UNIVERSITY OF GEORGIA

College of Agriculture & Environmental Science

Accountability & Reporting

Hatch Project Outline Submission

Project Title Freeze protection in perennial fruit crops

Project Director Name Rachel Itle

Use this document to write out your Non-Technical Summary and Methodology before uploading to the Project Outline Submission Smartsheet form.

Helpful Hint: Project Outlines are most often rejected by the Associate Dean of Research due to insufficient detail and length of the Non-Technical and Methodology sections.

Non-Technical Summary (8,000 Character Limit)

A successful non-technical summary clearly states the project's focus and importance, using specific data to highlight the work's significance. Discuss the project's broader impact and alignment with larger goals, avoiding technical jargon and providing context where needed. Prioritize practical outcomes and benefits for broad understanding.

1. Issue and Importance

Spring Freeze/frost damage has affected blueberry production in Georgia on nearly an annual basis and these freeze events have cost the industry millions of dollars in losses in the last 10 years alone. Two large losses in recent years occurred in 2017 (approximately 85% crop loss) and in 2022 (approximately 50% crop loss). Current spring freeze protection methods include overhead irrigation and wind machines. Producers are currently employing each of these protection methods, with selection based on initial investment needs and the effectiveness of the freeze protection method. Most of the freezes that occur for blueberry production in Georgia can be mitigated using these methods.

The most widely used freeze protection method is overhead irrigation. This method relies on overhead sprinklers turned on right before freezing conditions begin and running until temperatures are above freezing. The continual coverage of plants with water allows for coating the plant in liquid water that will solidify into ice. The change of state of water from liquid water form to the solid ice form releases heat which protects the flower bud structures. Wind machines are also used to prevent freeze damage. This method rotates the air when temperatures from ground level to approximately 6ft are colder than air temperatures above the 6ft mark. This temperature phenomenon is known as a temperature inversion. Fans on the wind machines are used for air rotation / air mixing to raise the temperature surrounding the plant to non-freezing conditions (Williamson et al., 2012; Smith, 2019).

There are two major issues facing the Georgia blueberry industry for freeze protection. First, there are limited insurance discounts, and second are limitations offered by current freeze protection methods. Insurance companies offer growers coverage discounts for farms based on various aspects of a farm. One area that has discounts associated with it is the area of freeze protection methods used. Despite the use of wind machines as a documented method of freeze protection in blueberries for use in Florida and Georgia (Williamson et al., 2012; Smith et al., 2017; Smith, 2019), insurance companies continue to limit and drop coverage discounts when wind machines are used as a freeze protection method on blueberry farms as compared to overhead irrigation. Overall, this can increase a grower's insurance coverage upwards of 30% (S. Wade, personal communication). With insurances continuing to reduce or eliminate coverage discounts for wind machines as freeze protection method, a large financial burden is placed on the growers. There are limited data collected comparing the effectiveness of the various freeze protection methods. Gathering data comparing the two main freeze protection methods in Georgia, overhead irrigation and wind machines, would give the growers some baseline data to use in their conversations with insurance companies to help in recovering coverage discounts.

The second major issue facing freeze protection in blueberry production in Georgia is that current methods are limited in their deployment due to initial cost of establishment and amount of acreage that can be protected at one time, in addition to inherent limitations of the current methods used. For example, high wind levels that do not allow for adequate water coverage and freezing on the plants with overhead irrigation methods. Also, very low temperatures that are outside of the range of protection offered by overhead irrigation and wind machine air movement, among others. Researching new freeze protection strategies would be very impactful and of great importance for the industry overall. Among possible new freeze protection methods are the use of new natural, biodegradable compounds which can be sprayed in the orchards before the freeze begins. These compounds can reduce implementation costs and increase the deployment of protection before a freeze.

Once the biodegradable compounds are sprayed on the plants they create a barrier, similar to a person putting on a heavy winter coat when temperatures drop, that protects the plant from freezing temperatures. The compounds are made from naturally occurring plant-based material called cellulose. Cellulose is the most abundant natural substance that can be extracted from plants and trees, such as wood pulps, cotton, among other natural plant sources. The cellulose is then processed into very tiny particles, to what is known as a nanoscale. Once this process is completed, the cellulose is referred to as “nanocellulose”. Nanocellulose works to protect the plant from freezing temperatures through its very low thermal conductivity. A low thermal conductivity means that the nanocellulose has a low ability to transfer heat, and it acts as an excellent barrier (insulating material) between the plant and the freezing temperatures (Uetani and Hatori, 2017). It is also important to note that nanocellulose is highly water soluble making it biodegradable.

Nanocellulose is currently being tested on fruit crops including sweet cherry, grape (Clark, 2021) and apple (Whiting, 2019) by colleagues at Washington State University. Once the nanocellulose is sprayed onto the plant, it dries and leaves a thin film over the plant that creates a protective layer against cold temperatures. Their research on these crops currently suggests that the nanocellulose sprays “improves cold-hardiness of sweet cherry and grape buds by about 2 to 4 °C [3.6°F to 7.2°F] compared to non-treated buds” (Clark, 2021). These technologies have the potential to reduce or eliminate devastating crop losses as a result of late spring freezes. Currently, there is no known work on how nanocellulose sprays may be used to protect blueberry flowers from freezing temperatures. These technologies have the potential to reduce or eliminate devastating crop losses as a result of late spring freezes.

2. Goals and Objectives

There are two main overall goals of this project:

a) Evaluate and compare the effectiveness of overhead irrigation and wind machines as freeze protection methods
b) Identify new frost protection strategies through nanocellulose spray applications

3. Target Audiences and How They Will Benefit

The target audiences are blueberry growers and members of the commercial blueberry industry. Results from these projects will be able to be used to: 1) aid in decision making on which traditional freeze protection method to purchase, 2) provide baseline information on effectiveness of traditional freeze protection methodology to insurance companies, and 3) begin exploring new and alternative freeze protection methods instead of or in addition to traditional overhead and wind machine methods.

