

S1070 Regional Research Project Agenda

Thursday-Friday, November 21–22, 2022

Stefan Jaronski, Chair
Julie Graesch, Vice-chair
Anamika Sharma, Member-at-large
Shaohui Wu, Secretary
Paula Agudelo, Administrative Advisor

November 21, 2022

(EST)

- 10:00 AM PRELIMINARY BUSINESS MEETING
1. Introductions and general information
 2. Minutes of 2021 (Shaohui Wu and Anamika Sharma)
 3. Sub-project Leads
- 10:30 AM Funding Opportunities from NIFA, Vijay K. Nandula
- 11:15 AM NEW PROJECT REVIEW AND PLANNING-Large acreage crops Annual Crops
[Shaohui Wu]
- 12:30 PM NEW PROJECT REVIEW AND PLANNING-Orchard Systems [David Shapiro-Ilan]
- 1:15 PM NEW PROJECT REVIEW AND PLANNING-Small Fruits and Vegetables [Jimmy Klick]

November 22, 2022

(EST)

- 10:00 AM NEW PROJECT REVIEW AND PLANNING-Urban and Natural Landscapes,
Rangelands, and Nurseries [David Oi]
- 11:00 AM DISCUSSIONS
1. Theme for 2023
 2. Discussion of collaborative projects
 3. New business
- 1:00 PM ADJOURN

Attendees 2022

Name	Affiliation	Email
1. Byron Adams	Brigham Young University	byron_adams@byu.edu
2. Paula Agudelo	Clemson University	pagudel@clemson.edu
3. Pasco Avery	University of Florida	pbavery@ufl.edu
4. Robert Behle	USDA-ARS, Illinois	robert.behle@usda.gov
5. Surendra Dara	Oregon State University	surendra.dara@oregonstate.edu
6. Emily Duren	University of Florida	duren.ebliss@ufl.edu
7. Julie Graesch	BioWorks Inc.	jgraesch@bioworksinc.com
8. Stefan Jaronski	Virginia Tech University	thebugdoc01@gmail.com
9. Navneet Kaur	Oregon State University	navneet.kaur@oregonstate.edu
10. Jimmy Klick	Driscoll's, California	jimmy.klick@driscolls.com
11. Albrecht Koppenhöfer	Rutgers University	a.koppenhofer@rutgers.edu
12. Sriyanka Lahiri	University of Florida	lahiris@ufl.edu
13. Vijay K. Nandula	REE-NIFA	vijay.nandula@usda.gov
14. David Oi	USDA-ARS, Florida	david.oi@usda.gov
15. O. P. Perera	USDA-ARS, Mississippi	op.perera@usda.gov
16. Lorenzo Rossi	University of Florida	l.rossi@ufl.edu
17. David Shapiro-Ilan	USDA-ARS, Georgia	david.shapiro@usda.gov
18. Anamika Sharma	Florida A & M University	anamika.sharma@fam.u.edu
19. Colin Wong	USDA-ARS, Georgia	Colin.Wong@usda.gov
20. Shaohui Wu	University of Georgia	shaohui.wu@uga.edu

BUSINESS MEETING

1. *Introductions:* Anamika Sharma (2022 Member-at-large): Welcomed all and began with introductions. Attendees introduced themselves including a short introduction about their affiliation and work.

2. *Minutes of 2021* (prepared by Shaohui Wu and Anamika Sharma): A copy of the 2021 minutes was circulated electronically prior to the meeting. A motion to approve the 2021 minutes was made by Anamika Sharma and was passed unanimously. Minutes of the 2022 meeting are required to be posted within 60 days.

3. *NIFA administrators report (Dr. Vijay K. Nandula):*

Dr. Nandula reported the deadlines for NIFA grant applications:

<https://www.nifa.usda.gov/grants/upcoming-request-applications-calendar>

The **Crop Protection and Pest Management (CPPM)** RFA has been released, and the application deadline is February 13, 2023, 5 pm EST.

<https://www.nifa.usda.gov/sites/default/files/2022-11/FY23-CPPM-RFA-508.pdf>

A CPPM Applied Research and Development webinar on Thursday, December 01, 2022, 12-1 pm EST is available to guide through the application process (recording available from the web link below).

<https://www.nifa.usda.gov/cppm-applied-research-development-rfa-technical-assistance-webinar>

Specialty Crop Research Initiative (SCRI): Pre-application deadline on January 12, 2023.

https://www.nifa.usda.gov/sites/default/files/2022-10/FY23-SCRI-Pre-App-RFA-508_0.pdf

Biotechnology Risk Assessment Research Grants Program: Application deadline on January 19, 2023.

<https://www.nifa.usda.gov/sites/default/files/2022-10/FY23-BRAG-RFA-508.pdf>

Food and Agriculture Service Learning Program: Application deadline on December 08, 2022.

<https://www.nifa.usda.gov/sites/default/files/2022-11/FY23-FASLP-RFA-508.pdf>

Assistive Technology Program for Farmers with Disabilities (AgrAbility): Application deadline on January 19, 2023.

https://www.nifa.usda.gov/sites/default/files/2022-11/FY23-AgrAbility-RFA-508_0.pdf

From Learning to Leading: Cultivating the Next Generation of Diverse Food and Agriculture Professionals: Application deadline on December 14, 2022.

