed plants differing from healthy plants was clearly achieved based on Principle Component Analysis (PCA) with the two PCs accounted for 86.7% classification. The changes of VOC profiles for infested tomato plants were quantitatively determined by GC-MS. Results indicated that a group of new VOC bio-markers (linalool, carveol, and nonane 2,2,4,4,6,8,8-heptamethyl-) played info-chemical roles in the tomato-aphids interaction. More importantly, the variation of the concentration of sesquiterpene VOCs (e.g., caryophyllene) and the appearance of new terpene alcohol compounds explained the sensor behaviors during E-nose tests, certificating the reliability and accuracy of the developed E-nose system. The VOCs of tomato plants growing in spring show similar profiles as that of plants growing in winter, except several terpenes of spring plants presenting a slightly higher intensity.

FY 2020 Presentations

- 1. Abbott, J. and Zhu, H. The effects of impacting droplet adhesion due to adjuvant type, concentration, and leaf surface roughness. The 40th Symposium on Pesticide Formulation and Delivery Systems: Formulation, Application and Adjuvant Innovation, Houston, Texas. 2019.
- Chen, L., and Zhu, H. Evaluation of laser-guided intelligent sprayer to control insects and diseases in ornamental nurseries and fruit farms. The 40th Symposium on Pesticide Formulation and Delivery Systems: Formulation, Application and Adjuvant Innovation. Houston, Texas. 2019.
- Zhang, Z., and Zhu, H. Integration of premixing in-line injection system into variablerate orchard spraying systems. The 40th Symposium on Pesticide Formulation and Delivery Systems: Formulation, Application and Adjuvant Innovation. Houston, Texas. 2019.
- 4. Zhang, Z., Zhu, H., Hu, C., Wei, Z. and Salcedo, R. Graphical user interface design for premixing in-line injection system attached to a variable-rate orchard sprayer. At the Virtual Annual ASABE meeting in Omaha, Nebraska. 2020.
- 5. Wei, Z., Zhu, H., Salcedo, R., Zhang, Z., and Duan, D. Effect of Pressure Fluctuation on Droplet Size Distribution from Pulse Width Modulated Nozzles. At the Virtual Annual ASABE meeting in Omaha, Nebraska. 2020.
- 6. Salcedo, R., Zhu, H., Zhang, Z., Wei, Z., Chen, L., and Ozkan, E. Evaluation of PWM technologies for pesticide spray applications in a two-year old apple orchard. At the Virtual Annual ASABE meeting in Omaha, Nebraska. 2020.
- Chen, L., Zhu, H., Horst, L., Wallhead, M., & Reding, M. Control of insects and diseases with laser-guided intelligent sprayers in fruit and ornamental nursery farms. At the Virtual Annual ASABE meeting in Omaha, Nebraska. 2020.

- 8. Abbott, JP., & Zhu, H. Leaf aging effects on sprayed droplet impact and adhesion. At the Virtual Annual ASABE meeting in Omaha, Nebraska. 2020.
- 9. Yan, T., Zhu, H., Wang, X., & Ling, P. Quantify spray deposition of a laser-guided boom spray system for greenhouse applications. At the Virtual Annual ASABE meeting in Omaha, Nebraska. 2020.

FY 2020 Publications

- Zhang, Z., Zhu, H., Hu, C., Wei, Z. and Salcedo, R. Graphical user interface design for premixing in-line injection system attached to a variable-rate orchard sprayer. ASABE Paper No. 2000099. (American Society Agricultural and Biological Engineers, St. Joseph, MI 49085). 2020.
- Wei, Z., Zhu, H., Salcedo, R., Zhang, Z., and Duan, D. Effect of Pressure Fluctuation on Droplet Size Distribution from Pulse Width Modulated Nozzles. ASABE Paper No. 2000124. (American Society Agricultural and Biological Engineers, St. Joseph, MI 49085). 2020.
- Salcedo, R., Zhu, H., Zhang, Z., Wei, Z., Chen, L., and Ozkan, E. Evaluation of PWM technologies for pesticide spray applications in a two-year old apple orchard. ASABE Paper No. 2000079. (American Society Agricultural and Biological Engineers, St. Joseph, MI 49085). 2020.
- Chen, L., Wallhead, M., Zhu, H., and Fulcher, A. Control of insects and diseases with intelligent variable-rate sprayers in ornamental nurseries. Journal of Environmental Horticulture. 37(3): 90–100. 2019.
- Cui, S., Inocente, E.A.A., Acosta, N., Keener, H.M., Zhu, H., and Ling, P. Development of fast e-nose system for early-stage diagnosis of aphid-stressed tomato plants. Sensors. 2019; 19: 3480. <u>https://doi.org/10.3390/s19163480</u>.
- Abbott, J., and Zhu, H. 3D optical surface profiler for quantifying leaf surface roughness. Surface Topography: Metrology and Properties. 2019; 7(4): 045016 (17 pages).
- Chen, L., Wallhead, M., Reding, M., Horst, L., and Zhu, H. Control of insect pests and diseases in an Ohio fruit farm with a laser-guided intelligent sprayer. HortTechnology. 30(2): 168– 175. 2020.
- Manandhar, A., Zhu, H., Ozkan, E., and Shah, A. Techno-economic impacts of using a laser-guided variable-rate spraying system to retrofit conventional constant-rate sprayers. Precision Agriculture. 21: 1156–1171. 2020.
- Zhang, Z., and Zhu, H. Hardware and software design for premixing in-line injection system attached on variable-rate orchard sprayers. Transactions of the ASABE. 63(4): 823-831. 2020.
- Fessler, L., Fulcher, A., Lockwood, D., Wright, W., and Zhu, H. Advancing sustainability in tree crop pest management: improving spray application rate with a laser-guided variable-rate sprayer in 'golden delicious' apple trees. Horticultural Science. 55(9): 1522–1530. 2020.

Strategies for Carbon Sequestration and Reducing Greenhouse Gas Emissions from Nursery Production Systems

Dr. Stephen A. Prior, USDA-ARS-National Soil Dynamics Laboratory, Auburn, AL; *Collaborators:* Drs. G. Brett Runion and H. Allen Torbert, USDA-ARS-National Soil Dynamics Laboratory, Auburn, AL; Dr. Glenn B. Fain, Horticulture Department, Auburn University, Auburn, AL

Background: Climate change and its potential global impacts are receiving significant attention from the scientific community. Increases in atmospheric carbon dioxide (CO₂) concentration, along with other trace gases [i.e., methane (CH₄) and nitrous oxide (N₂O)] are widely believed to be the driving factors behind global change. Much of the work on reducing greenhouse gas (GHG) emissions and increasing carbon (C) sequestration has been conducted in row crop and forest systems; however, virtually no work has focused on contributions from sectors of the specialty crop industry such as ornamental horticulture. Ornamental horticulture impacts rural, suburban, and urban landscapes. Since little is known about the impact of the horticulture industry on these driving factors, the National Soil Dynamics Laboratory has an on-going joint effort with the Horticulture Department at Auburn University to determine baseline GHG emissions, develop strategies to reduce these emissions, and develop strategies to increase soil C storage. The specific objectives are to: (1) Determine the carbon sequestration potential within nursery systems; (2) Develop strategies for increasing carbon sequestration above current levels; (3) Measure greenhouse gas emissions from nursery production systems. **Progress and Accomplishments in FY 2020**

Progress and Accomplishments in FY 2020

- A long-term study assessing landscape carbon dynamics (belowground C storage and atmospheric CO₂ efflux) for horticultural media (Pine Bark, Whole Tree, and Clean Chip Residual each with and without standard sand and fertilizer amendments, plus a soil only control) was established in Spring, 2018. Initial and yearly C samples have been collected to monitor C changes over time. In addition, Automated Carbon Efflux Systems (ACES) have been installed to continuously monitor CO₂ efflux. Collectively, these data will be used to develop C budgets for different media.
- A manuscript was published on a study investigating the interaction of fertilizer placement (broadcast vs. incorporated) and irrigation method (overhead vs. drip) on growth and GHG emissions from Japanese boxwood.
- A study on growth and GHG emissions from a shade tolerant (hasta) and a sun tolerant (day lily) species with three fertilizer placement methods (broadcast, incorporated, and dibble) has been completed and a journal article has been published.
- A greenhouse study examining growth and GHG emissions (using standard GRACENet protocols) from three common annuals grown in 3 different media (80:20 peat: vermiculite; 80:20 peat: Whole Tree; and 60:40 peat: Whole Tree) has been completed and a publication is in journal review.
- A preliminary short-term study examining the effects of varying amounts of biochar in peatperlite growth media on leachate (N) and trace gas efflux in violets indicated that efflux was unaffected by biochar while N in leachate was reduced with biochar addition.