4. How Activities Lead to Outcomes

The results generated from this project will help to provide the blueberry industry with objective data comparing the two main freeze protection methods, which currently is not available. Results will also help to guide the use of alternative freeze protection methods.

References

Clark, B. 2021. Cellulose nanocrystals insulate crops against frost damage. 9 Feb. 2021. Accessed 19 Mar. 2021. https://news.wsu.edu/2021/02/09/cellulose-nanocrystals-insulate-valuable-crops-frost-damage/

Smith, E.D. 2019. Cold hardiness and options for the freeze protection of southern highbush blueberry. Agriculture. 9(1) 13pp.

Smith, E.D., T. Coolong, and P. Knox. 2017. Commercial freeze protection for fruits and vegetables. UGA Cooperative Extension Bulletin 1479. 12pp.

Uetani, K. and K. Hatori. 2017. Thermal conductivity analysis and applications of nanocellulose materials. Sci Technol Adv Mater. 18(1):877-892.

Whiting, M. 2019. Reducing cold damage in apple and sweet cherry. Feb. 2019. Accessed 19 Mar. 2021. http://treefruit.wsu.edu/article/reducing-cold-damage-in-apple-and-sweet-cherry/

Williamson, J.G., P.M. Lyrene, and J.W. Olmstead. Protecting blueberries from freezes in Florida. UF IFAS Cooperative Extension Bulletin HS968. 7pp.

Methodology (8,000 Character Limit)

A successful methodology statement describes the ways in which the project will be conducted, emphasizing scientific methods and any unique aspects or significant departures from usual methods. This includes research settings, equipment, and tools—particularly any specialized instruments—research objectives and tasks, experimental procedures, data collection methods, and statistical analysis techniques for each task, aligning outcomes with project goals.

1. Comparing Traditional Freeze Protection Methods: Overhead Irrigation and Wind Machines

Plantings of major commercial cultivars of the two main blueberry types, southern highbush and rabbiteye, will be established at the Westbrook Farm on the University of Georgia Griffin Campus. Three separate field plots will be established under three conditions: 1) overhead irrigation freeze protection, 2) wind machine freeze protection, and 3) no freeze protection method will be compared for their ability to protect flower bud development as well as fruit set. Plots will be established outside of the coverage area of the opposing freeze protection method to prevent one influencing the other. Plants will be subjected to natural freezes occurring throughout the winter/spring season (February – April) yearly and will be evaluated before and after each freeze event. On each plant, five branches will be randomly selected for evaluation. Branches will be evaluated from tip of the branch downward for approximately 8in in length to capture the majority of the flower buds present. Flower buds will be counted and staged following the standard commercial blueberry flower bud staging guide from Michigan State University (MSU Blueberry Growth Stages). For each freeze event, an initial healthy flower bud assessment (pre-freeze) will be conducted. After each freeze event, branches will be evaluated three to five days after the freezing temperatures have finished to assess damage. Damage will be assessed following each freezing event in the same way. Fruit set on each branch will be counted when the fruit are in green fruit to fruit coloring stage. The three conditions of 1) overhead irrigation freeze protection, 2) wind machine freeze protection, and 3) no freeze protection method will be compared for their ability to protect flower bud development as well as fruit set. All evaluations will be non-destructive, visual observations on the plant and will not be cut or removed from the plant for assessment. During harvest season, plants will be evaluated for yield. In addition, fruit evaluated for basic fruit quality measurements including berry size, fruit texture (skin strength and fruit firmness), total soluble solids content (°brix), total titratable acid content (%TTA), and sugar acid ratio (°brix/%TTA).

2. Evaluating Potential New Freeze Protection Methods: Nanocellulose

To evaluate nanocellulose compounds in a laboratory setting to reduce cold damage of blueberry, branch cuttings from the two main blueberry types, southern highbush and rabbiteye, will be collected from UGA Blueberry Research and Demonstration Farm in Alma, GA. Plants are managed following recommended commercial guidelines. At least one southern highbush and one rabbiteye variety will be selected for evaluations. Samples will be collected during the winter/spring seasons, with collections starting in January and continuing until March, and collections will be performed weekly to capture different stages of floral bud development. For each sampling date, approximately 100 stems per variety/type will be collected. Samples will be transported to the UGA Griffin Campus, Griffin, GA in a cooler with ice or shipped overnight. Samples will be then kept in a refrigerator at 4 °C for no longer than a day before processing. Samples will be prepared in 5cm stems sections with buds. Stems will be cut into desired lengths and wrapped in a damp tissue paper at the cut end to prevent wilting. Based on previous initial work, nanocellulose applications and treatments will be selected for further study to evaluate if blueberry freezing tolerance can be improved upon. Nanocellulose treatments will focus on further examination of number of applications, spray drying time, and combination with hydrophobic compounds. All samples will undergo the freezing tolerance test in the UGA cold hardiness laboratory in Griffin, GA.

Freezing tolerance testing will be done with at least ten temperature treatments ranging from 4 °C (control; 39°F) to -24 °C (-11°F). Treated branch sections will be prepared and sealed in a plastic bag, with each plastic bag representing a temperature treatment. Bags containing samples will be hung on metal rods and set in the freezing chamber (Temperature and humidity chamber PR-3FPH, Tabai ESPEC; Osaka, Japan). Control samples will be left in the refrigerator at 4 °C. Freezing tests will be started by decreasing the temperature in the freezing chamber at a rate of 3 °C/hour (as in nature air temperature usually decreases 1-2 °C/hour) from -2 °C until it reaches -24 °C. Each bag will be rapidly removed from the freezing chamber when the temperature inside the chamber reaches their assigned temperature treatment. After removal, bags will be placed in a refrigerator at 4°C to thaw.