<https://www.nifa.usda.gov/sites/default/files/2022-11/FY22-NG-RFA-508-MOD2.pdf>

Food Safety Outreach Competitive Grant Program: Application deadline on February 16, 2023.

<https://www.nifa.usda.gov/sites/default/files/2022-11/FY23-FSO-RFA-508.pdf>

Methyl Bromide Transition Program: Application deadline on February 13, 2023.

<https://www.nifa.usda.gov/sites/default/files/2022-11/FY23-MBT-RFA-508.pdf>

AFR webinars are available for many of these programs and can be used to assist with application process.

Dr. Nandula mentioned that NIFA always looks for qualified reviewers. If anyone is interested in serving as a volunteer as a NIFA panelist, the information can be found via:

<https://prs.nifa.usda.gov/prs/volunteerPrep.do>

Accomplishments/Outcomes 2021-2022

Symposium was organized ‘Incorporating Microbials into the Culture of IPM’

Session Date: Sunday, November 13, 2022

Session Time: 1:30 PM - 4:30 PM

Location: Meeting Room 122, Vancouver Convention Centre

Large acreage crops

Shaohui Wu (University of Georgia): Previously, the new strain of entomopathogenic fungus (EPF) *Cordyceps javanica* wf GA17 was isolated from whitefly epizootics in Georgia and was found to be highly virulent against whiteflies. The new fungal strain was then tested for field persistence and efficacy against whiteflies. However, without formulations the fungus lost over 90% viability within 24 h after application and the field efficacy was not satisfactory in cotton fields as expected. Hence, in collaboration with Robert Behle (USDA-ARS, Peoria, IL) and David Shapiro-Ilan (USDA-ARS), several formulations are being tested for improving the fungal persistence and field efficacy against whiteflies.

In addition, in collaboration with Israeli researchers, Drs. Dana Ment and Guy Mechrez (Volcani Center, Agricultural Research Organization), nanoparticle formulations for entomopathogenic nematodes (EPNs) were tested for their tolerance against ultraviolet (UV) radiation. A TiO₂ based pickering emulsions showed excellent protection of EPNs from UV radiation, and the technology has potential to be used for pest management in any crop system. The nanoparticle formulation for EPNs is currently under a pending patent application. A paper has been published based on this research. The work was conducted under a BARD grant in David Shapiro-Ilan’s lab.

Stefan Jaronski (USDA-ARS retired; Virginia Tech): APHIS in Phoenix, Arizona conducted additional *Metarhizium* bait trials for control of grasshoppers in rangeland and generated much better results than in the past (control mortality was minimal). The technology used a wheat bran carrier and an experimental *Metarhizium* strain DWR-2009 (ARSEF 10343) with good spore production and shelf-life as bait, which achieved good results and demonstrated the feasibility of using wheat bran as carrier delivering the fungus. The behavior fever of grasshoppers inhibits the activity of most *Metarhizium* and *Beauveria* strains to a greater or less extent, except for *M. acridum*. The grasshoppers were exposed to the fungal bait in the field for 3 days using field cage and then incubated to observe the infection rates so as to avoid effects of behavioral fever, and thus measure extent of infection from the bait.

For *Melanotus communis* wireworm control in potato, Virginia Tech graduate student, Mika Pagani, presented her mesocosm data from 2022 at this year’s ESA meeting. She tested three granular baits that incorporated the commercial strain, *Beauveria bassiana* strain ANT-03 –

coarse corn meal coated with a methyl carboxymethyl cellulose binder, spent solid substrate of millet, and similar spent rice substrate. In previous assays the strain showed moderate efficacy, best of all the currently commercial strains. In non-sterile soil, indigenous *Metarhizium* strains infected many wireworms. (Pagani isolated those *Metarhizium* strains and will deposit them into the ARSEF collection. For years, Kabaluk in Canada has also observed indigenous *Metarhizium* that interfered with wireworm field trials.) Pagani noted that among the granular baits the spent rice substrate was superior. Larval *Tenebrio molitor* (a more susceptible host) was tested in parallel soil pots with much greater efficacy. The rice spent substrate was also superior to others. Sharma asked if the indigenous *Metarhizium* strains were from Virginia, which was confirmed by Jaronski. Jaronski added that in a survey conducted two years ago the field soil contained up to a few thousand CFUs/g soil which interferes with field trials. The *M. communis*, attacking potato in Virginia, was not very susceptible to any commercial strains of EPF including strain PPRI5339 (BASF), ANT-03 (Anatis Bioprotection), GHA (Certis Bio) (no infection), ATCC74040 (Lallemand) (no infection), Met-52 (Lallemand) (very poor infection) when assayed with 10^7 CFUs/g soil. In an immersion bioassay conducted 2 years ago, none of the strains was effective by immersing insects in fungal suspension of 10^7 CFUs/ml; at 10^8 CFUs/ml, BASF strain produced 30% infection after two weeks; ANT-03 strain 50%; remainder 0% (spore viability was >90% for all strains). Pagani is going to look at spore attachment on the cuticle of wireworms, spore germination, penetration through the cuticle, in an effort to understand the overall low susceptibility of this species to the commercial strains.