• A study examining the effects of varying amounts of biochar in pine bark based growth media on growth and trace gas efflux in day lilies is currently underway.

FY 2020 Presentations

- 1. Murphy, A.M., Prior, S.A., Runion, G.B., Torbert, H.A., Sibley, J.L., Fain, G.B., and Pickens, J.H. 2019. Greenhouse gas emissions as impacted by high wood fiber substrate in the production of three annual crops. 2019 Southern Nursery Association Research Conference, Baltimore, MD.
- Murphy, A.M., Prior, S.A., Runion, G.B., Torbert, H.A., and Sibley, J.L. 2018. Trace gas emissions from a sun and shade grown ornamental crop. HortScience 53(9): S483. (From Proceedings of the Southern Region American Society for Horticultural Science Annual Meeting, Jacksonville, FL)

FY 2020 Publications

- Murphy, A., Runion, G.B., Prior, S.A., Torbert III, H.A., Sibley, J.L., Fain, G.B., Pickens, J. 2019. Effects of fertilizer placement on greenhouse gas emissions from a sun and shade grown ornamental crop. Journal of Environmental Horticulture. 37(3):74-80.
- Murphy, A.M., Prior, S.A., Runion, G.B., Torbert, H.A., Sibley, J.L., Fain, G.B., and Pickens, J.H. 2019. Greenhouse gas emissions as impacted by high wood fiber substrate in the production of three annual crops. Southern Nursery Association Research Conference 63:51-56. (Proceedings)

Evolving and Integration of Management Plans for Major Taxonomic Groups and Potential New Invasive Pests of Ornamental and Floriculture Production

Cindy McKenzie, ARS, Subtropical Insects and Horticultural Research, Fort Pierce, FL Lance Osborne, University of Florida, MREC, Apopka, FL

Background: The neonicotinoid class of insecticides is widely used for controlling whiteflies in many cropping systems including ornamental and floriculture crops and are in very grave danger of being removed from the marketplace in the United States due to concerns over toxicity to pollinators. This class of insecticides has already been banned from use in the European Union and the state of Oregon has recently taken measures to do the same. Environmental groups want the entire neonicotinoid class removed which would be catastrophic not only to the ornamental and floriculture industry but also to cotton, field, and vegetable production as they all rely heavily on this class to manage many economically important plant sucking pests. We will evaluate alternatives to this class of insecticides by working closely with the chemical industry to identify registered pesticides and those near registration that could fill the void created if the neonicotinoid class was banned. We will focus on whitefly control, since this is the primary pest affected and will cause the greatest amount of damage/cost to the ornamental and floriculture industry if neonicotinoids are lost. We will continue to evaluate commercially available predatory mites (Amblyseius swirskii, Amblyseius cucumeris and Amblyseius andersonii) for development and integration into BMPs including their susceptibility to alternative insecticides and their utility in banker plant systems currently under development. We have developed BMPs for whitefly targeted at propagated ornamentals and plants for planting intended for export as well as BMPs for thrips targeted at plants for planting. We have initiated new projects aimed at enhancing biological control for citrus and madeira mealybugs and are developing a biological control program for poinsettia thrips using the predatory thrips Franklinothrips vespiformis. All management programs are evolving, and new data will be integrated into existing programs and programs yet to be developed for potential new invasive pests of ornamental and floriculture production.

Progress and Accomplishments in FY 2020

- During the life of this NACA, 112 whitefly samples representing 1,532 individual whitefly have been molecularly biotyped for growers/researchers free of charge so that management programs could be tailored to an individual nursery or greenhouse grower's whitefly population (MEAM1 vs MED / B vs Q).
- During the life of this NACA, 15 thrips samples 110 individual thrips have been both molecularly biotyped and traditionally identified using morphological techniques to include in a *Scirtothrips dorsalis* (chilli thrips) national survey which will conclude this year. We have identified two members of the cryptic species complex in the U.S.: South Asia 1 and East Asia 1. East Asia 1 is capable of overwintering on hydrangea in the northeast (NY and MA).

- Management plans for thrips (<u>https://edis.ifas.ufl.edu/pdffiles/IN/IN114500.pdf</u>) and whiteflies <u>http://edis.ifas.ufl.edu/pdffiles/IN/IN117100.pdf</u> are continually updated/available online.
- Non-neonicotinoid rotation greenhouse trials (2) with new chemistry insecticides that were previously determined to be compatible with *Amblyseius swirskii* are complete. Seven rotation regimes were evaluated and the trial was repeated. Third rotation trial with the top three insecticides were evaluated to determine if insecticide placement within the rotation regime affected MED whitefly control. Data is being analyzed and a manuscript is in preparation.
- Libraries for 23 populations of predatory mites representing seven species: *Phytoseiulus persimilis* (5), *Neoseiulus californicus* (4), *N. cucumeris* (4), *N. fallacis* (2), *Amblyseius swirskii* (4), *A. andersoni* (3), *Amblydromalus limonicus* (1) have been constructed and Illumina sequencing is currently being finished and evaluated for this barcoding project. Benefits to stakeholders include new tools to identify/validate current and new predator populations and association of traceable genetic markers w/effective predator populations being used in commercial settings.
- Chilli thrips true host range is complete and manuscript is in preparation.
- Chilli thrips national survey will be completed in 2020 and manuscript is in early preparation.
- Completed laboratory studies comparing the potential of the predatory mite species *Amblyseius swirskii* and *Amblydromalus limonicus* to control chilli thrips; manuscript is *in press*.
- Determined the efficacy of a pepper-based banker plant system utilizing *Amblyseius swirskii* and *Amblydromalus limonicus* for control of chilli thrips on rose; manuscript is currently under review.
- Assessing the suitability of *Artemia* cysts for development and reproduction of *Franklinothrips vespiformis* and determined preference for cysts over the target prey *Echinothrips americanus*. Data collection is still ongoing.
- Conducted greenhouse studies to determine if the application of *Artemia* cysts as an alternative food source for *Franklinothrips vespiformis* enhanced biological control of *Echinothrips americanus*. Trials are finished and data is currently being analyzed.

FY 2020 Presentations

- Kumar, V., Dickey, Aaron, Seal, Dakshina, Shatters, Robert Jr., Osborne, Lance, McKenzie, Cindy, Osborne, L. 2018. Unexpected high intragenomic variation in two of three major pest thrips species does not affect ribosomal internal transcribed spacer 2 IITS2) utility for thrips identification. Entomological Society of America, St. Louis, MO Nov 17-20, 2019.
- McKenzie, Cindy L., Osborne, Lance, Polston, Jane. 2019. Can Aleurotrachelus trachoides Back Acquire and Transmit Tomato Yellow Leaf Curl Virus? Entomological Society of America, St. Louis, MO Nov 17-20, 2019.
- 3. Osborne L.S. and C.L. McKenzie. 2019. Industrial Hemp Research Update: Adapting IPM for Ornamentals to Industrial Hemp Production in Protected Agriculture. Open

House for Florida Nursery Grower Landscape Association and Local Growers Hemp Industry, UF/IFAS-MREC, Apopka, FL October 1, 2019.

