**Multi-State S1083**

**Ecological and genetic diversity of soilborne pathogens and indigenous microflora**

**Report for project period: October 1, 2020, to September 30, 2021**

**Report date: December 01, 2021**

**Annual Meeting Date: October 5, 2021**

**Participants:**

Dr. Nathan Slaton (Administrative Advisor), University of Arkansas

Dr. Sydney Everhart (Secretary), University of Connecticut

Dr. William Kingery, Mississippi State University

Dr. Alejandro Rojas, University of Arkansas

Dr. Terry Spurlock, University of Arkansas

Dr. Sean Sabanadzovic, Mississippi State University

Dr. Nina Aboughanem, Mississippi State University

Dr. Soledad Benitez Ponce, The Ohio State University

Dr. Rachel Koch Bach, University of Connecticut

Dr. Sharifa Crandall, Pennsylvania State University

Dr. Shankar Ganapathi Shanmugam (Chair), Mississippi State University

Dr. Fulya Baysal-Gurel, Tennessee State University

Dr. Richard Baird, Mississippi State University

**Brief summary of annual meeting**

The S1083 2020-2021 meeting was held on October 05, 2021. Due to the pandemic restrictions, the group met virtually through Zoom. The meeting lasted for 3 hours (10:30 am to 1:30 pm ET) and the agenda included a) Research updates b) Discussion about opportunities to collaborate, c) Brainstorming on the ingoing effort developing a review paper by the group, and d) Electing the secretary for 2022. The meeting was led by Dr. Ganapathi Shanmugam, with Dr. Sydney Everhart as secretary. All the group members actively participated and presented their research updates covering a wide area of expertise. Some of the general themes that were addressed included soil disease suppression, microbiome associated soil disease control, cover crops for soilborne disease control and virus-fungi association in soilborne diseases. Dr. Benitez Ponce initiated the discussion regarding the ongoing effort to develop a review paper as a potential outcome of this group. A follow-up meeting was planned to discuss about development and writing of a review paper, as an output from this team. Dr.Alejandro Rojas was nominated for the secretary position. He accepted the nomination, and without any other nominations, was elected as the incoming secretary for 2022. Finally, possible future meeting venues were discussed. The group planned to meet on Saturday prior to the annual meeting of the American Phyto pathological Society in Pittsburg, PA in August 6-10, 2022. Annual meeting video link <https://vimeo.com/624741504/2b6b2d217d>

**Research Updates:**

Dr. Terry Spurlock, University of Arkansas

**Accomplishments:**

Short-term outcomes: Soilborne disease incidence and severity were high in both cotton and soybean in 2021. A wet year following a wet year in 2020, calls to farmers fields revealed seedling diseases caused by *Rhizoctonia solani* and *Pythium* spp. Phytophthora root and stem blight and aerial blight were impactful on soybean. Taproot decline was also impactful to many growers’ fields in the southeastern portion of the state, specifically Chicot, Ashley, and Desha counties. An increase in sudden death syndrome above previous years was also observed.

Outputs: Fields calls indicated these diseases continue to be economically impactful. Growers and consultants were advised of management options through phone calls, text messages and post to social media (Twitter). Training of county agents to identify the (relatively new) disease taproot decline was continued in 2021 through field training.

**Activities:**

Objective 2: Evaluate the efficacy of soil-borne disease management strategies (chemical, biorational/biological, cultural) and characterize the associations among microbial community profile, soil physicochemical properties, environmental factors, and disease suppression.

Corn - a new product brought to market by FMC was evaluated in on-farm trials as well as traditional replicated small plot research on experiment stations. The product was determined to cause phytotoxicity in fields where corn was planted relatively early by AR standards. Efficacy against both soilborne and foliar diseases were inconclusive.

Soybean - variety tests and seed treatment and in-furrow fungicide trials continued to determine best management practices for taproot decline. Due to severe flooding, these trials at the Southeastern Research and Extension Center near Kelso, AR were completed but damaged. Symptoms of taproot decline were highly variable across the tests and therefore no varieties were determined to be tolerant of the disease. Some chemistries applied as seed treatments and in-furrow continue to show limited efficacy.

Cotton - numerous seed treatment fungicide trials were conducted at the Southeastern Research and Extension Center where inoculation using a virulent isolate of *R. solani* AG4 demonstrated some efficacy among commercial and experimental formulations of products.

**Publications:**

Teddy Garcia-Aroca, Paul P. Price, Maria Tomaso-Peterson, Tom W. Allen, Tessie H. Wilkerson, Terry N. Spurlock, Travis R. Faske, Burt Bluhm, Kassie Conner, Edward Sikora, Rachel Guyer, Heather Kelly, Brooklyn M. Squiers & Vinson P. Doyle (2021) Xylaria necrophora, sp. nov., is an emerging root-associated pathogen responsible for taproot decline of soybean in the southern United States, Mycologia, 113:2, 326-347, DOI: 10.1080/00275514.2020.1846965

T.N. Spurlock, J. A. Rojas, Q. Fan, A.C. Tolbert, and R. Hoyle. 2021. Understanding Taproot Decline. Arkansas Soybean Research Studies. pp. 96 - 101.

Faske, T., Smith, S., Spurlock, T., Wamishe, Y. MP154 Arkansas Plant Disease Control Products Guide. 2021.