Sharma brought up that in a previous meeting Jaronski or Graesch mentioned that in Africa it was planned to use airplane or drone to release fungal products for control of locusts. Graesch said the plan was to release the mixture of azadirachtin with *Metarhizium acridum* using aerial application to large areas, especially breeding grounds with locust nymphs to be more susceptible than adults. Jaronski added that the fungal application in Africa has been made via air spraying with mineral oil formulation (standard Green Muscle type of products), over several hundred thousand hectares in east Africa. *M. acridum* successfully controlled the locusts but it is unavailable in the U.S.

Orchard Systems

Collin Wong (USDA-ARS, Byron, GA) (working with Shapiro-Ilan's group):

Entomopathogenic nematodes (EPNs) in Barricade gel formulation were sprayed for controlling the flatheaded apple borer, which attacks a variety of trees in US and Canada. The trial was conducted on maple trees in Tennessee and in walnut orchards in California. Initial tree damage data did not show significant treatment effects for reducing maple tree damage in nurseries; follow-up observations will be made to assess the larval reduction and mortality. In the laboratory tests, the EPNs were able to kill the larvae of this pest, and hence the field trials were pursued to evaluate the efficacy in controlling this pest.

Codling moth granular virus available on the market was sprayed against shuckworms in pecan. Also, Entrust was sprayed in pecan orchards for potential synergistic effects with other organic products.

Pasco Avery (University of Florida): Emily Duren from Avery's team reported that laboratory bioassays were conducted to screen the products against the citrus red mite. Among them, BotaniGard Maxx was the best product, and BotaniGard ES also showed good efficacy. These two products were then sprayed on citrus trees growing in citrus under protection screen (CUPS) as well as not under protective screens, i.e. in open air. Trees inside and outside CUPS were naturally infested with citrus red mites and biocontrol assessment of the mites was determined by comparing reduction of the population per treatment over time. Leaf samples were taken from trees prior to spraying and on a weekly basis post-spray to check the persistence of fungal spores on leaves over time using leaf presses. In addition, coverslips were pinned onto leaves to check spore deposition on leaves. Spore persistence was assessed both inside and outside CUPS. Samples from inside CUPS showed the best results with longer spore persistence and the mite population was reduced; viable spores were still detected on leaves at 21 days post application. The research team has finished the 1st trial for both the field (CUPS) and a 2nd trial is planned in the spring, 2023.

Also, a similar test was conducted using 3 small young cohort lemon trees in mesh cages in the greenhouse per treatment. Destructive samplings of the 3 cohort trees were conducted to count eggs, nymphs and adults to check if the treatments reduce pest densities. The leaves have been collected for the 1st trial for the greenhouse study; however, they have not been assessed. Another greenhouse trial as before will be conducted in the spring 2023 using mesh cages.

Compatibility studies were conducted on different ag-chemicals, insect growth regulators, insecticides, miticides from different companies with different *Beauveria bassiana* strains and also *Cordyceps javanica*. Shapiro-Ilan asked what strains of *Isaria (Cordyceps)* were tested and was told that it was the Apopka strain from PFR-97. Sharma followed to ask that if all strains tested are commercially available. Avery confirmed it and said that it is important to test the available products and let the growers know whether the products are compatible or not with the agrochemical.

Duren added that problems might occur with rain washing spores off leaves, and they plan to do a field study soon. Avery followed that they were going to expose the treated leaves to rain using a rain simulator to check if spores would adhere to the leaf surface after a rain event. Positive results have been obtained from a preliminary test, and an intern will be expected to join in February to take over the test with the rain simulator.

Behle asked Avery if he has had spore viability issues with commercial products. Avery said spore viability was always checked before use and had not had any problems. Duren added that compatibility was always checked before combining products. Behle asked if viability was checked by germination or CFUs; Avery said both because germination might be more reliable but CFUs reflect the spore coverage on leaf surface; they do leaf press on PDA with dodine or only PDA with antibiotics for washed leaves to count CFUs on underside or top side. Graesch asked if they checked mycosis on mites after infection; Duren confirmed it and said that it was checked individually in bioassays and BotaniGard Maxx worked the best, killed the insects in 2 days and had 90% mycosis. Avery added that the results were un-published but were presented by Duren who gave a presentation in 2022 ESA meeting on the mite project.

Lorenzo Rossi (University of Florida): Rossi collaborated with Pasco Avery on endophyte establishment in citrus rootstocks in the greenhouse with *Beauveria bassiana*. Three different methods were used to inoculate the fungus: foliar spray, seed coating, and soil drenching. The preliminary results showed that endophyte establishment depended on inoculation methods. The leaf spray was most successful; fungal endophytes were detected in leaves and moved to stem. But there were no good results in soil drench and root; seed treatment was ineffective. The *B. bassiana* strain used in the study is a commercial strain (BASF strain Velifer) available for growers to use. So far, the tests have been conducted on rootstocks only, and an intern will be recruited to work on grafted plants in the field next year. Behle asked about the seed treatment. Rossi answered that the seeds were soaked in spore suspension for 12 h and allowed to germinate and no CFUs were recovered on leaves, and he suggested that sculping the seed cuticle or increasing the soaking time might help.