- Osborne L.S. and C. L. McKenzie. 2019. Research on the Management of Invasive Species. Tour and lectures. Valencia College Survey of Pest Control course. UF/IFAS-MREC Apopka, FL Oct 3, 2019.
- Osborne L.S. and C. L. McKenzie. 2019. Biological Control using banker plants. Wellington Garden Club, Wellington, FL Oct 7, 2019
- 6. Osborne L.S. and C.L. McKenzie. 2019. Managing Pests in the Garden. Wellington Garden Club. UF/IFAS-Wellington, FL Oct. 7, 2019.
- Osborne L.S. and C.L. McKenzie. 2019. Development and Use of Insect Fungal Pathogens on Pests of Ornamentals. Biopesticides North America Summit. Orlando, FL. October 9-10[,] 2019.
- 8. Osborne L.S. and C.L. McKenzie. 2019. Managing Pests in Roses. Greater Palm Beach Rose Society. West Palm Beach, FL. Oct. 28, 2019.
- 9. Osborne L.S. 2019. Pests of Hemp. UF-IFAS Hemp working group. Gainesville, FL. November 5, 2019.
- 10. Osborne L.S. and C.L. McKenzie. 2019. Annual Scout Training Program. UF/IFAS-MREC Apopka, FL Nov. 7, 2019.
- Kumar, V., Dickey, Aaron, Seal, Dakshina, Shatters, Robert Jr., Osborne, Lance, McKenzie, Cindy, Osborne, L. 2018. Unexpected high intragenomic variation in two of three major pest thrips species does not affect ribosomal internal transcribed spacer 2 IITS2) utility for thrips identification. Entomological Society of America, St. Louis, MO Nov 17-20, 2019.
- McKenzie, Cindy L., Osborne, Lance, Polston, Jane. 2019. Can Aleurotrachelus trachoides Back Acquire and Transmit Tomato Yellow Leaf Curl Virus? Entomological Society of America, St. Louis, MO Nov 17-20, 2019.
- 13. Osborne L.S. and C.L. McKenzie. 2019. Biological Control of Pests. Plant Health On the Go! UF/IFAS-MREC Apopka, FL Dec. 3, 2019.
- Osborne L.S. and C.L. McKenzie. 2019. Banker Plants. University of Maryland Extension and Maryland Nursery, Landscape and Greenhouse Association Biological Control Conference. Baltimore, MD. Dec.17, 2019.
- Osborne L.S. and Cindy McKenzie. 2020. Banker Plant Systems and Biological Control for Ornamental Crops. February Flora and Fauna Festival. Gainesville, Florida. February 8, 2020.
- Osborne L.S. and Cindy McKenzie. 2020. Banker Plant Systems for Crops Grown in Greenhouses. FNGLA Citrus Nursery Workshop. University of Florida, Citrus Research and Education Center. February 10, 2020.
- Osborne, L.S. and Cindy McKenzie. 2020. Pesticide and Biological Control Tactics for Common and Invasive Pest of Hemp. Syngenta Flowers – Alva, Florida. February 28, 2020.
- 18. Osborne, L.S. and Cindy McKenzie. 2020. Biological Control Tactics for Common and Invasive Pest Ornamental Pest. Lee County Extension. February 29, 2020.
- 19. Osborne, L. S. and Cindy McKenzie. 2020. Biological Control of Ornamental Plant Pests. South-West Horticulture Agents Green Team. Virtual meeting, April 17, 2020.
- 20. McKenzie, C.L. and L.S. Osborne. 2020. *Invited* by AmericanHort and the Horticultural Research Institute to present our project "Evolving and Integration of Management Plans

for Major Taxonomic Groups and Potential New Invasive Pests in Ornamental and Floriculture Production" for their tHRIve webinar series to showcase whitefly and exotic invasive pest research funded through the Floriculture, Nursery and Research Initiative. Both presentations are available for viewing at <u>https://www.hriresearch.org/thrive-webseries</u>.

- Osborne, L.S. and Cindy McKenzie. 2020. Whiteflies, Thrips and Other Invasive Pests. HRI Thrive Web Series - HRI Coffee Chat: FNRI Research on Invasive Insect Pests. (https://www.hriresearch.org/thrive-web-series). June 17, 2020.
- 22. Osborne, L. S. and Katherine Houben, Cindy McKenzie. 2020. Resistance Management A Systems Approach. WinField Cultivate Happy Hour. Virtual Meeting, July 9, 2020. <u>https://www.hriresearch.org/thrive-web-series</u>
- 23. Osborne, L. S. and Cindy McKenzie. 2020. HRI Chat Session (Follow-up to HRI Thrive Web Series). Virtual meeting, July 28, 2020.
- 24. Osborne, Lance, Erich Schoeller and Cindy McKenzie. 2020. Ornamental plant IPM. New Pests, New Tools and New Tactics: Whiteflies, Thrips and Other Invasive Pests. Florida Ornamental IPM. Virtual Workshop. Virtual meeting, August 26, 2020.

FY20 Publications (proceedings, journal, magazine that are in press or published):

- Ahmed, M.Z., Diepenbrock, L.M., Hodges, G., Whilby, L. Miller, D., Burrow, J.D, McKenzie,
 C. Field guide of lebbeck mealybug. Florida Department of Agriculture and Consumer
 Services, Division of Plant Industry, FDACS-P-02117, December 2019, 8 pp.
- *Colmar, Serra*, McKenzie, Cindy L., Luo, Weiqi, Osborne, Lance S. First report of *Bemisia tabaci* Mediterranean (biotype Q) (Hemiptera: Aleyrodidae) in the Dominican Republic. Florida Entomol 102(4): 778-782. 2019. <u>https://doi.org/10.1653/024.102.0417</u>
- McKenzie, Cindy L., Sparks, Jr., Alton N., Roberts, Phillip, Oetting, Ronald, Osborne, Lance S. Survey of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in agricultural ecosystems in Georgia. J Entomol Science 55(2): 163-170. 2020. https://doi.org/10.18474/0749-8004-55.2.163
- Kumar, Vivek, Mehra, Lucky, McKenzie, Cindy L., Osborne, Lance S. Functional response and prey stage preference of *Delphastus catalinae* Horn and *D. pallidus* LeConte (Coleoptera: Coccinellidae) on *Bemisia tabaci* (Hemiptera: Aleyrodidae). Biocontrol Science and Technology. 2020. <u>https://doi.org/10.1080/09583157.2020.1749833</u>
- *Kumar, Vivek*, McKenzie Cindy L., Osborne, Lance S. Predator-In-First: A preemptive biological control approach for sustainable management of multiple pepper pests in Florida. Sustainability. 12(18); 7816. 2020. <u>https://dx.doi.org/10.3390/su12187816</u>
- Avery, Pasco B., Kumar, Vivek, Francis, Antonio, McKenzie, Cindy L., Osborne, Lance S. Compatibility of the predatory beetle, *Delphastus catalinae*, with an entomopathogenic fungus, *Cordyceps fumosorosea*, for biocontrol of invasive pepper whitefly, *Aleurothrixus trachoides* in Florida. Insects 11(9), 590. 2020. <u>https://doi.org/10.3390/insects11090590</u>
- *Kumar, Vivek*, McKenzie, Cindy L., *Avery, Pasco B.*, Osborne, Lance S. Suitability of ornamental pepper cultivars as a banker plant for the establishment of predatory mite Amblyseius swirskii in controlled production. Sustainability 12 (19), 8031. 2020. https://doi.org/10.3390/su12198031
- Schoeller, Erich N., McKenzie, Cindy L., Osborne, Lance S. Comparison of the Phytoseiid Mites Amblyseius swirskii (Athias-Henriot) and Amblydromalus limonicus (Garman and McGregor) for Biological Control of Chilli Thrips Scirtothrips dorsalis Hood (Thysanoptera: Thripidae). Experimental and Applied Acarology (accepted 9/28/2020; In press)

Identification of Methods for Quarantine Certification of Imported Fire Ant in Nursery Production Systems and Establishment of Biological Control Agents to Reduce Fire Ant Populations

Dr. Jason Oliver, Dr. Karla Addesso, Dr. Anthony Witcher, and Fulya Baysal-Gurel, Tennessee State University, McMinnville, TN

Dr. Lisa Alexander, USDA-ARS National Arboretum, ADODR

Background: Imported fire ants (IFA) infest over 325 million acres in North America and their presence affects shipments of nursery stock from infested to non-infested areas via requirements of the Federal Imported Fire Ant Quarantine (FIFAQ). Currently, only three IFA quarantine treatments are approved for field-grown nurseries: 1) a pre-harvest broadcast bait (e.g., Amdro, Award II, Distance, Extinguish, or Siesta) followed 3-5 days later with granular chlorpyrifos, 2) a post-harvest root ball dip in chlorpyrifos or bifenthrin, or 3) a post-harvest drench, plant rotation, and drench in chlorpyrifos. The broadcast pre-harvest granular option is the most practical treatment method, but is very expensive (>\$200/acre/year applied multiple times in a typical 5-year growing cycle) and is limited by only one current label registration. The postharvest dip or drench methods are labor intensive and difficult with large numbers of plants, potentially damaging to the environment, and hazardous to labor due to large volumes of insecticide and worker reentries into pesticide treated areas. A heavy FIFAQ reliance on one primary active ingredient, chlorpyrifos, further increases uncertainty about the availability of certification options, especially since cancellation decisions are still pending between the Ninth Circuit Federal Court and the U.S. Environmental Protection Agency. With the exception of bifenthrin plant dips, most FIFAQ options for field-grown plants have relatively short certification periods (30 to 84 days), which do not fit well with current nursery production practices. New treatment options are needed that have lower cost, longer certification, potential for rapid field application, and less worker exposure.