T.N. Spurlock, A.C. Tolbert, S.F. Pennington, and R. Hoyle. 2021. Evaluation of cotton seed treatments against *Rhizoctonia solani* AG-4 in southeast AR, 2020. PDMR15:CF200.

T.N. Spurlock, A.C. Tolbert, S.F. Pennington, and R. Hoyle. 2021. Evaluation of cotton seed treatments at two seeding rates against Rhizoctonia solani AG-4 in southeast Arkansas, 2020. PDMR15:CF201.

T.N. Spurlock, A.C. Tolbert, S.F. Pennington, and R. Hoyle. 2021. Evaluation of experimental cotton seed treatments against Pythium ultimum at Kelso, Arkansas, 2020. PDMR15:CF202.

T.N. Spurlock, A.C. Tolbert, S.F. Pennington, and R. Hoyle. 2021. Evaluation of cotton seed treatments against *Pythium ultimum* in southeast AR, 2020. PDMR15:CF204.

Bradley, C. A., Allen, T. W., Sisson, A. J., Bergstrom, G. C., Bissonnette, K. M., Bond, J., Spurlock, T, et al. 2021. Soybean Yield Loss Estimates Due to Diseases in the United States and Ontario, Canada, from 2015 to 2019. Plant Health Progress. :PHP-01-21-0013-RS.

Dr. Alejandro Rojas, University of Arkansas

**Major goals of the project**

(1) Evaluate the biology and diversity of soil-borne pathogens, associated antagonistic microorganisms, and environmental conditions in the context of the whole-system phytobiome. This objective includes traditional, metagenomics, and spatial/temporal methodologies to understand microbial community dynamics that determine soil-borne disease incidence and severity on economically important crops in the U.S.

(2) Evaluate the efficacy of soil-borne disease management strategies (chemical, biorational/biological, cultural) and characterize the associations among microbial community profile, soil physicochemical properties, environmental factors and disease suppression.

**Accomplishments**

The research has been focused on three main areas: evaluation of chemical and biological agents for control of soilborne pathogens, soybean seed quality issues and their management, and ecology of soilborne pathogens in horticultural and field crops.

The evaluation of chemical and biological agents for control of soilborne pathogens is focused on rice. We are conducting evaluations of chemical recommendations provided by industry to control common soilborne pathogens: *Pythium* spp., *Rhizoctonia solani* and *Fusarium* spp. In rice, eight chemical treatments were evaluated including sedaxane, azoxystrobin, metalaxyl and fludioxonil individually and in different combinations. All assays were conducted in vitro using a seed assay with the respective pathogens and in the greenhouse using seedling cup assay. We are repeating the experiments and summarizing the findings of this research. With respect to the development of biocontrol agents is a project in collaboration with Dr. Clemencia Rojas, and the aim is to characterize the potential biocontrol range of bacteria against rice fungal and oomycete pathogens. These bacteria were previously identified as potential biocontrol agents of rice panicle blight. The project is funded by the Arkansas Rice Promotion Board and we identified *Burkholderia* and *Pseudomonas* as a potential biocontrol agent of fungal pathogens that affect mainly rice, but also caused an impact on other field crops. The genomes of these bacteria have been sequenced and we are focused on the characterization of these bacteria in vitro and as seed treatments.

With respect to seed quality, we have different lines of investigation that converge on a similar goal: determining the factors that drive seed quality and efficient and effective use of fungicides to control foliar pathogens. The first line is establishing a baseline sensitivity of *Cercospora* against several foliar fungicides, which is a proposal with Dr. Travis Faske funded by the Soybean Promotion Board. The goal is to determine the extent of resistance of *Cercospora* spp. and *Corynespora cassiicola* in the field to different chemistries now being used by growers, mainly triazoles (FRAC 3 - DMI) and Azoxystrobin (FRAC 11 – QoI). A collection of *Cercospora* spp. isolates was established from six counties in Arkansas, and those isolates were initially tested for resistance against Tetraconazole (DMI). We are testing the same set of isolates against Azoxystrobin (QoI). Part of this project is also contributing to ongoing collaborations with Dr. Terry Spurlock, Dr. John Rupe, Dr. Nick Bates on understanding the role of foliar fungicides on impacting seed infection and stinkbug damage. Reduced seed quality is associated with *Cercospora* and *Phomopsis*. Seed collected from plots with different fungicide and insecticide applications were collected and evaluated for seed quality and seed infection. In addition, in collaboration with Dr. Rupe, soybean plots with foliar fungicide treatments applied at R3, R5 and R3+R5 were evaluated and developing pods and mature seeds were collected for the detection of fungal pathogens using DNA based tools, especially those species associated with reduced seed quality.

In the area of the ecology of soilborne pathogens, we are actively working on research of Taproot decline (TRD), *Xylaria* *necrophora* in collaboration with Dr. Spurlock. We aim to characterize how cover crops could improve or cause issues with soilborne diseases using TRD as model to understand this potential interaction. In this area, we have been working on testing cover crops as alternative hosts for this pathogen and we are working on developing molecular diagnostic methods to track this pathogen. A field trial was established at Milo Shult Research & Extension center at Fayetteville. The aim is to study the relation between plant infection and severity of symptoms using isolation and PCR. The trial is arranged split design (cover crop and no cover crop) and within each split, there is randomized block design with three cultivars inoculated or non-inoculated and each treatment combination has four replications. Each plot is 4-rows, 36 ft long and the planting rate was 6 seed/ft. Inoculum was grown on millet, colonized material was air dried and placed with seed at 2.5 g/row-ft. Soil samples were collected, and roots were also collected at three time points at reproductive stages. Aerial images and SPAD measurements were also taken at each time point (July 13th, August 9th and September 13th).