In addition, Rossi worked with grower collaborators to increase soil quality with soil amendments, composts, mulch, and cover crop in citrus. Using cover crop for citrus is new because it is usually used for annual crop, while citrus trees and cover crops grow at the same time. Rossi was interested in evaluating if soil quality increases with EPF and other microbes in soil by using cover crops, or what cover crops act on soil, particularly in sandy soil. Shapiro-Ilan asked if cover crops increased the persistence of *Beauveria* in soil. Rossi answered that a technician in his lab is still looking into that and they also collected soil samples at different seasons (summer and winter) to check seasonality of EPF and other microbes. Shapiro-Ilan added that his lab used cover crops in pecan a while ago and observed enhanced fungal persistence by using cover crops.

Stefan Jaronski (USDA-ARS retired; Virginia Tech): Continued to work with APHIS people in Mission Texas on efficacy testing using whole trees and mesocosm trees of Jasmine or citrus in 40-gallon pots with two EPF strains, No-Fly (*Cordyceps* strain) and BioCeres (ANT-03 strain of *Beauveria*). Jaronski also comparing wettable powder and ES formulations of BioCeres products. BioCeres was superior to No-fly so far. Spore viability varied with products. However,

neither fungus worked in summer in southern Texas due to heat (leaf temperatures 39-44° C). Jaronski will continue to work with APHIS for another full season. Both No-Fly and BioCeres are far superior to PFR-97.

David I. Shapiro-Ilan (USDA-ARS, Byron, GA): Shapiro-Ilan's lab tested fungal endophytes in pecan via seed treatment rolling with fungal spores or soaking in suspension, drenching and foliar spray. Successful establishment was confirmed in all approaches by molecular detection in plant tissues and plating. Wu participated in the study. They are continuing the endophyte project in pecan and interested in looking at the suppression of pecan aphids in endophytic plants.

EPN pheromones enhanced activity of nematodes in infection and dispersal. Previously, in the laboratory and greenhouse pheromones increased EPN efficacy in biocontrol. They recently finished the 2nd year field trial against pecan weevil and found an increased level of control of the pest. Also, pheromone biomass extracts increased bioactivity of beneficial fungi and other microbial components in a peach system. A patent has been submitted on this microbiome effect (collaborating with Fatma Kaplan, Pheronym, Inc.)

The ambrosia beetle is a major pest problem in pecan and nursery crops. A postdoc Kyle Slusher has found good results in the laboratory showing the efficacy of different EPN species. The EPF will also be tested against the ambrosia beetle. The project is funded by a SCRI grant.

In addition, Shapiro-Ilan's lab has been working with Tracy Leskey's group on EPNs against spotted lanternfly. Some efficacy was observed against early instars, but the study was still going on; Laura Nixon (postdoc) was leading this study.

Sharma asked about compatibility of biochar with nematodes. Shapiro-Ilan answered that a paper was published several years ago. Behle added the function of biochar, which may act as fertilizer enhancer or soil amendment; it was tested in turf for increasing moisture and nutrients in greens and tees, and a company is producing biochar as a bio-product (contact Steve Vaughn, USDA-ARS-NCAUR, Peoria, Illinois, email: steven.vaughn@usda.gov).

Shaohui Wu (University of Georgia): In collaboration with Drs. Steven Arthurs (BioBee) and Anna Wallingford (University of New Hampshire), and Shapiro-Ilan (USDA-ARS), laboratory and field studies were conducted to evaluate the persistence of a novel capsule formulation of the EPN *Steinernema feltiae* ENO2 strain (Nemaplus[®]) with traditional aqueous applications. Nematode persistence was evaluated by baiting with the larvae of *Tenebrio molitor* at different times after inoculation. It was consistent in laboratory soil cup studies and field tests in pecan orchards that the capsule formulation persisted longer than the aqueous applications.

Metabolites of the EPN symbiotic bacteria *Photorhabdus luminescens* and *Xenorhabdus bovienii* were tested for toxicity to pecan aphids and lady beetles. It was found that both bacterial metabolites were highly toxic to pecan aphids while being safe to lady beetles. The results have been published, and a grant proposal has been submitted based on this project. Shapiro-Ilan added that this is the first time the bacterial metabolites were tested against the beneficial insects. **Ann Hajek (Cornell University):** For *Lycorma delicatula* (spotted lanternfly), studies were conducted and published describing results on bioassays with entomopathogenic fungi against spotted lanternflies of different instars. Analysis was completed documenting the genetic diversity of *Beauveria bassiana* isolates from *L. delicatula* and comparing their virulence with a commercially available isolate and this paper has been submitted for publication. Results from bioassays with the poorly known entomophthoralean pathogen *Batkoa major* were analyzed and published along with a short description of this species. Additional studies with the biology and ecology of *B. major* were completed and analyzed and a paper is being written. During 2022, we continued epizootiological studies, including evaluating the infection of spotted lanternflies by EPF at field sites from June through November. Many fungal isolates remain to be identified and Koch's postulates have been evaluated for some.