Progress and Accomplishments in FY 2020

- Fire Ant OnyxPro Band Test. An OnyxPro (plus other generic bifenthrin and lambdacyhalothrin treatments) band test at Magness Nursery was previously performed from Fall 2018 to May 2019 period (reported on FY2019 report). For plots that were 100% fire-antfree in May 2019, we continued making OnyxPro applications at 3 month intervals (i.e., May 2019, Aug. 2019, Nov. 2019, and Feb. 2020) to half of the plots and left the other half of the plots untreated. After Feb. 2020, we stopped treating all plots, but continued monitoring through Oct. 2020. <u>Major Outcomes</u>: OnyxPro sprayed sites remained nearly fire-ant-free (only a few colony incursions), but plots that were left untreated began to have significant fire ant colony incursions at ~4.5 months post-treatment (in both 2019 spray cessation plots and then in the 2020 spray cessation plots).
- Scimitar Band Test 1: A Scimitar GC band test was previously reported for the February 2019 to May 2020 period at LanTenn and Hidden Valley Nurseries, which involved banded rates of 5 (labeled ornamental rate), 10 (labeled turf rate), or 15 (experimental banded rate) fl oz / acre applied at 3 month intervals (see FY2019 report). At the Hidden Valley site, pre-emergent herbicides were tank mixed with the sprays and at the LanTenn site a Boominator

1404 floodjet nozzle was used to treat both sides of the nursery row in a single pass. Spraying of the site was terminated after Feb. 2020, but we have continued to follow mound invasion patterns into October 2020. <u>Major Outcomes</u>: a) The 10 and 15-fl oz banded nursery rows remained nearly fire-ant-free during the 3-month interval sprays (the lowest 5 fl oz rate was the least effective treatment), but ants returned within 4-months of spray cessation, b) tank mixing Marengo herbicide pre-emergent did not interfere with insecticide activity during Feb. 2019 or Feb. 2020 sprays, and c) the floodjet nozzle effectively treated both row sides in a single pass.

- Scimitar Band Test 2: During Feb. 2020, we repeated the Scimitar 5, 10, and 15 fl oz band test at Magness (3 farm reps) and Little River (1 farm rep) Nurseries. Sprays in this test have been made in Feb. 2020, May 2020, Aug. 2020 and are pending for Nov. 2020 and Feb. 2021. <u>Major Outcomes</u>: The 15 fl oz rate has remained 100% free through July 2020 (i.e., the last month the data were summarized), but the 5 and 10 fl oz rates have had some mound incursions. There have been some weed management issues at the nurseries that may be compromising the treatment efficacy of sprays.
- Band Test Chemical Assay and Laboratory Bioassays of Soil Samples: Before and after the August 2020 Scimitar band sprays, we began pulling soil samples at 0-1 and 1-2 inch depths from 4 replicates of each Scimitar and control treatments. The 0-1 inch depth sample was used in a chemical extraction assay, and the 0-1 and 1-2 inch depth samples were used in laboratory bioassays with worker fire ants. <u>Major Outcomes</u>: a) We were unable to detect lambda-cyhalothrin in chemical assays of band test soil samples that were 3 months old, but some of the highest rate (15 fl oz / acre) samples collected immediately after band sprays had detectable residues and b) preliminary laboratory bioassays with worker ants indicated little difference among treatments at 3 months post-spray, but a rate trend was evident in bioassays of the soil samples from 0 and 1.5 month post-spray bioassays, with the highest mortality at the 1.5 month post-spray timing. We plan to continue soil sample band spray plots at 0, 1.5, and 3 month post-spray periods for more laboratory bioassays with worker ants.
- August OnyxPro Band Spray: During OnyxPro band spray tests (described above), a new nursery block with fire ant mounds was sprayed with OnyxPro during August 2019 to determine if colonies could be eliminated with band spray-only treatments initiated in a summer month. The nursery block also was sprayed with OnyxPro at 3-month intervals in Nov. 2019 and Feb. 2020, and colony changes at the site were followed through October 2020. <u>Major Outcomes</u>: a) The August 2019 spray did not provide 100% fire ant control until after the February 2020 spray and b) after February 2020, fire ant colony control remained low through July 2020 (last date data were summarized).
- Biofumigant Cover Crops and Fire Ant Impacts: A plant pathology doctoral student in Dr. Fulya Baysal-Gurel's program is evaluating biofumigant cover crops for their effects on *Phytophthora* in boxwood plantings. Treatments include white mustard, astro argula, dwarf essex rape, oriental mustard, turnips, mighty mustard, amara mustard, Dominus (170 and 340 lb / acre), mustard meal, solarization, and inoculated and non-inoculated controls. These biofumigant crops were tilled into the soil, and soil samples subsequently pulled for imported fire ant laboratory bioassays in September 2020 (and planned for December 2020 and March 2021). Major Outcomes: Results are still pending, so we don't yet know if these biofumigant cover crops might be useful for reducing fire ants in nursery sites.
- **Spring Mound Drench Test 1 (2 April 2020):** Previous laboratory studies indicated a pyrethroid (Scimitar) could kill individual fire ant workers more quickly when combined

with some plant oils. A field test was performed to evaluate Scimitar and plant oil preharvest individual tree treatments that could potentially eliminate fire ant colonies more rapidly for quarantine certification (i.e., 24 to 48 hours). Individual fire ant colonies were treated with nootka tree oil (1% solution), Essentria IC3 (3% solution) and Ultra-Pure Hort oil (3% solution) all combined with Scimitar (10 fl oz / acre rate). <u>Major Outcomes</u>: a) The nootka oil and Essentria provided 99 and 99.1% colony control at 2 days after treatment (DAT), hort oil provided 99.9% at 4 DAT, and Scimitar alone had 100% control at 11 DAT, b) treatments maintained colony control at 99.7 to 100% for the 22 day post-evaluation, and c) oil and Scimitar combinations greatly improved speed of control. However, the cost of the oil treatments may be prohibitive for practical grower use.

- Spring Mound Drench Test 2 (12 May 2020): A second mound drench test was performed using a lower cost principal ingredient of Essentria IC3 (i.e., wintergreen oil). Plant oil treatments were again paired with Scimitar (10 fl oz / acre) and included wintergreen oil (1% solution), Damoil dormant oil (3% solution), or Damoil (3%) and wintergreen oil (0.5%) combination treatment. <u>Major Outcomes</u>: a) The three oil plus Scimitar treatments provided 99.1, 99.9, and 98.9% control by 4 DAT, respectively, while Scimitar alone achieved 99.9% control at 6 DAT, b) unfortunately, all treatments regained colony activity between 15 and 21 DAT, and c) warmer May weather may have negatively influenced treatment efficacy compared to the first April mound drench test or wintergreen oil treatments may be less effective than Essentria IC3 due to other non-wintergreen oil ingredients in the Essentria product. The cost of these wintergreen oil treatments would be lower than the 2 April mound drench test.
- SINV-3 Hybrid Variant Relocation Efforts: On 3 Feb. 2020, 16 fire ant colonies were excavated from a pasture on the Brooks Farm in Polk County, TN with the assistance of Greg Paxton (Univ. TN Extension) and Kelley Fradey (TSU Extension). The pasture site had previously yielded hybrid fire ants infected with a genotype variant of Solenopsis invicta virus-3 (SINV-3 hybrid). Our goal was to attempt to relocate this apparently hybrid-fire-antspecific virus to a new hybrid location. We also sampled workers from colonies at our proposed release site in Hamilton Co. on Highway 111 right-of-way to ensure the site was still negative for SINV-3. Worker samples from each colony were sent to Dr. Steven Valles (USDA-ARS Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL) for determination of SINV-3 infection status. Major Outcomes: All of the ant samples from both the Polk Co. and Hamilton Co. sites were negative for SINV-3, but two colonies had weak detections of SINV-1. Therefore, we were unable to relocate hybrid variant SINV-3 to the Hamilton Co. site in February 2020. We decided to re-sample the site during summer 2020, when virus levels may be higher based on past experience. However, the CMAVE facility was closed during summer 2020 due to the covid-19 pandemic, so our relocation efforts were postponed until a future date. We hope to determine in the future if this new hybrid virus is more suitable for redistribution efforts in states like Tennessee with large hybrid fire ant populations.

FY 2020 Presentations

1. Oliver, J., K. Addesso, A. Witcher, N. Youssef, P. O'Neal, A. Dismukes, R. Weeks, L. Alexander, D. Oi, S. Valles, and M. Halcomb. 2020. Imported fire ant updates.

Virtual webinar for growers arranged by Nursery Extension Amy Dismukes. Presentation was subsequently re-recorded after the virtual event for public posting.

- Oliver, J., K.M. Addesso, A. Witcher, L. Dant, N. Youssef, P.A. O'Neal, A. Dismukes, R.D. Weeks, and L. Alexander. 2019. Development of pre-harvest fieldgrown nursery treatment alternatives to chlorpyrifos for the Federal Imported Fire Ant Quarantine. National Entomological Society of America Annual Meeting, 17-20 Nov. 2019. St. Louis, MO. (Conference Poster Presentation).
- Oliver, J., K. Addesso, N. Youssef, and P. O'Neal. 2019. Fire ant management in pastures. Ag in the Foothills Field Day. Little River Unit of the East Tennessee Research and Education Center. 3 Oct. 2019. Maryville, TN. (Oral Field Day Presentation). (10 Hours Non-Traditional Teaching).