Samples visually scored after damage has been allowed to develop after approximately one week. Samples will be kept in a refrigerator until they will be dissected and visually rated as alive or dead. Buds and stems of each temperature treatment will be cut with a razor blade and visually inspected for brown discoloration under the stereo microscope. The buds with browning color will be scored as dead, while buds that remained green and without any discoloration will be scored as alive. For stems, a small piece of each stem will be sliced off longitudinally with a razor blade to evaluate the discoloration of the cambium and the phloem. Stems with yellow and brown colored cambium and phloem will be rated as dead, while stems that remain green will be considered alive and free from injury. For each temperature treatment, 12 buds (three buds per stem) and four stems will be evaluated for each cultivar and temperature treatment. The number of dead or alive buds or stems of each temperature treatments will be recorded. Data will be analyzed to determine the LT50, i.e. the temperature at which 50% of buds or of stems were killed by freezing temperatures.

Continue Methodology

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Hatch Proposal Checklist

Put an X in the blue box for the checklist below that applies to your Project Outline situation.
Faculty, notice your PI responsibilities.

PUT X IN BOX

X	New Hatch Project	
✓	WHO	STEP
	PI	Prepare Project Outline
	PI	Peer-Review Approval of Project Outline
	DH	Dept Head Reviews/Approves Project Outline
	PI	Submit Project Outline & Dept Head Approval to ABO
	AD	AD Research Reviews/Approves Project Outline
	ABO	ABO Submits Project Outline in NIFA NRS
	NIFA	NIFA Reviews/Approves Project
	PI	Submit Progress Report Annually to ABO
	ABO	ABO Submits Progress Report in NIFA NRS
	PI	Submit Final Report to ABO
	ABO	ABO Submits Final Report in NIFA NRS

PUT X IN BOX

	New Multistate Project	
✓	WHO	STEP
	PI	Get Dept Head Approval to Join MRP
	PI	Petition MRP Committee to Participate
	PI	Request AD Office Submit Appendix E
	PI	Submit Project Outline & Dept Head Approval to ABO
	AD	AD Research Reviews Project Outline
	ABO	ABO Submits Project Outline in NIFA NRS
	NIFA	NIFA Reviews/Approves Project
	PI	Submit Progress Report Annually to MRP Committee
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PUT X IN BOX

	Renewing Hatch Project	
✓	WHO	STEP
	PI	Make Minor Updates to Existing Approved Project
	DH	Dept Head Reviews Updated Project
	PI	Submit Dept Head Approval & Updated Project Outline to ABO
	AD	AD Research Reviews Updated Project
	ABO	ABO Submits Project Outline in NIFA NRS
	NIFA	NIFA Reviews/Approves Project
	PI	Submit Progress Report Annually to ABO
	ABO	ABO Submits Progress Report in NIFA NRS
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PUT X IN BOX

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ACRONYM KEY:

ABO .....Ag Business Office Accountability & Reporting
AD.....Associate Dean of Research
DH.....Dept Head
MRP.....Multistate Research Project
NIFA.....National Institute of Food and Agriculture
PI .....Principle Investigator

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HATCH PROJECT OUTLINE

GEORGIA AGRICULTURAL EXPERIMENT STATIONS

Submit completed form to: **nifahelp@uga.edu**

NOTE: CAES's ABO Accountability & Reporting submits your proposal into NIFA's National Reporting System on your behalf.

Choose one: X Hatch

__ Hatch Multistate → Multistate # from NIMSS _____

Find # at www.nimss.org.

NOTE: For Multistate Projects, Appendix E must be processed for Project Director and an Co-PIs with the Associate Dean for Research Office **BEFORE** submitting this form.

Primary Critical Issue – Choose ONLY ONE that most closely aligns with your research. (These are the primary reporting categories used for CAES and CFACS NIFA reporting.) **See Critical Issues here for descriptions.**

__ Animal Production	X Plant Production
__ Food Safety & Quality	__ Sustainability, Conservation & the Environment
__ Health & Wellness	__ Urban Agriculture
__ Community, Home & Life Skills	__ Youth & Family Development

Project Title: Field Applied Management Solutions for Control of Bitter Rot and Glomerella Leaf Spot of Apple

Date of Initiation: 10/01/2024 (MM/DD/YYYY)

Estimated Date of Completion: 09/30/2029 (MM/DD/YYYY) Not to exceed 5 years

Project Director: Phillip Brannen
List first and last name

UGA Co-Project Directors:
If applicable, provide first and last name of co-project directors.

Department: Plant Pathology

Non-Technical Summary — In lay terms, briefly describe the following: (1) the issue and why it is important, (2) your goal and objectives, (3) the target audiences and how they will benefit, and (4) how your activities lead to the outcomes described in the goal statement or objectives. *Limit of 8,000 characters (including spaces).*

Justification

Though apple production in Georgia ranks relatively low in total acreage, apples are an important economic driver in the northern Georgia region due to agritourism, especially in the autumn months. One of the primary threats to this industry is the rapid spread of Glomerella leaf and fruit spot (GLS), a fungal disease caused by *Colletotrichum fructicola*, as well as other *Colletotrichum* species. This pathogen has recently caused significant damage to apple cultivars with 'Golden Delicious' heritage. Without adequate control, leaf spots can result in complete and premature defoliation of the tree. The pathogens can also spread to the fruit, covering the apples in black spots which reduce grade and sales. Bitter rot (BR), also caused by several *Colletotrichum* species, remains one of the primary diseases of apple in hot, wet environments, so this disease will continue to be a major determinant of apple yields in Georgia.