For *Halyomorpha halys* (brown marmorated stink bugs), infection of *H. halys* by the microsporidian *Nosema maddoxi* from before overwintering through after overwintering were conducted over the 2020-2021 and 2021-2022 winters. During these studies, they discovered another fungal pathogen of *H. halys*. It has been identified and Koch's postulates have been conducted to prove pathogenicity and a publication is being prepared. A paper is also being prepared reporting fungal infection from before to after overwintering.

Small Fruits and Vegetables

Albrecht M. Koppenhöfer (Rutgers University): Study on use of EPNs for control of plum curculio in highbush blueberries was concluded in 2021, and the results have been published. No additional research was conducted in 2022. Further research on EPN use against plum curculio including other components is planned for 2023 onwards.

Graesch asked if the EPNs used were commercial strains. Koppenhöfer confirmed and the *S. riobrave* strain 355 was obtained from Shapiro-Ilan's lab and it is produced by BASF; for quality consistency commercial strains were produced via wax worm larvae for one generation in the laboratory for experimental use.

David Shapiro-Ilan commented that the results that *S. riobrave* was most effective against plum curculio were consistent with his studies in peaches.

Stefan Jaronski (USDA-ARS retired; Virginia Tech): In grapes, Virginia Tech graduate student Jason Bielski did a 2nd year test on treating egg masses of the spotted wing lanternfly

with *Beauveria bassiana*. The newly hatched neonates sit on the surface of egg masses for several days while their cuticle hardens, which provides opportunities for the insects to be infected by the fungus previously applied to the surface of the fibrous egg mass. To overcome the challenge of keeping neonates alive long enough to become patently infected, the insects were incubated for only 3 days after exposure and then macerated in acid fuchsin stain to look for *B. bassiana* blastospores and hyphae, which worked moderately well. Additional nymphs were killed after the three day incubation period, by freezing, surface disinfected and then plated on selective agar to check for outgrowth of *B. bassiana*. The two methods correlated well in result findings. These may be used for other small insects which are difficult to keep alive long enough for the fungal infection. He tested 0.5x, 1x and 3x of the labeled rate of BotaniGard® (1 lb/acre); 1 and 3 lb/acre rates achieved >50% infection. Also, ANT-03 strain of *B. bassiana* was tested in both formulations with similar results. The data were presented in the ESA meeting by Bielski, and a manuscript will be expected after completing another replication this spring.

Jimmy Klick (Driscoll's): An outbreak of the cyclamen mite in strawberry was observed in organic production in Northern California. An epizootic of EPF in cyclamen mites was noticed. The fungus was slow growing on the media, and mass production has been explored for potential biocontrol. The taxonomy of the fungus is unknown and needs to be identified. Jaronski suggested it might be *Hirsutella*, a very slow growing fungus, or *Isaria*. He suggested sending the fungal culture to authority such as the current curator of the ARSEF collection of EPF for morphological and molecular identification.

There have been issues with supplies such as PFR-97® and Mycotrol for growers. BoteGHA had steady supplies and achieved exciting results. In cages with beans, high mortality (80%) of the mite was induced after 3 or 4 days. Field trials were tested and had good results. Growers in Mexico lost personnel in nematode production, which is important for white grub control. Alternatives are needed, and mustard seed meal has been tested by incorporating in soil for controlling grubs. Different commercial products of EPNs were applied post planting using the appropriate rates (misused inappropriate rates in the past). There were severe problems with chilli thrips in berry production in FL. BotaniGard (*B. bassiana*) with azadirachtin combinations applied in the field weekly at standard rates caused 70% reduction in chilli thrips, which was better than Spear-T that did not work. The work on chilli thrips was in collaboration with Sriyanka Lahiri. Growers in Mexico have been trying to produce their own fungi for biocontrol.

Sharma asked if BoteGHA was used for mites or thrips. Klick replied that BoteGHA was tested for cyclamen mites and BotaniGard was used for chilli thrips. Graesch commented that ownership of BotaniGard has been switched from Bioworks to Certis Bio and it is no longer available through Bioworks. Jaronski added that BotaniGard and BoteGHA have the same material and formulation. Sharma commented that mites are not listed on BoteGHA label in California, and Graesch explained that is because California registration needs supporting data

generated specifically in the region. Klick asked if there is potential to add mites on the label for California, and Graesch answered that mites are listed in the liquid formulation but not in the wettable powder formulation and there is potential to work with industry to generate the data for additional crops, pests and locations. Jaronski suggest contacting Certis if there is an interest for this product.

Graesch added that Certis may come up with new formulations, both liquid and dry, to be launched in 2023, and it is unknown whether they will phase out the old formulations. Jaronski said that the spore concentration in the products will be decreased, confirmed by Graesch that the liquid will drop from 11% to 2% and the wettable powder from 22% to 4%. Jaronski added that a Canadian company has picked up the registration of Naturalis[®] and Met-52[®], which will be brought back to the market in Canada and probably also in the U.S. in 2023-2024. Avery asked if Met-52 will be produced in the same formulations; Graesch said from what she heard the formulations will be the same, liquid and granular (in rice). Shapiro-Ilan asked if that will be conidia-based formulations, which was confirmed by Jaronski. Graesch commented that it is easier to keep the same formulations for bringing products back to the market; although formulations will be expected to improve, it takes time and budget.