**What do you plan to do during the next reporting period to accomplish the goals?**

The overall aim is to mitigate the impact of plant diseases in Arkansas field crops and reduce unnecessary fungicide inputs. Hopefully, combining variety selection based on disease resistance, followed by well-judged use of proper fungicides or biologicals, we could minimize the impact of pathogens. However, pathogens are constantly evolving or being introduced, so there is a continued need to screen crop varieties for resistance, chemical control and biological agents to determine their efficacy. In parallel, it is key to understand the factors that drive the pathogen biology and epidemiology to better use those tools and strategies to manage diseases.

For the chemical and biological control of fungal pathogens, in collaboration with Dr. Rojas, we will continue the evaluation of rice seed chemical treatments and compare those to the potential biocontrol agents that have been characterized. The research is focused on the used cell-free extracts as simplifies the validation and delivery of the potential molecules doing the disease control. With the genomes, some clusters have been identified but so far, we are using crude extracts to determine the activity in vitro and later evaluate those in greenhouse.

Ecology of soilborne pathogens and improvement of soil health is another focus in the research. In this area, we are also using microbiome and cultural approaches to study soilborne fungi and beneficial fungal communities in field and horticultural crops. In this aspect, supported by the Arkansas Department of Agriculture and in collaboration with Dr. Amanda McWhirt, we are studying strawberry production systems in Arkansas and cover crop as a mechanism to increase soil health. A year worth of samples were collected in strawberry demonstration plots in the Vegetable Station (Kibler, AR) and we are processing to characterize the dynamics of soil microbial communities under different management. Regarding taproot decline, we will continue characterizing the host range of the pathogen. In collaboration with Dr. Spurlock, we will continue to study the ecology and epidemiology of this pathogen in the field using traditional and DNA based detection methods using field trials and in-farm trials.

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| --- | --- | --- | --- |
| **Role**  | **Non-Students or faculty**  | **Students with Staffing Roles**  | **Computed Total by Role**  |
| **Undergraduate**  | **Graduate**  | **Post-Doctorate**  |
| Scientist  | 1 | 3 | 2 | 0 | 6 |
| Professional  | 0 | 0 | 0 | 0 | 0 |
| Technical  | 2 | 0 | 0 | 0 | 2 |
| Administrative  | 0 | 0 | 0 | 0 | 0 |
| Other  | 0 | 0 | 0 | 0 | 0 |
| Computed Total  | 3 | 3 | 2 | 0 | 8 |

**Student Count by Classification of Instructional Programs (CIP) Code**

|  |  |  |  |
| --- | --- | --- | --- |
| Undergraduate  | Graduate  | Post-Doctorate  | CIP Code  |
| 2 | 0  | 0  | 01.00 Agriculture, General.  |
| 0  | 2 | 0  | 01.11 Plant Sciences.  |

**Target Audience**

Growers, crop consultants, private industry persons, and those interested in further education and outreach.

**Products
Conference**

Fan Q., Faske T., Spurlock T., Rojas A., and Roberts T.“The impact of different cover crop rotations on soilborne microbial diversity and disease emergence of Soybean-Corn cropping system” Southern Soybean Disease Workers Meeting 2021.

Delgado L, Winters S, Rojas C. and Rojas A. Bacterial biocontrol agents against fungal and oomycete pathogens of rice and other field crops. South Division APS 2020 Meeting.

Smith A., Moscoso J., Winters S., McWhirt A., and Rojas A. “Friend or foe: exploring the diversity of fungi associated with strawberry production in Arkansas” Mid-Atlantic Mycology Conference.

Gil J., and Rojas A. Comparative analyses of effector and CAZyme profiles in binucleate Rhizoctonia isolates. Plant Health Meeting APS 2021.

Lancaster A., Faske T., Rupe J., and Rojas JA. Determining baseline fungicide sensitivity in Cercospora spp. from Arkansas to DMI and QoI Fungicides. Plant Health Meeting APS 2021.

Sharfadine S., and Rojas JA. Evaluation of chemical seed treatment against fungal and oomycete pathogens in managing rice diseases. Plant Health Meeting APS 2021.

Fan Q., and Rojas JA. The colonization of various cover crops by the Xylaria necrophora – causal agent of soybean Taproot decline disease. Plant Health Meeting APS 2021.

**Publications**

Gil, J., Ortega, L., Rojas, J.A. and Rojas, C.M., 2021. Genome Sequence Resource of Burkholderia glumae UAPB13. *PhytoFrontiers*, (ja).

Buckner, E. and Rojas, A., 2021. Baseline Sensitivity to Demethylation Inhibitors Fungicides In Cercospora spp. and Corynespora spp. in Arkansas Soybeans. *Discovery, The Student Journal of Dale Bumpers College of Agricultural, Food and Life Sciences*, *22*(1), pp.8-14.