In 1998, GLS was first identified in the United States when a large outbreak occurred in eastern Tennessee orchards (González and Sutton 1999). Although this was the first official description of GLS in the USA, a strain of *Colletotrichum* described by Taylor (1971) as causing similar leaf spots and defoliation was widespread much earlier in the Piedmont and Coastal plain of Georgia. While the leaf symptoms of Taylor's disease are consistent with the GLS common in Brazil, this disease produced much larger lesions on the fruit (up to 20 mm). However, the fruit symptoms observed today in Georgia orchards generally appear consistent with the disease commonly described in Brazil.

To reiterate, bitter rot has been a primary and major pathogen of Georgia apples throughout the history of the industry. In contrast, though GLS has been periodically observed in Georgia for over 30 years, it had not impacted the vast majority of apple orchards. This changed dramatically in 2022, as virtually every orchard in Georgia was impacted to some degree. Losses in some of the most widely planted cultivars like 'Golden Delicious' and 'Gala' were near 100%. Without better control strategies, the apple industry in Georgia is now threatened with potentially severe annual losses. The combination of these two *Colletotrichum* diseases creates a significant threat to the viability of Georgia apple production, so there is a strong need for applied research to address both diseases.

Currently, the primary method of controlling both BR and GLS is through fungicide applications, especially quinone outside inhibitors (QoI). Although these fungicides are effective, they come with a high risk that fungal populations will develop resistance and, therefore, leave growers with few options to combat the disease. Preliminary data from one Georgia orchard in 2022 found the G143A resistance mutation in 16 out of 17 *Colletotrichum spp.* isolates. Based on this information, it is hypothesized that the G143A mutation is at least in part contributing to the increasingly severe outbreaks in Georgia. At a minimum, resistance to QoI and other fungicide classes should be monitored over time, as control failures can occur when fungicides cease activity.

In summary, *Colletotrichum* is a diverse genus that contains species pathogenic to several economically important fruit crops, of which apple is one. More than a dozen *Colletotrichum* spp. have been found to cause both bitter rot and/or GLS. Because they thrive in hot, humid weather, Georgia provides an ideal climate for survival and spread. The impact of these pathogens to the Georgia apple industry must be addressed with new fungicides, and for those that are currently utilized, the potential for resistance to fungicides makes monitoring an important part of the disease management strategy.

Objectives

Based on the need for field-applied solutions to manage both bitter rot and Glomerella leaf spot of apple, this study includes the following objectives:

1. Evaluate the effectiveness of new fungicides and fungicide programs against bitter rot and Glomerella leaf spot in an orchard setting.
2. Monitor the pathogens causing bitter rot and Glomerella leaf spot in Georgia orchards for fungicide resistance development over time, especially to the QoI fungicide class.

Methodology — Describe the ways in which the project will be conducted, with emphasis on the general scientific methods and any unique aspects or significant departures from usual methods. *Limit of 8,000 characters (including spaces).*

Methodology

Background

One way of controlling both BR and GLS is through resistant cultivars. For BR, though all apples seem to be susceptible to some degree, there are cultivars that are less susceptible. For GLS, this can be a simple solution, since most apple cultivars without 'Golden delicious' in their heritage are completely resistant. Additionally, a few cultivars with 'Golden delicious' heritage have been bred for resistance to GLS while having similar agronomic traits as susceptible cultivars. While resistant cultivars are a promising solution, they do not solve the current problem with either disease, as susceptible cultivars make up a significant portion of the apple trees in the ground today. While Georgia producers grow a wide variety of apples for the fresh market, roughly half of the production area is planted to susceptible cultivars. Therefore, growers must use other forms of management to keep BR and GLS at bay.

The main method of controlling BR and GLS on susceptible cultivars is through fungicide applications. Typically, growers use a combination of multi-site and single-site fungicides in rotation or as tank mixes. The multisite fungicides captan and mancozeb are widely used, since they are effective against a number of apple diseases and carry low potential for resistance development. These fungicides must be applied preventatively since they work by inhibiting conidial germination on the leaf surface. Since captan and mancozeb are non-systemic, most of the product will be washed off by rain and needs to be reapplied frequently. Although mancozeb is highly effective against *Colletotrichum* diseases, its use is limited to the spring due to the 77-day pre-harvest interval. Captan is therefore the backbone of many growers' late-summer and fall spray programs, often mixed with phosphorous acid or ziram for added protection.

Several single-site fungicides are also commonly used against BR and GLS. Due to the systemic nature of these fungicides, each application lasts longer than the protectant fungicides previously mentioned. These products can be highly effective, but because of their single-site mode of action, they carry a risk of resistance development in the pathogen population. The Fungicide Resistance Action Committee (FRAC) has categorized fungicides based on their specific modes of action, providing FRAC codes for various classes. For control of BR and GLS, the FRAC 11 quinone outside inhibitor (QoI) class is the most widely used and most efficacious fungicide class; it includes pyraclostrobin, trifloxystrobin, and kresoxim-methyl. These fungicides can provide curative control for the diseases, but they are commonly applied prior to infection, since they also inhibit conidial germination. In fact, these have been shown to be some of the most effective fungicides in field trials in Georgia. The G143A mutation has been consistently associated with qualitative resistance to QoI fungicides, whereas F129L and G137R have been found to provide partial resistance. As reported above, preliminary data from one Georgia orchard revealed in vitro resistance to pyraclostrobin and found the G143A mutation in 16 out of 17 samples tested. Because GLS incidence has increased despite the use of fungicides, we hypothesize that the G143A mutation is prevalent throughout Georgia orchards and is potentially leading to these outbreaks.

Approach

Objective 1. Evaluate the effectiveness of new fungicides and fungicide programs against bitter rot and GLS in an orchard setting.