Sriyanka Lahiri (University of Florida): Lahiri has been working on strawberry pest management, especially the control of chilli thrips, an invasive pest of strawberries, with EPNs. Chilli thrips do not pupate in soil, with the entire life cycle above ground. The challenge was to find an EPN strain that effectively controls the pest in winter strawberry in FL. In a preliminary field study, *S. feltiae* and *H. bacteriophora* applied at 100 million infective juveniles (IJs)/acre twice (5 days apart) in December using a backpack sprayer caused significant suppression of the immature stages of chilli thrips. The test will be repeated this year. There is a potential to use EPNs for chilli thrips management in organic strawberry production. A number of growers are concerned that the data were generated from organic strawberry with no fungicide applications, which may be a limitation although they are interested in trying the EPNs in their commercial fields. Lahiri has been working with growers to simply release predators and EPNs recently in a field heavily infested with chilli thrips and will check the results of using EPNs in strawberry.

Graesch asked if any adjuvant was added for foliar application of EPNs, and if any laboratory test was conducted and infection was observed (chilli thrips have very small body size, < 1 mm). Lahiri said they have not done the laboratory test and the study was implemented in the field using the most promising species (*S. feltiae* and *H. bacteriophora*). Shapiro-Ilan commented that most EPN products are not desiccation tolerant but there is a chance that the nematodes would infect the thrips if they are close; nematode infection may occur even if the host size is small but with only a few IJs invade the host and produce for only one generation; the efficacy might not be caused by nematode infection directly but rather the function of the symbiotic bacteria. Shapiro-Ilan agreed with Graesch to look for adjuvants to anti-desiccance, such as Barricade, to

protect EPNs in above-ground applications and improve the EPN persistence. The fungicides may not affect the activity of EPNs but can affect EPF like *Beauveria*, but applying fungicides and EPF at different times (e.g., 1 week apart) should minimize the effect. Klick asked if Barricade is organic compatible; Shapiro-Ilan said no, especially in fruit production. Graesch commented that other than Barricade, there are some common options of adjuvants such as capsules and Silwet L-77; Bioworks is also looking at different firegel formulations. BreakThru may be also used as an adjuvant for EPNs. Kaur was concerned about the potential effect of adjuvants for microbial biopesticides on pollinators. Graesch suggest applying them in the early morning or late evening when pollinators are not as active to minimize the impact on pollinators, and also the time is most suitable for EPN/EPF applications because UV light (detrimental to EPN/EPF) is weak. Graesch mentioned a study on hibiscus bud weevil control with EPNs and Barricade (conducted by Alexandra Revynthi from University of Florida). Shapiro-Ilan added that the adjuvants sometimes may improve the dispersion of EPNs, but the big issue for applying nematode above-ground is UV and desiccation. Klick mentioned a previous study they conducted in China for using *Metarhizium* in drenching through irrigation against chilli thrips in blueberry; the fungal application was combined with predatory mites and significant reductions in chilli thrips numbers and damage levels were observed. Lahiri commented that there was no negative effect on predators; *Beauveria* may affect the predators; Klick said that was probably because the drenching application avoid most contact with predatory mites. Graesch said that could depend on the formulations used; the wettable powder formulation typically does not affect predatory mites, but the oil formulation may reduce the predatory mite population because of the oil component.

Urban and natural landscapes, rangelands, and nurseries

Albrecht M. Koppenhöfer (Rutgers University): Native persistent EPNs may provide longer lasting suppression of various turf insect pests than commercial EPN strains that have been selected for high virulence and effective mass-production at the cost of reduced field persistence. Surveys on golf courses recovered mostly the EPNs *Steinernema carpocapsae* and *Heterorhabditis bacteriophora*. Isolates were mixed within species to increase genetic diversity and used to inoculate field plots at 2 golf courses in June 2020 at a rate of 1.25 billion per ha for each species alone and half of that rate for each species in a combination treatment. Plots measured 20 m x 10 m and were half in the fairway and half in the rough. Populations of EPNs, annual bluegrass weevil (ABW), surface-active insects, and soil insects were monitored regularly from June 2020 through October 2022. Overall, EPN numbers were higher in the rough than the fairway and higher in treated than untreated plots for the EPN species the plots were treated with. ABW larval densities in the fairway in mid-June of the three years were 47-89% lower in plots treated with both EPN species than in untreated plots. Among surface-active insects, ABW adult numbers in the fairway were lower in plots treated with *S. carpocapsae* and the species combination than in untreated plots; black turfgrass ataenius numbers were lower in all EPN

treatments than in untreated plots. White grub densities in the rough were lower in plots treated with *H. bacteriophora* than in untreated plots; similar reduction in the combination treatment were not statistically significant.