Hatlen, R.J., Higgins, D.S., Venne, J., Rojas, J.A., Hausbeck, M. and Miles, T.D., First report of halo blight of hop (Humulus lupulus) caused by Diaporthe humulicola in Quebec, Canada. *Plant disease*.

Milani, T., Hoeksema, J., Jobbágy, E., Rojas, J.A., Vilgalys, R. and Teste, F., 2021. Co-invading ectomycorrhizal fungal succession in pine-invaded mountain grasslands. *Authorea Preprints*.

**Extension Publications**

*J.C. Rupe,* *J.A. Rojas,* *and T.R. Roberts*. Effects of Cereal Rye Termination Date on Seedling Diseases and Soil Nutrient Content. Pest Management: Disease Control. Arkansas Soybean Research Studies 2020.

T.N. Spurlock, J. A. Rojas, Q. Fan, A.C. Tolbert, and R. Hoyle. Understanding Taproot Decline. Pest Management: Disease Control. Arkansas Soybean Research Studies 2020.

**Dr. Soledad Benitez Ponce, The Ohio State University**

The activities performed in the Benitez Lab at The Ohio State University, as they pertain to the S1083 multistate project are described below by project objective.

Research in the Benitez Lab is focused on understanding the impact of production practices, in crop health and its associated microbiome and function, with particular emphasis on corn and soybean production, and practices that promote soil health through crop diversification.

**Accomplishments:**

*Objective 1. Evaluate the biology and diversity of soil-borne pathogens, associated antagonistic microorganisms, and environmental conditions in the context of the whole-system phytobiome. This objective includes traditional, metagenomics, and spatial/temporal methodologies to understand microbial community dynamics that determine soil-borne disease incidence and severity on economically important crops in the U.S.*

In 2020-2021, we continued to evaluate relationships between communities of fungi in soils and incidence of soybean cyst nematodes, across the state of Ohio. For this, during the 2021 growing season a total of 102 soil samples were collected across 19 counties. The majority of samples were from farms under a corn-soybean rotation, with 2021 being in soybean. The soil samples are currently being processed to determine SCN-egg counts, and update current distribution of SCN in Ohio. This work is in collaboration with the soybean pathology team at OSU. Counties in which SCN was previously not reported were particularly targeted in this survey. Fungal community characterization of these samples will be performed using culture-based and culture-independent approaches. From past surveys we identified that the most common fungi recovered through culturing belong to the genus Fusarium, whereas through amplicon metabarcoding we recover greater number of reads of Mortierella. Finally, a culture-independent survey of fungi associated to SCN cysts also indicates presence of Fusarium in cysts, but also other fungal species such as Exophiala sp., Talaromyces, Zygomycota, and Trichoderma sp.

*Objective 2. Evaluate the efficacy of soil-borne disease management strategies (chemical, biorational/biological, cultural) and characterize the associations among microbial community profile, soil physicochemical properties, environmental factors and disease suppression.*

The successful application of biological control products and other microbial inoculants is often dependent on environmental variables and management practices, as complex interactions often occur in the plant-soil interface. The Benitez Lab is continuing research on beneficial fungi, with emphasis on the arbuscular mychorrizal fungi (AM). During the 2020-2021 reporting period, the work was focused on testing molecular tools to detect AM colonization in the field. For this we used samples from the 2020 field season, which were treated with AM-fungal inoculants, a conventional seed treatment (nematicide and fungicide mix) or non-treated. The two approaches tested were an amplicon-metabarcoding approach using the PacBio technology, targeting AM-specific ribosomal genes; and a commercially available synthetic long-read sequencing technology, through Loopgenomics. In addition, we used standard PCR, with published primers for different species of Glomeromycota. For the standard set of primers offered by Loopgenomics, no AM reads were detected. In addition, preferential amplification of plant material was observed, suggesting the standard primers offered by the service are not ideal for root DNA extracts. However, previous bioinformatic analysis of the primers suggested potential amplification of AM species. Like the results from Loopgenomics, successful recovery of AM reads was not observed with the protocol used for PacBio sequencing. These results contrast with some of the field colonization data, which indicate that AM colonization in untreated roots ranged between 0-25%; in seed treated roots between 25-50%, in AM-only treated between 10-35%, and in AM-treated + seed treatment between 5-65%. The next steps for this project are to continue working on a molecular protocol that can be used to monitor if the inoculant successfully colonized soybean roots, compared to native AM populations.

**Impacts**

Training

One PhD students is being trained as part of this experiments; as well as one undergraduate summer interns (2021), and two research associates.

Dissemination

 Results have been presented through multiple venues, including meetings with growers and industry partners.

Target Audience

 The target audience for this work is the scientific community in general. In addition, some of the material and examples of methods applied in our research are presented to undergraduate and graduate students in the two courses I teach/co-teach: Phytobacteriology and Current topics in Plant Microbiome and its application in agriculture. Finally, we share our results with industry, in particular Groundworks BioAg, and the Ohio Soybean Council. We also partner with OSU soybean pathology team, to further disseminate our results to the community of soybean producers.