Treatments utilizing various fungicides and/or regimens with varying modes of action will be evaluated for control of BR and GLS. The trials will take place in blocks of 'Royal Empire' and 'Golden Smoothie' apple trees located at the Georgia Mountain Research and Education Center (Blairsville, GA), with the 'Royal Empire' used for BR and the 'Golden Smoothie' utilized for GLS. All treatments will be identical for the first seven applications, but beginning at the first cover spray, treatments will be separated into 1) an untreated control, 2) a highly varied standard treatment regimen including fungicides with known efficacy against BR or GLS while incorporating good resistance management techniques of rotation and tank-mixing, 3) a fungicide or fungicide regimen with presumptive activity against the pathogen of interest, and so on for up to 6-7 treatments per trial. Treatments will be applied with an airblast sprayer (100 gal/A spray volume) at each application date. The experimental design will be a randomized complete block, with each plot consisting of five trees and treatments replicated five times. To reduce the effects of off-target spray drift, unsprayed rows will be left between each spray row and only the center three trees of each plot will be used for disease evaluation. All cultural practices will be in keeping with apple production methods commonly observed throughout the Southeast. Precipitation will be recorded for the duration of the trial. Incidence and severity of BR and GLS will be recorded for a total of 25 fruit or leaves randomly collected from the three center trees from each plot. Incidence will be measured as the number of fruit or leaves with one or more spots and severity will be measured as the average number of spots per fruit or leaf. JMP Pro 16 (SAS Institute, Inc., Cary, NC) will be utilized for data analysis, and Student's t test will be utilized for treatment means separation. Appropriate statistical transformations will be utilized as needed.

Objective 2. Monitor the pathogens causing both bitter rot and Glomerella leaf spot in Georgia orchards for fungicide resistance development over time, especially to the QoI fungicide class.

Fungicide resistance will be monitored by collecting fruit and/or leaves with symptoms of BR or GLS from commercial orchards. QoI fungicides will be prioritized for resistance testing. Resistance will be tested through the Molecular Diagnostic Lab in Tifton, GA. The lab uses the following basic protocol, provided by Dr. Alejandra Maria Jimenez-Madrid. Pathogens will be isolated from symptomatic tissue and grown on artificial nutrient media. Media plates will be incubated for several days at the ideal temperature to obtain pure cultures. Pure isolates will be transferred to fresh fungicide amended media plates with a predetermined discriminatory dose. A non-amended media will be used as a control. The plates will be incubated for 3-4 days to evaluate the fungal growth on fungicide amended and non-amended control. For QoI fungicides, sequence analysis of the cytochrome b gene will be carried out to test for known mutations (G143A, F129L, G137R) responsible for resistance phenotypes (Yin et al. 2023). Additional monitoring for resistance (e.g. SDHI) will be coordinated on an ad hoc basis with the Molecular Diagnostic Lab.

References

González, E., and Sutton, T. B. 1999. First report of Glomerella leaf spot (*Glomerella cingulata*) of apple in the United States. Plant Dis. 83:1074.

Leite, R. P., Tsuneta, M., and Kishino, A. Y. 1988. Ocorréncia de mancha foliar de Glomerella em maicieira no estado do Paraná. Fundação Institito Agronômico do Paraná. Informe de Pesquisa, 81.

Sutton, T. B., and Sanhueza, R. M. 1998. Necrotic leaf blotch of Golden Delicious-Glomerella leaf spot: A resolution of common names. Plant Dis. 82:267-268.
Taylor, J. 1971. A necrotic leaf blotch and fruit rot of apple caused by a strain of *Glomerella cingulata*. Phytopathology 61:221-224.
Yin, Y., et al. 2023. Fungicide Resistance: Progress in Understanding Mechanism, Monitoring, and Management. Phytopathology. 113:707-718.

Integrated Activities

Is this an **integrated** research and extension project? X YES ___ NO

AREERA 204 (Integrated Research-Extension activities: A jointly planned, funded, and interwoven activity between research and extension to solve a problem; this includes the generation of knowledge and the transfer of information and technology.)

If **YES**, leave below statement unedited or provide a brief description of **what makes this an integrated activity.** Explain how research findings/tools will be shared with farmers, industry, homeowners, etc. *(1-2 sentences/limit of 4,000 characters including spaces)*

Research findings will be shared through in-service trainings and field days for extension personnel. Once appropriately trained, extension personnel will disseminate information via workshops, presentations, e-studies, educational materials, and audio-visual mediums.

Research Effort Categories *(Applies to this project specifically, not your overall appointment — **Must total 100%.**)*

Basic Research ___ % Applied Research 100 % Developmental Research ___ %

Animal Health Component ___ % This is subject to PI's discretion. See Animal Health Research explanation here.

Multistate Activities

Is this a multistate project? *AREERA 104 (Research multistate project)* ___ YES X NO

If **YES**, leave the statement below unedited or provide a brief description of what makes this a multistate activity.
(1-2 sentences/limit of 4,000 characters including spaces)

This project is made up of collaborative efforts of programs from institutions located in at least two or more states. Our state collaborates towards objectives and outcomes. Evidence of this multistate collaboration is documented in the NIMSS system (see Multistate# referenced).

Classifications — Your project must have **at least one row filled** with KA, SOI, and FOS. **Only one number per field**. Extra rows are provided, if you wish to break down your classifications further. ***Must total 100%.*** See this reference guide to identify the appropriate classification areas for your project. **NOT required for multistate project** *since already reported on NIMSS project.*

Knowledge Area	Subject of Investigation	Field of Science	Percent
212. Pathogens and Nematodes Affecting Plants	4020 Fungi (includes yeast)	1160 Pathology	100

Assurances

Are **human** subjects involved? ____ YES X NO

If **YES**, is the project exempt from Federal Regulations? ____ YES ____ NO

If **YES**, select the appropriate exemption category. ____ 1 ____ 2 ____ 3 ____ 4 ____ 5 ____ 6

See this Exemption Category descriptions here.