Planned Presentations Canceled Due to Covid-19 Pandemic:

 Oliver, J., K. Addesso, A. Witcher, L. Dant, N. Youssef, P. O'Neal, V. Ojha, A. Dismukes, R. Weeks, and L. Alexander. 2020. Continuing efforts to develop chlorpyrifos replacements for fire ant quarantine certification. Tenn. State Univ. 42nd Annual University-Wide Research Symposium, 23-27 Mar. 2020. Nashville, TN. (Poster Presentation).

FY 2020 Publications

- Pandey, M., K.M. Addesso, L. Alexander, N.N. Youssef, and J.B. Oliver. 2020. Relationship of imported fire ant (Hymenoptera: Formicidae) integument coloration to cuticular hydrocarbon and venom alkaloid indices. Environmental Entomology. Submitted 25 Sept. 2020 (Peer review in progress).
- Valles, S.M., J.B. Oliver, K.M. Addesso, and O.P. Perera. 2020. Unique *Solenopsis* venom protein 2 alleles in *Solenopsis invicta* × *Solenopsis richteri* hybrid fire ants. Toxicon. In Preparation.
- Oliver, J., K.M. Addesso, A. Witcher, L. Dant, N. Youssef, P.A. O'Neal, A. Dismukes, R.D. Weeks, and L. Alexander. 2019. Development of pre-harvest field-grown nursery treatment alternatives to chlorpyrifos for the Federal Imported Fire Ant Quarantine. National Entomological Society of America Annual Meeting, 17-20 Nov. 2019. St. Louis, MO. (Online Abstract).

Improved Pathogen Detection and Management of Bulb Diseases

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Dilip Lakshman, USDA-ARS, FNPRU/SASL

Background: The goals of this project continue to relate to the detection and management of diseases, particularly those caused by *Botrytis* spp., of peonies, tulips, and lilies. This approach includes evaluating the effectiveness of integrating cultural practices and biopesticides into *Botrytis* disease management programs.

Progress and Accomplishments in FY2019 & 2020:

- During the course of this project, genetic analysis has revealed an unprecedented diversity of *Botrytis* species in peony fields in the PNW and Alaska. More *Botrytis* species have been found on a single host than ever before reported before, including up to 10 potentially novel species. Some species found in Alaska have been described on other host plants in different parts of the world, indicating a wider geographic and host distribution than previously thought. Also, some of the recovered isolates share sequence similarity with unnamed species found living as endophytes in weedy hosts, suggesting that the species found on peony may have flexible lifestyles as recently discovered in the genus. Koch's postulates were performed to confirm pathogenicity of two (2) novel *Botrytis* species found on peonies.
- *Botrytis* spore loads were monitored in coolers at 12 peony farms using plates of *Botrytis* Selective Media (BSM). Spores were detected in all of the coolers, including those treated with ozone. These data indicate that additional studies are needed to determine the risk this inoculum poses in relation to disease development during storage.
- Ten fungal and oomycete plant pathogens species were identified as causing disease in surveys of peony plantings in 12 states. This included new disease reports for several states, including five genera not previously reported as pathogens of peonies in the United States. These results will provide plant disease diagnosticians and growers a more comprehensive resource for understanding the regional prevalence of peony diseases and subsequently improve disease management decisions. These results were also incorporated in the publication of a new 'Grower's Guide to the Most Common Diseases of Peony in the United States' to assist with the diagnosis and management of diseases on peonies.
- Five (FY19) to seven (FY20) field or postharvest trials were conducted on tulips and peonies to examine the effectiveness of new "reduced-risk" and biopesticides in controlling foliar, stem, or postharvest diseases on these crops. None of the biopesticides tested were effective in reducing the development of *Botrytis* and *Graphiopsis* diseases on field-grown peonies and tulips. Trials did demonstrate that a preharvest application of isolfentamid (Astun) to peony flower buds was as effective as a 5-second postharvest dip of buds in fungicides in providing effective control of *Botrytis* development during the storage and display of peony flowers.

- A summary of the pest management practices and preliminary results of this research was presented during a 2019 Keynote address at the XIII International Symposium on Flower Bulbs and Herbaceous Perennials in Seoul, South Korea. The presentation also provided a historical perspective on the changes in pest management strategies, highlighted challenges growers encounter, and provided an overview of recent advances in the protection of ornamental flower bulb crops against disease and arthropod pests.
- A summary of Botrytis research supported by this project was also presented in a Keynote (Zoom) address at the "2020 China (Luoyang) International Peony Industry Forum" in Luoyang, China.
- In addition to the publications, presentations, and news releases outlined below, two oneday educational workshops and one field-day were organized to disseminate information from this project to regional bulb and cut flower growers in 2019/20

FY 2020 Presentations

- 1. **Chastagner, G**. 2019. Overview of research on diseases of ornamentals at WSU Puyallup. NCERA224 Annual Meeting, San Juan, PR. December 9, 2019.
- 2. Chastagner, G. 2019. Management of Diseases on Peonies. NCSU Peony Webinar, Raleigh, NC. December 18, 2019.
- 3. **Chastagner, G**. 2020. Disease Management on Peonies and Tulips: New Tools and Approaches to Improve Control and Extend the Life of Flowers in Storage. Wilbur-Ellis Professional Markets Technical Seminar. Puyallup, WA. January 8, 2020.
- 4. Chastagner, G. 2020. Botrytis research update. Annual Alaska Peony Grower Association Conference, Fairbanks, AK. January 31-Februacy 1, 2020.
- Chastagner, G. 2020. Peony cut flower production and *Botrytis* research in the USA. 2020 China (Luoyang) International Peony Industry Forum. Luoyang, China. April 17-19, 2020 (Keynote Zoom Address)

FY 2020 Publications

- Garfinkel, A.R., K.P. Coats, D.L. Sherry and **G.A. Chastagner**. 2019. Genetic analysis reveals unprecedented diversity of a globally-important plant pathogenic genus. Scientific Reports 30 Apr 2019, 9(1):6671. DOI: 10.1038/s41598-019-43165-y
- Lakshman, D.K., Cloyd, R.A. and **Chastagner, G.A.** 2019. Integrated management of diseases and pests on ornamental geophytes: challenges and progress. Acta Hortic. 1237, 13-32

DOI: 10.17660/ActaHortic.2019.1237.3

Garfinkel, A.R. and Chastagner, G.A. 2019. Strategies to address emerging fungal diseases in peony (*Paeonia lactiflora*) in the United States. Acta Hortic. 1237, 199-206
DOI: 10.17660/ActaHortic.2019.1237.26

- Garfinkel, A.R., and **G.A. Chastagner.** 2019. Survey reveals a broad range of fungal pathogens and an oomycete on peonies in the United States. Plant Health Progress 20(4), 250-254
- Chastagner, G.A., and A. Garfinkel. 2020. Diseases Affecting Ornamental Geophytes and Their Control. Chapter 15, pp. 367-414. In: M. Reid (ed). Achieving Sustainable Cultivation of Ornamental Plants. Burleigh Dodds Science Publishing. DOI: <u>10.19103/AS.2020.0066.15</u>

Garfinkel, A., and **G. Chastagner**. 2020. A Grower's Guide to the Most Common Diseases of Peony in the United States. Washington State University FS338E

Media Stories

Truscott, Seth. December 2019. WSU scientists find a slate of unexpected diseases in peony https://news.wsu.edu/2019/12/19/wsu-scientists-find-slate-unexpected-diseases-peony/

Discovery and Management of Diseases of Floriculture Crops

Margery Daughtrey, Section of Plant Pathology and Plant-Microbe Biol., Cornell University LIHREC, Riverhead, NY