**Products**

Publications

1. Huo D, Frey T, Lindsey L, **Benitez MS**. Contrasting effects of soybean-wheat-corn rotation on corn and soybean production and soil health. Submitted to: “Crop, forage and turfgrass management” (June 2021, Accepted with revisions, Oct 2021).
2. Malacrino A; Abdelfattah A; Berg G; Benitez MS; Bennett AE; Böttner L; Xu S; Schena L. Exploring microbiomes to enhance plant health

Submitted to: Biological Control. Oct 2021.

Oral presentations

1. Frey T, Huo D, Lindsey L, Benitez MS. Corn-Soybean-Winter Wheat Rotation Reduces Corn Yields and Improves Soil Health in Ohio. Submitted for consideration to the North Central meeting of the American Phytopathological Society, June 2021
2. Benitez MS, Haden R, Malacrino A, Willman M. Bacterial and Fungal Communities Respond to Cover Crop Termination by Glyphosate but Recover After Soybean Establishment. The World Microbe Forum, June 2021.
3. Medina-Lopez, M, Abdullah J, Benitez MS. The fungal communities associated to soybean cyst nematode in Ohio. Ohio Soybean Board meeting, August, 2021.
4. Benitez, MS. Microbiome dynamics in agricultural systems. Ecuadorian Phytopathology Congress. Keynote session. October 2021.

**Plans for next year**

Objective 1.

1. Complete fungal community analysis of 2021 field soil survey, and from SCN cysts

2. Estimate SCN incidence in collected samples, through SCN egg counts.

3. Analyze soil physico-chemical characteristics for each collected sample

4. Determine relationship between SCN incidence, fungal communities and soil physico-chemical characteristics. In addition, incorporate information of environmental conditions and cropping history.

Objective 2.

1. Further analyze AM colonization rates in soybean roots to determine if there is a relationship between ability to recover AM through sequencing approaches is related to AM colonization status.

2. Continue testing other platforms and primers to detect AM and monitor inoculation.

3. Develop experiments to test the relationship between AM colonization and soybean seed treatments.

**Dr. Sharifa Crandall, Pennsylvania State University**

**Intended activities:** I focused my new soilborne research program at Penn State on researching several infamous fungal and oomycete plant pathogens that are a problem specifically in Pennsylvania and the North East (e.g., *Fusarium, Verticillium, Rhizoctonia*). I combined my own knowledge and expertise in microbial ecology and fungal pathology with what I found to be the current gaps and future directions for our discipline. I then created three large focal areas and questions for my research program: (1) disease dynamics of microbial communities: how do fungal and oomycete pathogens shape microbial community diversity, structure, and function within the root, soil, as well as host phenotype? (2) ecological interactions: how does stress drive the complex functional interactions between hosts, microbiomes, and the environment?, and (3) soil management: how can different sustainable soil farming strategies improve soil health? These are the intended areas I plan to conduct research in over the next few years.

**Short-term Outcomes:**

I joined the advisory board of PASA - Pennsylvania Sustainable Agriculture and served on various committees to help increase the diversity of farmers and students who will join the agricultural workforce as well as helped allocate COVID-19 relief funds to farmers in my region based on need.

**Outputs:**

2 publications

13 student-led webinars for the PA Master Gardner's program on Plant Diagnostics

4 graduate students

1 post-doctoral scholar

1 research laboratory technician

**Activities:**

Helped new graduate students start research projects on soilborne disease ecology

**Milestones:**

1) To find collaborators and grow *Verticillium dahliae* for downstream greenhouse and field experiments by December 2021

2) To conduct a soil steaming and Trichoderma and *Fusarium solani* biocontrol experiment by the end of September 2021

3) To collect and identify key soil microbes that could play a role in grape diseases

**Impacts:**

1) Develop diagnostic tools for soilborne pathogen *Verticillium dahliae* on potato and alternative hosts.

2) To identify the diversity of the soil microbiome after steaming treatments and the interaction of biocontrol and fungal pathogens in situ, model: soybean

3) Identify the source and role of soilborne microbes in causing disease of grapes during the growing season summer/fall 2021

**Indicators:**

1) Testing the feasibility and efficacy of the *V. dahliae* diagnostic assays across collaborating labs and diagnostic clinics.

2) Growing soy in sterilized soil, with and without biocontrol agent and monitor plant health.

3) Identify key indicator microbes that are involved in grape health.

**Publications:**

**Crandall, S.G.,**Ramon, M. L., Burkhardt, A. K., Bello, J. C., Adair, N., Gent, D. H., Hausbeck, M. K., Quesada-Ocampo, L. M. and Martin, F. N. 2021. A multiplex TaqMan qPCR assay for detection and quantification of clade 1 and clade 2 isolates of *Pseudoperonospora cubensis* and *P. humuli. Plant Disease.* https://doi.org/10.1094/PDIS-11-20-2339-RE

**Crandall, S.G.**, Spychalla, J., Crouch, U., Acevedo, F., Naegele, and T. Miles. (In Press). Rotting grapes don't improve with age: cluster rot disease complexes, management, and future prospects. Invited: Special Feature, *Plant Disease.*

Dr. Shankar Ganapathi Shanmugam and Dr. William l. Kingery - Mississippi State University.