IRB approval date _____ (MM/DD/YYYY)

Are **vertebrate animals** used? ____ YES ____ NO

If **YES**, IACUC approval date _____ (MM/DD/YYYY)

Submit your **completed Project Outline** and
approved Department Head Approval Form to:

nifahelp@uga.edu

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Hatch Proposal Checklist

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PUT X IN BOX

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	ABO	ABO Submits Project Outline in NIFA NRS
	NIFA	NIFA Reviews/Approves Project
	PI	Submit Progress Report Annually to MRP Committee
	PI	Submit Progress Report Annually to ABO
	ABO	ABO Submits Progress Report in NIFA NRS
	PI	Submit Final Report to ABO
	ABO	ABO Submits Final Report in NIFA NRS

PUT X IN BOX

	Renewing Hatch Project	
✓	WHO	STEP
	PI	Make Minor Updates to Existing Approved Project
	DH	Dept Head Reviews Updated Project
	PI	Submit Dept Head Approval & Updated Project Outline to ABO
	AD	AD Research Reviews Updated Project
	ABO	ABO Submits Project Outline in NIFA NRS
	NIFA	NIFA Reviews/Approves Project
	PI	Submit Progress Report Annually to ABO
	ABO	ABO Submits Progress Report in NIFA NRS
	PI	Submit Final Report to ABO
	ABO	ABO Submits Final Report in NIFA NRS

PUT X IN BOX

	Renewing Multistate Project	
✓	WHO	STEP
	PI	Make Minor Updates to Existing Approved Project
	DH	Dept Head Reviews/Approves Updated Project
	PI	Submit Dept Head Approval & Updated Project Outline to ABO
	AD	AD Research Reviews Updated Project
	ABO	ABO Submits Project Outline in NIFA NRS
	NIFA	NIFA Reviews/Approves Project
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	ABO	ABO Submits Progress Report in NIFA NRS
	PI	Submit Final Report to ABO
	ABO	ABO Submits Final Report in NIFA NRS

ACRONYM KEY:

ABO.....Ag Business Office Accountability & Reporting
AD .....Associate Dean of Research
DH .....Dept Head
MRP .....Multistate Research Project
NIFA .....National Institute of Food and Agriculture
PI.....Principle Investigator

College of Agricultural & Environmental Sciences
UNIVERSITY OF GEORGIA

HATCH PROJECT OUTLINE

GEORGIA AGRICULTURAL EXPERIMENT STATIONS

Submit completed form to: **nifahelp@uga.edu**

NOTE: CAES's ABO Accountability & Reporting submits your proposal into NIFA's National Reporting System on your behalf.

Choose one: X Hatch

Hatch Multistate → Multistate # from NIMSS

Find # at www.nimss.org.

NOTE: For Multistate Projects, Appendix E must be processed for Project Director and an Co-PIs with the Associate Dean for Research Office **BEFORE** submitting this form.

Primary Critical Issue – Choose ONLY ONE that most closely aligns with your research. (These are the primary reporting categories used for CAES and CFACS NIFA reporting.) **See Critical Issues here for descriptions.**

Animal Production	X Plant Production
Food Safety & Quality	Sustainability, Conservation & the Environment
Health & Wellness	Urban Agriculture
Community, Home & Life Skills	Youth & Family Development

Project Title: Tackling Soil-Borne Fungal and Oomycete Pathogens in Vegetable Production through Integrated Fundamental and Translational Studies

Date of Initiation: 10/01/2024 (MM/DD/YYYY)

Estimated Date of Completion: 09/30/2029 (MM/DD/YYYY) Not to exceed 5 years

Project Director: Miaoying Tian
List first and last name

UGA Co-Project Directors:
If applicable, provide first and last name of co-project directors.

Department: Plant Pathology

Non-Technical Summary — In lay terms, briefly describe the following: (1) the issue and why it is important, (2) your goal and objectives, (3) the target audiences and how they will benefit, and (4) how your activities lead to the outcomes described in the goal statement or objectives. *Limit of 8,000 characters (including spaces).*

Phytophthora capsici and *Fusarium oxysporum* f. sp. *niveum* (FON) are two devastating soil-borne plant pathogens that pose significant threats to vegetable production in Georgia and worldwide. *P. capsici* is a broad-host-range oomycete capable of infecting over 50 plant species in the Solanaceae, Cucurbitaceae, Fabaceae, and Brassicaceae families, with cucurbits and peppers being particularly susceptible. It can infect all parts of susceptible vegetable crops, leading to potential losses of up to 100%. Current disease management strategies rely heavily on fungicides and cultural practices, but these approaches have shown limited effectiveness and come with high production costs. The arsenal of fungicides available for *P. capsici* control is limited, and their repetitive use contributes to the emergence of fungicide-resistant strains. The use of disease-resistant varieties would be the most effective approach, but they are either scarce or entirely absent depending on the specific crop species. FON is a host-specific fungal pathogen responsible for Fusarium wilt in watermelon. Managing this disease is extremely challenging due to the long-term survival of chlamydospores in the soil and the emergence of new races. Currently, four races have been identified, including races 0, 1, 2, and 3, prevalent in major watermelon production regions. While watermelon cultivars resistant to races 0 and 1 exist, they do not confer resistance against races 2 and 3, which predominate in Georgia. The genetic basis underlying the increased virulence and aggressiveness of these new races remains unknown, hampering the

development of a rapid and reliable race-specific diagnostic assay and impeding progress in disease-resistant breeding efforts.