EPN, especially *S. carpocapsae*, can provide adequate control of ABW larvae on golf courses, but due to competition from several insecticides that are even more effective, may only be used on golf courses with insecticide resistant ABW populations. Another EPN species, *S. riobrave*, has shown superior control of several weevil species but has not been tested against ABW. In greenhouse tests, *S. carpocapsae* provided significant control even of highly insecticide resistant ABW but was less effective against populations with resistance ratios to the pyrethroid bifenthrin of 100x and 343x than against populations with 1-55x resistance ratios. We tested the field efficacy of *S. carpocapsae* and *S. riobrave* at three golf courses with ABW populations with bifenthrin resistance level of 55x, 100x, and 343x, respectively. There was no clear effect of resistance on the performance of the nematodes, albeit *S. carpocapsae* tended to be somewhat less effective against the 343x population. At the higher rate (2.5 billion nematodes per ha), *S. carpocapsae* provided 51 to 88% (average 68%) control compared to *S. riobrave*'s 8 to 57% (average 32%). Hence, *S. riobrave* does not seem to be an effective control option for ABW larvae whereas *S. carpocapsae* may be an effective alternative to insecticides against insecticide resistant ABW populations.

Shapiro-Ilan mentioned that several isolates were found in the field and asked if there is a plan to look at them further (hybridization, etc., to check which one is the best). Koppenhöfer said there were different isolates of *S. scarabaei* found in grubs in different areas; so far he has only looked at the virulence and efficacy in the laboratory and greenhouse, but the new isolates were not better than the original strain (21 years old) and there were no differences in IJ production. Another direction to look at is the persistence, and *S. scarabaei* persists for long time. Shapiro-Ilan suggest that if one isolate is superior to others in virulence, hybridization may be considered to screen them. Wong asked if the nematodes were passed through resistant pests for several generations to overcome insecticide resistance (e.g. pyrethroid resistance); Koppenhöfer said it was not done due to the difficulties in larval rearing. Kaur asked how the insecticide resistance ratios were determined in ABW, and if there was a susceptible population. Koppenhöfer replied that for laboratory tests ABW adults collected from the field were tested in Petri dishes at different concentrations of insecticides, and the greenhouse tests were conducted in grass pots for both adults and larvae using several different insecticides and ABW populations; there were susceptible population at Rutgers' Hort Farm and two county golf courses that receive few insecticide applications (2x resistance). In addition, they also did field tests in golf courses with different resistance ratios targeting different life stages, adults, young and old larvae and obtained similar results; the research has been published. Sharma asked if spinosad was tested, and it was confirmed by Koppenhöfer. Avery asked about the synergistic interaction of EPN and EPF; Koppenhöfer said no work was done in his lab but there were some works published

including the paper of Wu (mostly additive interactions), and the EPF need to be applied 2-4 weeks ahead of EPN. Klick mentioned his project on grub control (e.g., mustard seed meal with traps) and wondered about the regulatory of getting *S. scarabaei* across the border to Mexico, and if unlikely the possibility of conducting intensive survey on native EPNs in white grubs in Mexico. Koppenhöfer said a Canadian company is producing *S. scarabaei in vivo* with wax worms, but in samples he obtained, IJ numbers were much lower than claimed on label; the chance of getting the nematodes across the border depends on the regulation policy, and for surveying it may be worth trying to look for EPN-infected grubs, identify and produce nematodes with the assistance of specialists. Wu and Koppenhöfer further commented on the interaction of EPN and EPF.

Navneet Kaur (Oregon State University): For the project on endophyte-mediated resistance response, Kaur has one graduate student who is looking at the endophytic fungus (*Epichloe* sp.) in cool season turfgrass systems. Pear Intasin is investigating a PCR detection method to explore the viability of these endophytes in perennial ryegrass cultivars being evaluated in the National Turfgrass Evaluation Project (NTEP). Once she identifies the cultivars that produce lolines, permines, and other ergot alkaloids, she will perform a series of greenhouse experiments to evaluate the feeding response of a relatively new cutworm species that is a pest of turfgrasses in different settings namely commercial fields of grass grown for seed, golf courses, and lawns for the past decade. They will establish the association of endophytic presence with insect response and evaluate endophyte mediated insect resistance for this project.

For the evaluation of native EPN strains against cutworm and sod webworm pests of grass grown for seed, in 2021, they identified three native EPN strains from commercial grass fields that are being maintained *in vitro* for infectivity trials against target insect populations. Infectivity trials are now completed using three different cutworm species and one sod webworm species. She did not get a chance for data analyses, but preliminary analyses looked promising. They included some *Steinernema* and *Heterorhabditis* strains from Dr. Shapiro's lab as well in these trials. This was the first time they performed insecticide compatibility assays of the native strains in Petri dishes and none of the insecticides that they used seemed to affect the EPN survival and infectivity. They are going to publish these results sometime in 2023.

Kaur presented extension talks on the above research findings to her growers/stakeholder groups on a regular basis. Some of these efforts are steps to identify viable alternatives to chlorpyrifos research. Kaur organized the symposium on "Incorporating Microbials into the Culture of IPM" during the joint Entomological Society of America meeting in Vancouver, BC. Four talks included perspectives of a commercial grower, extension educator, biopesticide industry representative, and Agriculture and Agri Food Canada researcher were presenters. This session was followed by a panel discussion. Challenges regarding pesticide registrations in North America and the need for an improvement of delivery methods were key discussion topics.

Audience ~40 attendance taken three times during the session. Kaur and Dr. Surendra Dara were also invited to present the recent trends and history of our subdiscipline, invertebrate microbial control in "Recent Trends and Prospects for the Future in Several Key Plant-Insect Ecosystems Sub-Disciplines" symposium. Group effort for writing a review article in *Annals of the Entomological Society of America* was suggested.