**Accomplishments:**

Research in soil microbial ecology lab pertaining to S1083 multistate project focused on understanding how management practices in Mississippi soybean production systems (e.g., early-planting, precision seed placement, irrigation systems, residue management) relate to the occurrence of Soybean taproot decline (TRD) in order to verify the effects of altering management practices, and to determine the potential for enhancing the disease suppressive ability of these endemic soils. The aim is to broaden the range of cost-effective practices for TRD suppression that are amenable to incorporation into Mississippi soybean production systems.

**OBJECTIVE 1:** Survey of fields for TRD incidence and severity as well as distribution within agricultural fields that differ in farming system classification.

**OBJECTIVE 2:** Evaluation of the ecology of *Xylaria* spp. based on pathogen population in soil and microbiome structure and function.

**Accomplishments**

**Objective 1**: A total of 20 soybean fields across six Delta counties were evaluated for the field-scale characteristics of symptomology of Soybean Taproot Decline. Evaluations were made at the R5 stage of crop development. A two-tier rating system for characterization was utilized. Field-scale distribution of symptoms were rated with the use of a 7-category system extending from *no symptoms visible* to symptomology *present throughout*. The categories included descriptors for symptoms appearing in concentration, i.e., clusters of infected plants, through symptoms distributed throughout the field. Fields were divided into four quarters with an overall rating given as the average of the four. Infection intensity percentage was measured by counting the number of infected plants per 100 plants in a linear section of row. This was done at two randomly chosen positions within each of the field quarters mentioned above.

158 survey transects across twenty soybean fields were conducted to rate TRD severity and distribution. Plant growth stage and residue ratings were also documented at each survey transect.

**Objective 2:** Two fields in the Mississippi Delta were sampled at locations within the fields that represented three ranges of Soybean Taproot Decline intensity (0%, 1-50%, and > 50% of plants infected along one linear meter of row, i.e., infection intensity grouping of low, medium an high). From each site, soil core plugs were collected along the one meter of row, to a 10-cm depth and composited. Two additional Delta fields, each with nine geo- referenced location per field were measured for percentage infection along one linear meter of row and soil sampled as described above at three dates (June, July and August). At the final sampling, all plants at each of the nine locations were collected and returned to the lab to make a final determination of Soybean Taproot Decline infection and to determine grain yield.

**Preliminary Results:** Amplicons targeting bacterial 16S and fungal 18S rRNA genes (ITS2) were sequenced using Illumina MiSeq sequencing platform. The distribution of the 500 most abundant OTUs (operational taxonomic units) in the soil samples from the two locations indicated two primary patterns of soil bacterial community structure. The location has a significant effect on the bacterial community structure. The important part of the structural variability was related to differences associated with the sampling dates. There was no significant difference in the pattern of bacterial community structure with mid and late sampling dates (p=0.618 and 0.529, respectively) indicating that the disease severity had a significant effect on the soil bacterial community composition.

**Impacts**

Target Audience: Outcomes and information from this project will help soybean producers in both understanding and managing taproot decline disease. The increasing distribution and occurrence of the disease throughout the state suggests that project benefits may extend to all soybean acres in Mississippi. However, the monetary impact is difficult to project as the economics of TRD are somewhat unknown given the highly variable in-field distribution and infection intensity. Indirect benefits are associated with an increased understanding of taproot decline ecology, particularly in how the disease interacts and responds to crop and residue management practices. The survey and classification of farming systems in relation to TRD occurrence and severity is anticipated to provide direct benefits to soybean growers by identifying practical management decisions that may reduce the impact of TRD.

**Plans for next year**

1. Quantitative PCR with SYBR-Green detection will be used to estimate *Xylaria spp* gene copy numbers in soil
2. Identify crop management combinations that correlates with disease intensity and microbial community structure.
3. Network analysis to understand the relationship between TRD and soil microbiome abundance and distribution.
4. Functional diversity of microbiome and its role in disease suppression.

**Training**

A Post-Doctoral associate (Nisarga Narayana), an Undergraduate Research Scholar (Rachel Hill) and a Student Worker (Abigail Allison) are being training in soil microbial ecology lab (Mississippi State University).

**Funded Projects**

Shanmugam, S.G (Principal), W.L. Kingery (Co-Principal), Tom Allen, Teresa Wilkerson and Mark Shankle .“Determining Management-related Factors that Impact the Incidence and Severity of Soybean Taproot Decline (TRD)” . Mississippi Soybean Promotion Board- $67,139 (April 1, 2020 - March 31, 2021).

Shanmugam, S.G (Principal), W.L. Kingery (Co-Principal), Tom Allen, Teresa Wilkerson and Mark Shankle .“Determining Management-related Factors that Impact the Incidence and Severity of Soybean Taproot Decline (TRD)” . Mississippi Soybean Promotion Board- $67,414 (April 1, 2021 - March 31, 2022).

**Products**

**Poster Presentation**

W.L. Kingery, Dan Prevost and Shanmugam, S.G. In-season Survey of Soybean Taproot Decline Incidence and Severity on Selected Mississippi Delta Farms. The American Phytopathological Society, Southern division meeting, March 2021

**Dr. Fulya Baysal-Gurel, Tennessee State University**

**Major Goals and Objectives**

Overall Goal: Identify effective soil-borne disease management strategies for field nursery production of woody ornamentals to manage soil-borne pathogens that can be easily and readily adopted by field nursery growers.