Given the inadequacy of current control measures against these two soil-borne pathogens, this project aims to pioneer novel and effective control methods by deepening our understanding of host-pathogen interactions. The overall goals include unraveling the molecular and genetic basis underpinning plant resistance and pathogen virulence and meanwhile developing disease-resistant varieties. Specific objectives include: 1) Identification of pivotal pathogenicity factors of *P. capsici* and their corresponding hosts targets; 2) Discovery of genetic factors defining differential virulence in various races of FON, essential for precise molecular diagnostics and advanced disease management; 3) Screening of diverse plant germplasm to uncover new sources of disease resistance; 4) Identification and characterization of genes pivotal in conferring resistance or susceptibility against both *P. capsici* and FON; and 5) Breeding disease-resistant varieties using CRISPR/Cas mediated genome editing technology. Through these concerted efforts, the project aims to forge a pathway towards sustainable and effective management of these challenging agricultural pathogens.

The research targets a diverse audience including plant pathology researchers, seed companies, breeders, agricultural industry, smallholder farmers, and policy makers. The insights gained from this research will serve as a foundation for plant pathology researchers to advance future studies and enhance the overall understanding of plant-pathogen interactions. Seed companies and breeders will benefit from the developed information and tools to expedite the breeding and commercialization of new varieties resistant to *P. capsici* and specific races of FON. Agricultural industry and smallholder farmers will benefit from disease-resistant varieties developed through this research as adoption of these varieties is expected to reduce crop losses and production costs thus enhancing profitability and sustainability. Policy makers can utilize the findings to shape policies and recommendations related to genome edited crops for sustainable disease management.

The expected outcomes of this project include scientific advancement, new disease management solutions, and skilled STEM workforce. Through activities addressing specific objectives 1, 2 and 4, the project anticipates uncovering key genes involved in pathogenicity of *P. capsici*, genetic markers defining virulence traits of FON races, and genes pivotal in conferring resistance or susceptibility against both pathogens. These findings promise to substantially enhance our understanding of molecular mechanisms governing pathogen virulence and host resistance, not only for these two pathogens but also for related plant pathogenic oomycetes and fungi. Dissemination of these findings through conference presentations and open-access publications will benefit scientific communities worldwide, laying the groundwork for innovative, mechanism-based disease control strategies. The activities addressing specific objectives 3 and 5 are expected to yield tangible outcomes in the form of commercially viable varieties that reduce crop losses and production costs, thereby promoting sustainability in agriculture. Furthermore, the project will provide training opportunities to undergraduate, graduate students and postdoctoral researchers, contributing to a sustainable workforce capable of addressing future challenges in agriculture and life sciences.

Methodology — Describe the ways in which the project will be conducted, with emphasis on the general scientific methods and any unique aspects or significant departures from usual methods. *Limit of 8,000 characters (including spaces).*

Objective 1 identification of pivotal pathogenicity factors of *P. capsici* and their corresponding host targets.
Key pathogenicity factors and their host targets are ideal targets to develop novel mechanism-based chemical and genetic control. Many studies have shown that pathogen effectors play key roles in pathogenicity. The genome of *P. capsici* encodes hundreds of apoplastic and cytoplasmic effectors. We will select five to ten candidate effector genes to determine their role in pathogenicity. The genes selected will include those that are most abundantly expressed and highly induced during infection, and the orthologs of the conserved effectors with a confirmed role in virulence determined in other *Phytophthora* spp. Published and newly generated mRNA-seq data will be utilized to identify the candidate genes. Their expression during infection will be further tested by RT-qPCR. Mutants of the selected genes will be generated using CRISPR/Cas9-mediated gene editing via *Agrobacterium*-mediated transformation [1, 2], with which we have successfully transformed *P. capsici* using a construct expressing GFP. Single zoospore derived mutants together with the wild-type strain will be inoculated on seedlings and fruit of pepper and cucurbits to determine the roles of the target gene in pathogenicity. For the genes confirmed to play a significant role in *P. capsici* pathogenicity,

its host targets will be identified using a yeast two-hybrid screen or co-immunoprecipitation followed by confirmation of interactions using at least two independent approaches commonly used for detecting biomolecular interactions.

Objective 2 Discovery of genetic factors defining differential virulence in various races of FON.
Identification of the molecular underpinnings of differential virulence in various FON races is a critical step to precise diagnostics and targeted breeding and deployment of disease resistant varieties. Many studies on *F. oxysporum* species complex and other fungi suggest that variations of effectors determine host specific pathogen infection. We will collect over 150 isolates and phenotype their virulence and aggressiveness on an expanded panel of watermelon varieties that include the differential cultivars previously used for race identification. Around 100 isolates with at least 20 representing each of the four races will be selected for whole genome sequencing followed by analyses of effector profiles, i. e. presence/absence and sequence polymorphisms of 104 candidate effectors identified in *59 F. oxysporum* genomes containing diverse *formae speciales* [3]. Association analyses will be performed to identify specific effector sequences that are associated with races. Their role in race determination will be functionally determined via gene overexpression and knockout using the suitable isolates.

Objective 3 Screening of diverse plant germplasm to uncover new sources of resistance to *P. capsici* and FON.
A collaboration with the vegetable breeder Dr. Amol Nanker in Department of Horticulture at UGA has been established to phenotype the UGA-Capsi-Core collection of about 500 sweet pepper accessions for resistance against *P. capsici* in greenhouses. We will focus on root and crown rot of seedlings after inoculation. An aggressive *P. capsici* strain isolated from infected pepper plants in Georgia will be used to inoculate four-week-old seedlings by soil drenching with zoospore suspension at the stem base. A total of 16 plants per accession in four blocks (replicates) will be grown in 48-cell inserts in plastic trays following a randomized block design. Disease will be scored at 2- and 4-weeks post inoculation, based on a 0-5 scale as described previously [4]. The disease severity data will also be used to compute the area under disease progress curve (AUDPC) for each evaluated accession. These phenotyping data will be integrated with the genotyping data for identification of disease resistance/susceptibility genes by genome-wide association analyses.

In addition, a field trial to screen yellow squash cultivars and germplasm will be performed in a field plot infested with *P. capsici* to identify the cultivars with tolerance/resistance for growers' immediate need and/or entering the breeding program.