Melanie Filiatrault, USDA ARS, Ithaca, NY

Progress and accomplishments in FY 2020

- Samples of ornamentals from commercial greenhouses were processed in the Daughtrey lab and bacterial isolates from new disease problems were sent to the Filiatrault lab for identification. *Dickeya* spp. cause plant disease in many crops and ornamentals and are responsible for considerable economic losses world-wide. The genus *Dickeya* has been subjected to extensive taxonomic reclassifications. Last year we isolated a bacterium from New Guinea impatiens causing blackleg and based on PCR and sequencing we identified the pathogen as belonging to *Dickeya dianthicola*. We submitted this manuscript to Plant Disease as a First Report. This year we completed the closed genome sequence of this strain which further supports the strain's assignment as *D. dianthicola*. This closed genome provides a more accurate representation of the genetic potential of the organism and a resource for the community. This complete genome will enable better primer design for classical genetics, more comprehensive searches for virulence related genes, and more accurate bioinformatic analysis.
- Last year, a hibiscus leaf spot pathogen was identified as either *Pseudomonas savastanoi* or *Ps. syringae* pv. *actinidiae* using several housekeeping genes. To definitively determine the species, whole genome sequencing using nanopore and Illumina sequencing technologies was performed. The genomic sequence of the pathogen shares highest homology to *Pseudomonas amygdali*. The genus *Pseudomonas* continually undergoes extensive taxonomic reclassifications. *P. amygdali* (reported synonyms *P. ficuserectae, P. meliae*, and *P. savastanoi*) has been recently proposed to be a separate species, and includes, *P. amygdali* pv. hibisci. Our data is consistent with this new classification. We are preparing a manuscript describing these findings. The genomic information is being used to better understand the pathogenicity of this pathogen and closely related Pseudomonads that infect floriculture crops.
- As with many bacteria, species of *Xanthomonas* have been the subject of taxonomic reclassifications. Previously we had identified a bacterium from an oregano leaf spot as *Xanthomonas gardneri*. This year whole genome sequencing was performed using nanopore and Illumina sequencing technologies. The results revealed that the isolate is phylogenetically closely related to *Xanthomonas gardneri*. This isolate caused a hypersensitivity reaction on tobacco indicating it is pathogenic. A manuscript is in preparation.
- In 2019 a bacterial isolate from pepper was isolated. This MLST analysis with five housekeeping genes revealed that the bacterium is phylogenetically closely related to *Pantoea vagans*. This year whole genome sequencing was performed using nanopore and Illumina. *Pantoea vagans* is commonly found as an epiphyte and has been used as a biocontrol, but *Pantoea* sp. frequently causes disease. In addition to pathogenicity assays, comparative genomic studies are planned to investigate factors that can be used to

distinguish epiphytes from pathogenic strains. These studies will provide insights into the phylogenetic and phylogenomic relationships that exist between *P. vagans* strains.

- Bacteria isolated from vein blackening and dark, watersoaked leaf spots on New Guinea impatiens 'Magnum White' are currently being analyzed.
- Poinsettias with severe leaf spotting from a common unrooted cutting source in Ethiopia were received for diagnosis from growers in NY, FL, IA, and NJ and identified as *Xanthomonas arboricola* and *X. axonopodis* pathovars by MLST. Currently cuttings of five cultivars of poinsettia are being rooted to allow Koch's postulates to be tested on the isolates. We are performing metabolic profiling and whole genome sequencing to complete species identification.
- 500 samples of growing mix were collected from crops of 3 Long Island greenhouse growers to check for *Pythium* root rot pathogens on edible crops in FY2020. There were 100 tomato and 300 hemp plants sampled. Isolates of *Pythium* spp. (100 from hemp; 15 from tomato) were recovered by potato bioassay and the mycelium will be forwarded to collaborator Carla Garzon for molecular population analysis to allow pathogen tracking and determine whether *Pythium* will be moving from edibles to ornamentals.
- Impatiens from the Imara XDR series were tested in a trial at Cornell's LIHREC under conditions highly conducive to disease: plants highly resistant to *Plasmopara obducens* downy mildew were identified in multiple colors; these continued to flower for months after impatiens 'Accent White' had collapsed.
- Verbenas of 75 cultivars were compared for their susceptibility to the powdery mildew *Podosphaera xanthii*, and many of these showed high resistance to the disease. Only 8 of the 75 cultivars were susceptible. This information will help growers to choose mildew-resistant verbena cultivars.
- In a trial at Cornell's LIHREC comparing susceptibility of 11 impatiens highly resistant to downy mildew caused by *Plasmopara obducens*, the least signs of the disease (sporulation on the leaf undersurface) were seen in Beacon Violet Shades, Beacon White, Beacon Orange, Imara Red XDR, and Beacon Bright Red (0.6–12.8 leaves per plant showed sporulation); Beacon Coral, Imara Orange XDR, and Imara Violet XDR (13.2–15.8 leaves per plant with sporulation) were statistically similar to these best cultivars.

FY 2020 Presentations

- 1. Diseases of urban (and suburban) ornamentals. New England Chapter, International Society of Arboriculture, Springfield, MA, October 9, 2019.
- 2. Ornamental plant diseases. Master Gardener Training, Cornell Cooperative Extension (CCE) of Orange County, Middletown, NY. October 11, 2019.
- 3. Ornamental plant diseases for tree stewards. CCE of Dutchess County, Millbrook, NY, October 12, 2019.
- 4. Woodland Fungi: what are they up to? South Fork Natural History Museum, Bridgehampton, NY, October 19, 2019.
- 5. Plants without many problems. With Dan Gilrein. Managing Landscapes Sustainably Conference, Cornell Cooperative Extension of Suffolk County, Stony Brook, NY, November 7, 2019.
- 6. Managing Botrytis in bedding plants. Northeast Greenhouse Seminar. Penn State Extension. Wilkes-Barre, PA. November 12, 2019.

- 7. What is a plant doctor? Center for Creative Retirement, Long Island University, Speonk, NY, December 2, 2019.
- 8. Beech leaf disease. NYS Department of Agriculture and Markets Horticultural Inspectors. Webinar. Dec. 12, 2019.
- 9. Down with downy mildew! Greenhouse/Floriculture Session, LI Agricultural Forum, Speonk, NY. January 8, 2020.
- 10. Malicious mildews: downy and powdery. Chesapeake Green, Maryland Nursery and Landscape Association, Linthicum, MD, February 19, 2020.
- Winning the blight fight: one research project at a time. With Chuan Hong. Boxwood Symposium, American Boxwood Association, National Agriculture Library, Beltsville, Md, February 19, 2020.
- 12. Training for detection of *Ralstonia solanacearum*. US Customs and Border Protection, Webinar, August 27, 2020.

FY 2020 Publications

- Daughtrey M., Buitenhuis R. 2020. Integrated Pest and Disease Management in Greenhouse Ornamentals. In: Gullino M., Albajes R., Nicot P. (eds) Integrated Pest and Disease Management in Greenhouse Crops. Plant Pathology in the 21st Century, vol 9. Springer, Cham. https://doi.org/10.1007/978-3-030-22304-5_22
- Castroagudin, V. L, Weiland, J. E., Baysal-Gurel, F., Cubeta, M., Daughtrey, M., Ward, N. A., LaMondia, J., Luster, D. G., Peduto Hand, F., Shishkoff, N., Williams-Woodward, J., Yang, X., LeBlanc, N., and Crouch, J. 2020. One clonal lineage of Calonectria pseudonaviculata is primarily responsible for the boxwood blight epidemic in the United States. Phytopathology <u>https://doi.org/10.1094/PHYTO-04-20-0130-R</u>
- Salgado-Salazar, C., LeBlanc, N., Wallace, E. C., Daughtrey, M. L., and Crouch, J.A. 2020. *Peronospora bergamotii, Hyaloperonopora daughtreyae* and *H. iberidis*: New species associated with downy mildew diseases affecting ornamental plants in the United States. European Journal of Plant Pathology 157:311-326.
- Castroagudin, V. L., Yang, X., Daughtrey, M. L., Luster, D. G., Pscheidt, J. W., Weiland, J. E., and Crouch, J. A. 2020. Boxwood blight disease: a diagnostic guide. Plant Health Progress. <u>https://doi.org/10.1094/PHP-06-20-0053-DG</u>
- Daughtrey, M., Beckerman, J., Davis, W. J., Rane, K. and Crouch, J. A. 2020. Corroboration that highly resistant impatiens cultivars are not immune to downy mildew disease: a report of crop losses from two California producers. Plant Health Progress <u>https://doi.org/10.1094/PHP-05-20-0040-SC</u>
- Richardson, P. A., Daughtrey, M., and Hong, C. 2020. Indications of susceptibility to *Calonectria pseudonaviculata* in some common groundcovers and boxwood companion plants. Plant Dis. <u>https://doi.org/10.1094/PDIS-08-19-1582-RE</u>
- Yingyu Liu, Tyler Helmann, Paul Stodghill, and Melanie Filiatrault. 2020. Complete Genome Sequence of the Necrotrophic Plant-Pathogenic Bacterium *Dickeya dianthicola* 67-19

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New Technologies that Manage Profit-Limiting Diseases with a Focus on Botrytis

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Background: Producing top quality floral crops through the mitigation of plant diseases is the goal of this research. We propose to limit management costs by incorporating new technologies and strategies to protect valuable floricultural crops from disease. Greenhouse floral crops are at risk from foliar and root rot diseases causing poor plant quality, plant death, and increased production costs. Botrytis cinerea is identified as a major problem during production, shipping and postharvest, causing leaf spots, blighting, stem cankers and damping off. Controlling Botrytis is difficult because it can infect crops at almost any stage of growth and damage all plant parts. Botrytis has adapted to some fungicides rendering them ineffective, but the extent of this problem is not known. Downy mildews have re-emerged on some greenhouse crops causing defoliation and plant stunting on susceptible crops including impatiens. Downy mildew on impatiens was commonly observed in production greenhouses and at retail in 2020. Root rot results from different soil-inhabiting pathogens and can move among producers and greenhouses via plants and recirculated irrigation water. Fungicide resistance affects recommendations and control success and available biocontrols may offer relief. Reduced risk fungicides and biopesticides with novel/unique modes of action are particularly needed for rotation schemes designed to manage and delay resistance and reduce the release of harsh pesticides into the consumer's environment. The overall objective of this cooperative research project is to develop new technologies and strategies to manage costly diseases of greenhouse floriculture crops with a focus on Botrytis.