Objective 1. Evaluate the efficacy of chemical and biorational products for controlling soil-borne diseases with different application methods, intervals and reduced-rate applications in woody ornamentals.

Objective 2. Develop improved soil-borne disease management strategies based on cultural approaches for suppression of Rhizoctonia and (or) Phytophthora spp. and other soil-borne pathogens.

Objective 3. Characterize the associations between microbial community profile and soil-borne disease suppression expressed in different soil-borne disease management strategies.

Objective 4. Engage in outreach and technology transfer with woody ornamental nursery growers.

**Accomplishments**

**Evaluate the efficacy of chemical and biorational products for controlling soil-borne diseases with different application methods, intervals and reduced-rate applications in ornamentals.**

The purpose of this study was to evaluate fire ant venom alkaloids and an alarm pheromone analog against several plant pathogens, including *Botrytis cinerea*, *Fusarium oxysporum*, *Phytophthora nicotianae, P. cryptogea*, *Pseudomonas syringae*, *Phytopythium citrinum*, *Rhizoctonia solani*, *Sclerotonia rolfsii*, *Xanthomonas axonopodis*, and *X. campestris*. All pathogens were tested against red imported fire ant venom alkaloid extract and alarm pheromone compound for growth inhibition in in vitro assay. The venom alkaloid extract inhibited fungal and oomycete pathogens. Neither of the treatments were effective against bacterial pathogens. Three soilborne pathogens, *P. nicotianae*, *R. solani*, *F. oxysporum*, and one foliar pathogen, *B. cinerea* were selected for further in-vivo assays on impatiens (*Impatiens walleriana* 'Super Elfin XP violet'). Total plant and root weight were higher in venom alkaloid treated plants compared to an inoculated control. The venom alkaloid treatment reduced damping-off, root rot severity, and pathogen recovery in soilborne pathogen inoculated plants. Similarly, venom alkaloid reduced Botrytis blight. However, higher venom rates caused foliar phytotoxicity on plants. Therefore, additional work is needed to evaluate rates of venom alkaloids or formulations to eliminate negative impacts on plants. Overall, these results suggest that red imported fire ant venom alkaloids may provide a basis for new products to control soilborne and foliar plant pathogens.

**Develop improved soil-borne disease management strategies based on cultural approaches for suppression of Rhizoctonia and (or) Phytophthora spp. and other soil-borne pathogens.**

We studied the response of the major cover crops being used by woody ornamental growers in the Southeastern United States to *Phytopythium vexans, Phytophthora nicotianae*, and *Rhizoctonia solani* in greenhouse conditions to identify the effective cover crops that can be used in a nursery field production system. Data related to post-emergence damping-off and plant growth parameters (plant height increase and fresh weight) were recorded. Similarly, cover crop roots were assessed for root rot disease severity using a scale of 0–100% roots affected. Among the tested cover crops, the grass cover crops triticale (×Triticosecale Wittm. ex A. Camus.), annual ryegrass (*Lolium multiflorum* L.), Japanese millet (*Echinochloa esculenta* (A. Braun) H. Scholz), and the legumes Austrian winter pea (*Pisum sativum* var. arvense (L.) Poir) and cowpea ‘Iron and Clay’ (*Vigna unguiculata* (L.) Walp.), showed lower root rot disease severity and post-emergence damping-off in the soil inoculated with *P. nicotianae, R. solani*, or *P. vexans* compared to the other crops. Since these cover crops can act as non-host crops and benefit the main crop in one way or another, they can be used in the production system.

**Future Plans**

1. Evaluate the efficacy of chemical and biorational products for controlling soil-borne diseases with different application methods, intervals and reduced-rate applications in woody ornamentals. We will continue to evaluate chemicals and biorational products for use in soil-borne disease management at greenhouse and field conditions.

2. Develop improved soil-borne disease management strategies based on cultural approaches for suppression of soil-borne pathogens. We will continue to evaluate cultural approaches for use in soil-borne disease management at on-farms and on-station.

3. Characterize the associations between microbial community profile and soil-borne disease suppression expressed in different soil-borne disease management strategies. Microbial community analyses will be performed on field experiment root and soil samples.

**Products**

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**Target Audience**

**Graduate assistantship:** Two M.S. level and 3 PhD level (Advisor) and one M.S. level (co-Advisor) students participated into this project. The students involved in protocol development, experimental design, conducting experiments, data collections for the projects.

**Extension/Outreach:** As woody ornamental plant pathologist, I interacted directly with nursery producer, TSU extension agents and TDA regulatory officials to provide diagnoses of biotic and abiotic problems and management recommendations to support sustainable nursery production in Tennessee.

How have the results been disseminated to communities of interest?

Woody ornamental plant pathology lab interacted directly with nursery producer, TSU extension agents and TDA regulatory officials to provide diagnoses of biotic and abiotic diseases and management recommendations to support sustainable nursery production in Tennessee. Our lab diagnosed over 250 woody ornamental samples in spring 2021. Woody ornamental plant pathology lab also participated in the training of nursery growers on soilborne diseases and their management.