Koppenhöfer commented that there have been different *Oscheius* species described but not with much potential in biocontrol and commercialization. Shapiro-Ilan said that the limitation has been largely on production and these nematodes do not have the close association with the symbiotic bacteria as other EPNs; they do show some biocontrol potential but not commercializing potential so far.

David Oi (USDA-ARS, Gainesville, FL) (from Steven Valles and David Oi): *Solenopsis invicta* virus 3 (SINV-3) is a virulent and specific pathogen of red imported fire ant, *Solenopsis invicta*, colonies. Under laboratory conditions, it consistently killed colonies and was highly transmissible. In 2022, we published the results of field introductions of this virus into fire ant nests where there were significant reductions of 57% in the size of nests and in the number of nests (7-fold decrease compared to controls) after 77 days. SINV-3 also persisted for over 20 months and spread to adjacent uninoculated colonies. A caveat to this study was that it was small, encompassing inoculations of twelve active fire ant nests. Nevertheless, this is the first documentation of a fire ant virus eliminating fire ant colonies under field conditions.

Surveys in June 2022 for the spread of SINV-3 in the Coachella Valley (Palm Desert area) of California from sites where it was introduced into fire ant nests in 2014, indicated the pathogen was detected at least a ¼ mile, or 400 m beyond release sites. This is 4 times further than the 100 m detection made in 2017. Note that introductions were made in a desert climate and fire ants are generally restricted to irrigated urban landscapes such as golf course and parks. Thus, fire ant populations are smaller than populations typically found in the southern U.S. and may be a factor in its limited spread.

Surveys for viral pathogens for the biocontrol of the little fire ant, *Wasmannia auropunctata*, an invasive, stinging ant were conducted in Florida, Hawaii, Argentina, and Australia. Six new and complete positive sense, single stranded RNA and one negative sense, single stranded RNA virus genomes were identified, sequenced, and characterized from transcriptomes of *W. auropunctata* collected in Argentina. The virus genome sequences were absent from the transcriptomes of *W. auropunctata* collected in Hawaii and Florida. Additional field surveys corroborated the absence of viruses in regions where the ant is invasive (USA and Australia). The replicative genome strand of four of the viruses (Electric ant polycipivirus 2, Electric ant solinvivirus, Electric ant virus 1, and Electric ant virus 2) was detected in *W. auropunctata* from Argentina, indicating that the ant is a host for these viruses. These represent the first viruses from the little fire ant.

Ann Hajek (Cornell University): For *Deladenus* and *Sirex noctilio*, Hajek's research group had collected data on the parasitic nematodes infecting the Eurasian woodwasp *Sirex noctilio* from 2011-2019. At the same time, they amassed data on parasitoids from these same *S. noctilio* populations in 204 pine trees. Data were analyzed to evaluate the interactions between and within nematode and parasitoid populations, as well as describing the *S. noctilio* populations. Results have been analyzed and submitted for publication.

David I. Shapiro-Ilan (USDA-ARS, Byron, GA): The facility at USDA-ARS, SE Fruit and Tree Research Unit (Byron, GA) will get goats and sheep to look at the benefits of animals in pecan orchards and will also study the EPN efficacy against parasites such as ticks that vector diseases (cattle fever). In Texas, using the nematodes in small sprayers onto antelopes at the feeding station worked well against the cattle fever ticks (this work is in collaboration with John Goolsby, USDA-ARS, Texas). APHIS is interested, as there are regulatory concerns that although EPNs do not need registration the use on animals requires additional clearances. This is also part of the goal of this project on goats and sheep, which is to look at the possibility of controlling both internal and external parasites.

Discussions

1. The theme for the symposium at ESA 2023. The theme of ESA 2023 is 'Insects and Influence: Advancing Entomology's Impact on People and Policy'. Shapiro-Ilan suggested formulating microbial agents for enhanced efficacy, as there have been new formulations explored for EPNs and EPFs since last discussion years ago and the formulation carrier may prevent the products from organic use; he recommended Wu to give a talk on EPN/EPF formulations for the relative research she has done. Sharma brought up the regulation policy. Graesch said that Bioworks has a regulatory department and may help with that or provide additional resources. Wu suggested inviting someone from EPA; Wong mentioned a retired EPA personnel in Iowa. Klick suggested inviting speakers on genetically modified (possibly a *Bt* expert). Kaur suggested delivery methods of microbials (bees -entomovectoring). Graesch also mentioned topic on drone release of entomopathogens. Further discussion on the symposium will be made in January, 2023.
2. Collaboration projects. Sharma mentioned the use of social media, blogs and short extension articles in advocating microbial control. Wu also suggested education programs on entomopathogens as biocontrol agents since this area lacks public recognition. Insecticide resistance with microbial biocontrol was discussed.
3. Business discussion: The service term for officers (chair, vice-chair, member-at-large, secretary) is for two years. Since elections were made last year, there is no new election this year.

4. Paula Agudelo mentioned that official memberships for the project need to be registered on NIMSS. The official membership should be approved by the affiliated institutions (USDA or land-grant universities).

1 PM on November 22, 2022 meeting adjourned.

Microbial related publications (research and outreach) from group members (2021-2022):

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