Specific objectives include:

- 1. Determine cultivar susceptibility to *Botrytis*.
- 2. Test biological and conventional products against Botrytis.
- 3. Collect *Botrytis* isolates from infected plants in greenhouses representing different crops and test for resistance to the fungicides commonly recommended for protection.
- 4. Determine which fungicides can protect susceptible plants during propagation, shipping, and postharvest.
- 5. Identify effective new fungicides and protection programs that include different modes of action for impatient downy mildew for application in the greenhouse and determine longevity of protection in the landscape.
- 6. Test new plant-protection products, including reduced-risk fungicides, biopesticides, and biocontrols against root rot (e.g. *Thielaviopsis basicola*, *Phytophthora* spp., and *Pythium* spp.) and foliar spots and blights (e.g. downy mildew, powdery mildew).
- 7. Provide results and recommendations directly to producers and allied industries via publication in trade magazines, website updates, and presentations. YouTube videos and other novel approaches to communicate the highlights of this work broadly will be used to complement the more traditional extension activities.

Progress and Accomplishments in FY 2020

1. Determine cultivar susceptibility to Botrytis.

Trial were conducted and tested the relative susceptibility of geranium and petunia cultivars. These trials were repeated for each crop. Experiments were set up as a completely randomized design and were inoculated with *B. cinerea* (1 x 10^6 conidia/ml). Disease was evaluated and data were statistically analyzed and AUDPC was calculated.

Geranium Trials 1 and 2: Nine geranium (*Pelargonium xhortorum*) and one ivy geranium (*Pelargonium peltatum*) cultivars were evaluated: Horizon Coral Spice, Bullseye Red, Maverick Scarlet Picotee, Pinto Premium Orange, Quantum Salmon, Nano Deep Rose, Multibloom Lavender, Ringo 2000 Violet, Pinto Pink, and Ivy Tornado White. Inoculum was prepared from 11-day-old *Botrytis* cultures. Disease was assessed weekly (no. blighted leaves, no. foliar lesions, no. blighted leaves with sporulation). Highly susceptible cultivars were Ringo 2000 Violet, Maverick Scarlet Picotee. Least susceptible cultivars were Pinto Premium Orange, Horizon Coral Spice, Ivy Tornado White.

Petunia Trials 1 and 2: Thirteen petunia (*Petunia xhybrida*) cultivars were evaluated: Wave Purple Classic, Wave Lavender, Tidal Wave Cherry, Tidal Wave Silver, Shock Wave Red, Shock Wave Coconut, Easy Wave Blue, Easy Wave Mix Flag Improved, Easy Wave Red Improved, Sophistica Blackberry, Debonair Lime Green, Ramblin Red, Success Burgundy. Disease assessed visually on a 0-10 scale (0=healthy, 1=1-10% small/isolated lesions, 2=11-20% small lesions, 3=21-30% moderate-sized isolated lesions, 4=31-40% numerous, moderate-sized lesions, 5=41-50% large necrotic areas, 6=51-60% large necrotic areas, 7=61-70% large necrotic areas, 8=71-80% large necrotic areas, 9=80-90% necrosis/defoliation, 10=>90% necrosis/defoliation to plant death. Least susceptible cultivars were Shock Wave Red and Sophistica Blackberry. Highly susceptible cultivars were Tidal Wave Cherry and Success Burgundy.

2. Test biological and conventional products against *Botrytis*.

Trials testing the efficacy of 10 biorational products and 1 industry standard were carried out twice for each crop (petunia and geranium). Experiments were set up as a completely randomized design and were inoculated with *B. cinerea* (1 x 10⁶ conidia/ml). Disease was evaluated and data was statistically analyzed. Products tested included Actinovate (*Streptomyces lydicus* [FRAC code BM02]), Botector (*Aureobasidium pullalans* [NC=not classified]), BotryStop (*Ulocladium oudemansii* [NC]), EcoSwing (*Swinglea glutinosa* extract [BM01]), LifeGard (*B. mycoides* [P06]), Prestop (*Gliocladium catenulatum* [BM02]), PureCrop1 (soybean, corn oil [NC]), Serenade Opti (*Bacillus subtilis* [44]), Serifel (*B. amyloliquefaciens* [44]), Zio (*Pseudomonas chlororaphis* [BM02]), and industry standard Decree (fenhexamid [17]). FRAC codes are assigned by the Fungicide Resistance Action Committee and represent different modes of action.

Geranium Biorational Trials 1 and 2: Two geranium cultivars (highly susceptible Ringo 2000 Violet and moderately resistant Pinto Premium Orange) were treated 3 times at 7-day intervals, inoculated once at 1-day post 1st treatment. Disease was assessed 3 times at weekly intervals starting 7 days after 1st treatment (no. blighted leaves, no. blighted leaves with

sporulation), AUDPC). Botector, Prestop WG and Serifel were effective for both cultivars. Many of the biorational products effectively controlled *Botrytis* on 'Pinto Premium Orange.' Combining a less susceptible geranium cultivar with biorational products could be a successful strategy for growers desiring a sustainable strategy.

Petunia Biorational Trials 1 and 2: Susceptible 'Petunia Shock Wave Red' was evaluated with 10 biorational products for their effectiveness to control *Botrytis*. Plants were treated 3 times at 7-day intervals, inoculated once at 1-day post 1st treatment. Disease was assessed 3 times at weekly intervals starting 7 days after 1st treatment (0-10 rating scale). Prestop WP controlled *B. cinerea* on petunia compared to the untreated control. LifeGard, Zio and Botector limited disease and were similar to the fungicide standard. Biorational agents like Botector, Prestop, Serifel were effective for *Botrytis* disease control. Less susceptible cultivars coupled with biorational agents can effectively manage *Botrytis* disease in the greenhouse.

A greenhouse trial was established at MSU to evaluate the ability of foliar calcium sprays to prevent infection by *Botrytis* on poinsettia bracts with and without the addition of fungicides. Popular poinsettia cultivars 'Prestige Red,' 'Christmas Joy White,' and 'Mars Marble' received three weekly foliar applications of calcium chloride at a rate commonly used to control bract edge burn, a physiological disorder, on poinsettias. Prior to inoculation with a spore suspension of *B. cinerea*, poinsettia bracts were sprayed with site-specific fungicides with or without calcium chloride. Disease severity was assessed 12 days post-inoculation and results suggested that rate of calcium used did not provide significant protection against *Botrytis*.

3. Collect *Botrytis* isolates from infected plants in greenhouses representing different crops and test for resistance to the fungicides commonly recommended for protection.

Diseased poinsettia, geranium, and petunia plants were collected from the major production regions in the state of Michigan. Samples of diseased tissue were cultured, and a total of 288 isolates were single-spored and maintained in culture. A detached geranium leaf assay confirmed that 175 isolates were pathogenic.

An additional 52 isolates from the collection were screened for sensitivity to eight sitespecific fungicides using a germination-based assay. Results from both years indicate pathogen resistance to all major site-specific fungicides registered for use, with 50% of the isolates displaying resistance to five or more fungicides. Additionally, 17 isolates displayed resistance to every site-specific FRAC code registered for use. Resistance frequencies were highest for thiophanate-methyl [1] (95%), pyraclostrobin [11] (79%), iprodione [2] (71%), and boscalid [7] (63%), intermediate for cyprodinil [9] (43%) and fenhexamid [17] (39%), and lowest for fludioxonil [12] (24%) and fluopyram [7] (13%). Resistance frequencies were similar among production regions and crops.

DNA has been extracted from 288 isolates of *Botrytis* to date, with more in progress. The goal of this work is to ultimately identify common mutations that confer fungicide resistance. This information can also allow for the assessment of genetic differentiation based on crop, location, and time of year.

Control of gray mold includes sanitation, environmental manipulation, and fungicide applications. Due to high fecundity, genetic variability, and a wide host range, fungicide resistance has become common in *B. cinerea* populations. High resistance frequencies to several fungicides suggest that current resistance management strategies are insufficient for greenhouse-grown ornamentals.