**Student Count**

Lead researcher is faculty advisor for Ravi Bika M.S and Krishna Neupane M.S; Sandhya Neupane Ph.D., Madhav Parajuli Ph.D. and Bhawana Ghimire Ph.D. She is serving as a member at the committee of Vivek Ojha since fall 2018, M.S. This project provided also research and Extension experience for Dr. Farhat Avin as post-doctoral researcher.

**Impact:**

1. Develop improved soil-borne disease management strategies based on cultural approaches for suppression of Rhizoctonia and (or) Phytophthora spp. and other soil-borne pathogens.

Impact: Provided the woody ornamental nursery industry with an environmentally friendly disease management strategy that suppresses soil-borne disease population densities comparable to chemical products.

2. Characterize the associations between microbial community profile and soil-borne disease suppression expressed in different soil-borne disease management strategies.

Impact: Provided information regarding how specific management strategies, such as green manure or compost amendments, can suppress plant pathogens and increase the abundance of antagonist populations.

3. Engage in outreach and technology transfer with woody ornamental nursery growers.

Impact: We were able to provide significant outputs to the woody ornamental nursery industry, researchers, extension specialists and agents on the effectiveness of different application methods, intervals and reduced fungicides rates, efficacy of soil-borne disease management strategies such as organic matter amendment, cover crop and solarization for reducing soil-borne pathogen populations. This will lead to outcomes of growers changing their behavior to include more sustainable production practices and reduction in the amount of fungicides applied in field-grown nurseries of woody ornamental.

**Dr.Richard Baird, Mississippi State University**

Title: Volatile Biomarkers For Early-Stage Disease Diagnosis of Sweet Potato Fungal Soft Tissue Disease Within Warehouses (Rhizopus stolonifer and Macrophomina phaseolina-Mp)

Objective: Long-term objective of this project is to develop monitoring hardware that can identify the presence of key rot pathogens in sweet potato roots from microbial volatile chemicals (MVOC’s) produced by rot pathogens. We are at later stages development of appropriate chemical and physical methodology to assess rot microbe development following harvest and during storage.

 Since 2019, laboratory and incubator studies were conducted (current) to evaluate and determine chemical signatures for isolates of Mp and R. stolonifer compared to uninoculated controls. Tissues from select varieties of sweet potatoes namely, Beauregard-14 (B 14), Beauregard-63 (B 63) and Orleans were used across 3 years to investigate the fungal infection. Headspace solid phase micro-extraction method coupled with HS-SPME GC/MS was used to collect microbial volatile organic compounds produced by the pathogen isolate inoculated sweet potatoes.

Results:

 HS-SPME GC-MS untargeted metabolomics work identified a set of volatile metabolites related to progression of fungal soft tissue disease on sweet potatoes, especially while in storage conditions. The OPLS-DA model identified ethyl acetate ethyl crotonate, ethyl isovalerate, and anisole as specific for the later stage of the disease while 1-menthone emissions were associated with early stages of the disease. Ethyl alcohol, 1-propanol, 3-methyl, 3-buten-1-ol, prenol and ethyl propionate were presence in the volatile profile of both of early and later stage of the disease. Therefore, based on our observations, we hypothesized that the synthesis of high ethanol content in the fungal infected sweet potatoes could lead to more VOCs, especially ethyl acetate, ethyl crotonoate and isoprenol, being produced. Even though many discriminatory biomarkers were identified, only the above 10 were confirmed as level 1 identified metabolites. OPLSDA combined with ROC analysis identified 1-propanol, ethyl alcohol, 3-70 methyl, 3-buten-1-ol and ethyl propionate as putative volatile disease markers for the fungal soft tissue disease in sweet potatoes. Additionally, we found that the R. stolonifer fungi emitted many butane derived volatiles including ketones, alcohols and esters. Overall, these findings elucidate the volatiles related to the pathogenicity of R. stolonifer on sweet potatoes.

Title: Developing a rapid and portable assay for the diagnosis of seedling diseases caused by Rhizoctonia solani using Near Infrared (NIR) Spectroscopy :Mariana Santos-Rivera, Matt Harjes, Richard Baird, and Carrie K. Vance

Objectives:

 The work proposed in this SRI is expected to allow future development of an NIR-based diagnostic test that can be run at the farm in real time to diagnose the AGs from R. solani affecting crops more accurately in their roots and leaves. A cost effective and reliable diagnostic test would enable county agents and consultants to provide timely management and advice or treatment with fungicides, thus decreasing expenses resulting from inaccurate diagnosis of affected crops.

Identify characteristic NIR spectral signatures from solani anastomosis groups though in-vitro experiments

Identify characteristic NIR spectral signatures from solani anastomosis groups though in-vivo experiment

Results:

Four multinucleate solani AG isolates were detected and discriminated that were inoculated in basal liquid cultures using NIRS, chemometrics and aquaphotomics. Biochemical changes related to differences in metabolic growth generated distinct patterns in the WAMACs and WASPs based on dominant peaks in the wavelength range 1300-1600nm where OH, CH, and NH bonds interact with NIR light.NIRS data in combination with chemometrics and aquaphotomics has potential as a rapid laboratory assay for solani AGs detection and discrimination as soon as 1-day post-inoculation using liquid cultures, in comparison with the 8 days required with the traditional classification method using DNA sequence data collection. This will reduce the current detection time and the selection of an accurate treatment for a most or less destructive AGs before economic losses occur in the crops.