Objective 4 Identification and characterization of genes pivotal in conferring resistance or susceptibility against both *P. capsici* and FON.
Plant genes that play key roles in resistance/susceptibility serve as the targets to develop disease resistant varieties via CRISPR-based genome editing. We will explore the publicly available or newly generated mRNA-seq data of susceptible and resistant varieties during infection of *P. capsici* and FON to identify candidate genes required for disease development and resistance. In addition, homologs of plant susceptibility (S) genes characterized in other plant species and the host targets of effector genes identified in Objective 1 will be included as potential candidate genes. To determine their roles in resistance/susceptibility experimentally in a rapid and efficient manner, we will utilize an *Agrobacterium rhizogenes*–mediated root transformation system, which has been used as a fast and efficient method for functional analysis of plant genes during plant interactions with soil-borne pathogens and beneficial microorganisms [5, 6]. Root transformation of cucurbits will be performed as described [7] to overexpress and/or mutate the candidate genes via CRISPR, followed by infection assays. To facilitate the identification of transgenic roots, constructs including a GFP reporter gene will be used. For monitoring disease development and scoring disease in a convenient way, a hydroponic plant growth system will be used for pathogen infection. For determining the role of candidate genes in resistance/susceptibility against *P. capsici*, as the pathogen infects leaves, we will also modify the gene expression in leaves using virus-induced gene silencing, or *Agrobacterium*-mediated transient expression systems to deliver the overexpression and CRISPR gene editing constructs. Genes with a confirmed role in resistance/susceptibility will be subjected to further characterization to determine the mechanisms.

Objective 5 Breed disease-resistant varieties using CRISPR/Cas mediated genome editing technology.
CRISPR-mediated genome editing has become an effective and attractive strategy for crop improvement due to its ease to use and ability to produce transgene-free mutants with precise genetic modification in a short period of time.

In Georgia, the production of yellow squash is most affected by *P. capsici*. We will take advantage of this cutting-edge technology to generate resistant yellow squash varieties. We have started with editing of the homolog of DMR6, mutations of which in many plant species lead to enhanced disease resistance against a diverse range of plant pathogens, to establish the transformation and genome editing system for yellow squash. Once established, we will edit a substantial number of candidate susceptibility (S) gene and resistance-related genes identified in Objective 4. Diverse genome editing strategies will be used to fine tune their expression by editing the promoters, and mutate S genes in a way that their cooperation in disease development is crippled but not its intrinsic function(s) in plant growth and development to achieve disease resistance without a fitness penalty.

Key references:

1. Wu et al. 2016, *BMC Microbiol* 16(1):204.
2. Gumtow et al. 2018, *Mol Plant Microbe Interact* 31(3):363-373.
3. van Dam et al. 2016, *Environ. Microbiol.* 18(11):4087–4102.
4. Mmbaga et al. 2018, *Journal of Plant Pathology & Microbiology* 9:1-8.
5. Pereira et al. 2023, *PLoS One* 18(5):e0285504.
6. Aggarwal et al. 2018, *Plant methods* 14:55.
7. Geng et al. 2022, *Hortic Res* 9.

Integrated Activities

Is this an **integrated** research and extension project? [ ] YES [X] NO

AREERA 204 (Integrated Research-Extension activities: A jointly planned, funded, and interwoven activity between research and extension to solve a problem; this includes the generation of knowledge and the transfer of information and technology.)

If **YES**, leave below statement unedited or provide a brief description of **what makes this an integrated activity.** Explain how research findings/tools will be shared with farmers, industry, homeowners, etc. *(1-2 sentences/limit of 4,000 characters including spaces)*

Research findings will be shared through in-service trainings and field days for extension personnel. Once appropriately trained, extension personnel will disseminate information via workshops, presentations, e-studies, educational materials, and audio-visual mediums.

Research Effort Categories *(Applies to this project specifically, not your overall appointment — **Must total 100%.**)*

Basic Research 70 % Applied Research 30 % Developmental Research [ ] %

Animal Health Component [ ] % This is subject to PI's discretion. **See Animal Health Research explanation here.**

Multistate Activities

Is this a multistate project? *AREERA 104 (Research multistate project)* [ ] YES [X] NO

If **YES**, leave the statement below unedited or provide a brief description of what makes this a multistate activity.
(1-2 sentences/limit of 4,000 characters including spaces)

Classifications — Your project must have **at least one row filled** with KA, SOI, and FOS. **Only one number per field**. Extra rows are provided, if you wish to break down your classifications further. ***Must total 100%.*** See this reference guide to identify the appropriate classification areas for your project. **NOT required for multistate project** *since already reported on NIMSS project.*

Knowledge Area	Subject of Investigation	Field of Science	Percent
212. Pathogens and Nematodes Affecting Plants	4020 Fungi	1102 Mycology	20%
212. Pathogens and Nematodes Affecting Plants	4099 Microorganisms, general/other	1040 Molecular Biology	30%
212. Pathogens and Nematodes Affecting Plants	1461 Peppers	1081 Breeding	20%

212. Pathogens and Nematodes Affecting Plants	1429 Cucurbits, other (includes pumpkin, squash, gourd)	1081 Breeding	30%

Assurances

Are **human** subjects involved? ____ YES X NO

If **YES**, is the project exempt from Federal Regulations? ____ YES ____ NO

If **YES**, select the appropriate exemption category. ____ 1 ____ 2 ____ 3 ____ 4 ____ 5 ____ 6

See this Exemption Category descriptions here.

IRB approval date _____ (MM/DD/YYYY)

Are **vertebrate animals** used? ____ YES X NO

If **YES**, IACUC approval date _____ (MM/DD/YYYY)

Submit your **completed Project Outline** and **approved Department Head Approval Form** to:

nifahelp@uga.edu