4. Determine which fungicides can protect susceptible plants during propagation, shipping, and postharvest.

Research carried out for Objectives 1-3 has identified effective products for control of Botrytis blight. However, plans to incorporate them into trials testing their efficacy during propagation, shipping and postharvest activities were not carried out due to the COVID-19 pandemic.

5. Identify effective new fungicides and protection programs that include different modes of action for impatiens downy mildew for application in the greenhouse and determine longevity of protection in the landscape.

Current efficacy testing compared new and experimental fungicides against highly effective products identified in previous testing including Orvego (ametoctradin/dimethomorph [45/40]), Segovis (oxathiapiprolin [49]), Segway (cyazofamid [21]), and/or Subdue MAXX (mefenoxam) [4]). Broadform SC (fluopyram/trifloxystrobin [7/11]), picarbutrazox [U17, U=unknown], and two experimentals were tested against industry standards; 3 rates of Experimental 5 reduced sporulation of impatiens downy mildew. Three greenhouse spray programs were compared for disease control using highly effective products. High input program 1: Weekly treatments for weeks 1-5 alternating Subdue MAXX, Segovis, Segway, Orvego, Subdue MAXX + Segovis. Medium input program 2: Treatments every other week for weeks 1,3,5 alternating Subdue MAXX, Segovis, Subdue MAXX + Segovis. Low input program 3: One treatment on week 5, Subdue MAXX + Segovis. A trial compared impatiens cultivars treated in the greenhouse and planted into the landscape; cultivar Imara SCR remained disease-free at the end of the trial. These results were incorporated into the updated 2020 impatiens downy mildew control recommendations: Day 1-Subdue MAXX, Day 7-Segovis, before shipment-Subdue MAXX + Segovis. A downy mildew resistant cultivar may allow for one single fungicide application before shipping.

6. Test new plant-protection products, including reduced-risk fungicides, biopesticides, and biocontrols against root rot and foliar spots and blights.

Total trials testing products for efficacy against ornamental plant diseases are summarized in Objectives 2, 5, 6, and included 8 experimentals, 11 biopesticides, 5 reduced-risk products and 3 other registered products. In this Objective 6, five trials tested products for control of Phytophthora, Pythium, Thielaviopsis and Rhizoctonia root rots on 4 crops, calibrachoa, snapdragon, pansy, petunia. Trials evaluated varied dosages, application intervals and application methods (drench vs spray). Calibrachoa Phytophthora trial 1: No rate response was observed among all dosages of Experimental 5 which were similar to the untreated inoculated, while Segway and Segovis were similar to the untreated uninoculated. Calibrachoa Phytophthora trial 2: Broadform was not effective regardless of rate, interval or application method; Subdue MAXX and Segovis prevented plant death. Snapdragon Pythium trial: No rate response was observed among all dosages of Experimental 5 which were similar to the untreated inoculated, while Segway was similar to the untreated uninoculated but with some stunting. Pansy Thielaviopsis trial: Broadform SC and Experimental 6 were not effective regardless of rate, interval, or application method (drench vs. spray); Medallion (fludioxonil [12]) was moderately effective and 3336 EG (EG (thiophanate-methyl [1]) prevented disease/death. Petunia Rhizoctonia trial: Obtego (*Trichoderma asperellum/T. gamsii* [BM02]) was similar to the untreated inoculated regardless of dosage, number of applications or application interval; 3336 EG prevented disease (disease severity and plant death were statistically lower than the untreated inoculated).

7. Provide results and recommendations directly to producers and allied industries via publication in trade magazines, website updates, and presentations. YouTube videos and other novel approaches to communicate the highlights of this work broadly will be used to complement the more traditional extension activities.

Research was presented in 7 scientific publications included 2 scientific journal abstracts, 2 scientific journal articles (one in press), three Plant Disease Management Reports. Seven online extension publications included 1 article in the Proceedings of the Great Lakes Fruit, Vegetable and Farm Market Expo/Michigan Greenhouse Growers Expo, and 4 articles in the Floriculture & Greenhouse Crop Production and 1 in the Landscaping sections of the Michigan State University Extension News for Agriculture. Seven presentations included 1 international and 1 national scientific meetings, 3 extension meetings and 2 seminars. Due to the COVID-19 pandemic, 4 of these were virtual presentations.

The COVID-19 pandemic prevented sampling of diseased ornamental plants in Michigan commercial greenhouses in 2020; however, research was shifted to focus on the isolates that were already in the *Botrytis* collection. Sampling will resume in 2021.

Research has identified effective products for control of Botrytis blight. However, plans to incorporate these products into trials testing their efficacy during propagation, shipping and postharvest activities were not carried out due to the COVID-19 pandemic. Trials will resume in 2021.

FY 2020 Presentations

- 1. Harlan, B., and Hausbeck, M. 2020. Downy mildew: It's complicated. Michigan State University Plant Trials Virtual Field Day, Zoom Meeting, East Lansing, MI, 4 Aug.
- Shrestha, S. 2020. Management of Botrytis blight through host resistance and biorational products. Plant Pathology Master's Thesis Defense, Zoom Meeting, Michigan State University, East Lansing, 18 Aug.

- 3. Lukasko, N. and Hausbeck, M. 2020. Fungicide resistance frequencies in *Botrytis cinerea* from poinsettia greenhouses and calcium as an alternative disease control measure. Plant Health 2020, 10-14 Aug. Online.
- 4. Hausbeck, M.K., Harlan, B.R., and Brisco-McCann, E.I. 2019. Advances in *Botrytis* control for flower crops. IX International Symposium on New Ornamental Crops, Guadalajara, Mexico, 30 Sep-3 Oct.
- 5. Hausbeck, M. 2019. New solutions for floriculture problems. Great Lakes Farm, Fruit and Vegetable Expo/Michigan Greenhouse Growers Expo, Grand Rapids, MI, 10-12 Dec.
- 6. Lukasko, N., and Hausbeck, M. 2019. Managing Botrytis blight in greenhouse crops. Metro Detroit Flower Growers Association Meeting, Macomb, MI, 2 Oct.

FY 2020 Publications

- Hausbeck, M.K., and Harlan, B.R. 2020. Evaluation of the fungicide Broadform for the control of Phytophthora root rot on calibrachoa, 2019. Plant Disease Management Reports 14:V145.
- Shrestha, S., Harlan, B.R., and Hausbeck, M.K. 2020. Evaluation of experimental fungicides and biopesticides against Botrytis blight on poinsettia, 2020. Plant Disease Management Reports 14:OT017.
- Lukasko, N.T., Harlan, B.R., and Hausbeck, M.K. 2020. Evaluation of Broadform residual control of gray mold on poinsettia, 2020. Plant Disease Management Reports 14:OT016.
- Harlan, B.R., and Hausbeck, M.K. 2019. Residual control of downy mildew and Phytophthora root rot on ornamental crops with the novel fungicide oxathiapiprolin. Acta Horticulturae (in press).
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Shrestha, S. 2019. Susceptibility of geranium cultivars to Botrytis cinerea in the greenhouse. Abstract. Phytopathology 109(10S):S2.93.

- Lukasko, N., Harlan, B.R., and Hausbeck, M.K. 2019. Susceptibility of geranium and snapdragon cultivars to Pythium irregulare. Abstract. Phytopathology 109(10S):S2.93-94.
- Byrne, J., Hausbeck, M., Willbur, J., and Hammerschmidt, R. 2020. Ralstonia solanacearum race 3 biovar 2 detected in greenhouse geraniums. Michigan State University Extension News for Agriculture-Floriculture & Greenhouse Crop Production: 22 Apr. Online at https://www.canr.msu.edu/news/ralstonia-solanacearum-race-3-biovar-2-detected-in-greenhouse-geraniums.
- Hausbeck, M., Harlan, B., and Lopez, R. 2020. Don't let downy mildew on impatiens surprise you. Michigan State University Extension News for Agriculture-Floriculture & Greenhouse Crop Production: 9 Apr. Online at https://www.canr.msu.edu/news/dont-let-downy-mildewon-impatiens-surprise-you.
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- Hausbeck, M., and Harlan, B. 2019. Botrytis control tips for greenhouse ornamentals. Michigan State University Extension News for Agriculture-Floriculture & Greenhouse Crop Production: 24 May. Online at https://www.canr.msu.edu/news/botrytis-control-tips-forgreenhouse-ornamentals.
- Hausbeck, M.K., and Harlan, B.R. 2019. New solutions for floriculture disease problems. Pages 2-3 in: Greenhouse Session Summaries, Great Lakes Fruit, Vegetable and Farm Market Expo, Grand Rapids, MI, Dec. Online at https://glexpo.com/wpcontent/uploads/2019/12/Greenhouse-II-Tuesday-PM.pdf.