S1070 Regional Research Project Agenda
Saturday, November 4, 2023

Gaylord National Resort & Convention Center, Chesapeake B-C
National Harbor, Forest Heights, MD 20745
and virtually

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

November 4, 2023

(EST)

9:00 AM	**REGISTRATION**
9:30 AM	**PRELIMINARY BUSINESS MEETING**
	Local arrangements report
	Introductions
	Minutes of 2022 [Shaohui Wu]
	Sub-project Leads
10:00 AM	**Funding Opportunities from NIFA, Vijay Nandula - REE-NIFA (Virtually)**
10:30 AM	**NEW PROJECT REVIEW AND PLANNING**
	Large acreage crops Annual Crops [Shaohui Wu]
11:15 AM	**NEW PROJECT REVIEW AND PLANNING**
	Orchard Systems [Colin Wong and David Shapiro-Ilan]
12:00 PM	**LUNCH BREAK (on your own)**
1:15 PM	**NEW PROJECT REVIEW AND PLANNING**
	Small Fruits and Vegetables [Jimmy Klick]
2:00 PM	**NEW PROJECT REVIEW AND PLANNING**
	Urban and Natural Landscapes, Rangelands, and Nurseries [David Oi]
2:45 PM	**DISCUSSIONS**
	Theme for 2023
	Discussion of collaborative projects
	New business
3:00 PM	**ADJOURN**

Attendees 2023

	Name	Affiliation	Email
1.	Shaohui Wu	The Ohio State University	wu.6229@osu.edu
2.	Albrecht Koppenhöfer	Rutgers University	a.koppenhofer@rutgers.edu
3.	Rogelio Trabanino	Zamorano University	rtrabanino@zamorano.edu
4.	Vijay K. Nandula	USDA-NIFA, Missouri	vijay.nandula@usda.gov
5.	Pasco Avery	University of Florida	pbavery@ufl.edu
6.	Anamika Sharma	Florida A&M University	anamika.sharma@famu.edu
7.	Jimmy Klick	Driscoll's, California	jimmy.klick@driscolls.com
8.	Marco Toapanta	Agrinova, LLC	marco.toapanta@hotmail.com
9.	Eric Clifton	BioWorks, Inc.	eclifton@bioworksinc.com
10.	Lorenzo Rossi	University of Florida	l.rossi@ufl.edu
11.	Jinbo Wang	USDA-APHIS, Maryland	jinbo.wang@usda.gov
12.	Julie Graesch	BioWorks Inc.	jgraesch@bioworksinc.com
13.	Julien Levy	Texas A&M University	julienlevy@tamu.edu
14.	Brian Lovett	USDA-ARS, New York	brian.lovett@usda.gov
15.	Edwin Lewis	University of Idaho	eelewis@uidaho.edu
16.	Abigail Kropf	Iowa State University	alkropf@iastate.edu
17.	Jermaine Perier	USDA-ARS, Georgia	jermaine.perier@usda.gov
18.	Kyle Slusher	USDA-ARS, Georgia	eddie.slusher@usda.gov
19.	Colin Wong	USDA-ARS, Georgia	colin.wong@usda.gov
20.	M. Eric Benbow	Michigan State University	benbow@msu.edu
21.	Ann Hajek	Cornell University	aeh4@cornell.edu
22.	Suzanne Wainwright	Buglady Consulting	sw@bugladyconsulting.com

Accomplishments/Outcomes 2022-2023

Symposium organized: Tuesday, November 7, 2023, 9:00 AM – 12:00 PM
Title Current and Future Impact of Microbial Control Agents; Room: Chesapeake B-C
Organizers: Anamika Sharma– Assistant Professor, Entomology, Florida Agricultural & Mechanical University; Julie Ann Graesch– Technical Services Manager, Technical Services, BioWorks Inc; Stefan Jaronski – Retired, Entomology, USDA – ARS

BUSINESS MEETING

1. *Introductions:* Julia Graesch (2023 Vice Chair): Welcomed all and began with introductions. Attendees introduced themselves including a short introduction about their affiliation and work.

2. *Minutes of 2022* (prepared by Shaohui Wu): A copy of the 2022 minutes was circulated electronically prior to the meeting. A motion to approve the 2022 minutes was made by Shaohui Wu. Revisions were requested by Pasco Avery and the amended minute was passed unanimously. Minutes of the 2023 meeting are required to be posted within 60 days.

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

Dr. Nandula provided link for NIFA-funded projects search
https://www.nifa.usda.gov/data/data-gateway
https://www.nifa.usda.gov/data/cris-current-research-information-system

Additional useful links can be found here:

Signing up for NIFA Updates
https://public.govdelivery.com/accounts/USDANIFA/subscriber/new?qsp=USDANIFA_2

Searching for Funding Opportunities
https://www.nifa.usda.gov/grants/funding-opportunities

List of NIFA's Competitive RFAs
https://www.nifa.usda.gov/grants/request-for-application-list-rfa

Upcoming NIFA RFA Calendar
https://www.nifa.usda.gov/grants/upcoming-request-applications-calendar

Dr. Nandula also 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/preLogin.do?page=welcome

Dr. Nandula mentioned that grant application deadlines for this year would be similar to those of last year, as indicated in the RFAs. He suggested aligning objectives as closely as possible with the statements in RFAs and recommended discussing research ideas with NIFA project leaders before submitting an application. It was encouraged to participate in grant review panels (with potential involvement of postdocs), as this could help in building networks and acquiring grant-writing skills. Dr. Ann Hajek emphasized the importance of time allocation in grant writing and suggested that studying the structures of successful grants (available on the NIFA website) could serve as excellent models.

NEW PROJECT REVIEW AND PLANNING

Large acreage crops

Shaohui Wu (The Ohio State University): In previous work at the University of Georgia, a new strain of the entomopathogenic fungus (EPF) *Cordyceps javanica* wf GA17 was isolated from whitefly epizootics in Georgia and found to be highly virulent against whiteflies. The new fungal strain was then tested for field persistence and efficacy against whiteflies. The field efficacy of C*. javanica* wf GA17 was not different from the commercial strain Apopka97, and both showed low and short-term efficacy in the field. This was attributed to two main factors: (1) the short persistence of fungal spores (both conidia and blastospores), with over 90% of spores losing viability within 24 hours post-application; (2) inadequate contact of spores with the target hosts, as most spores landed on the upper leaf surface while whiteflies were predominantly active on the lower leaf surface. Future improvements will be necessary to enhance formulation and spore delivery (to the lower surface) to increase field persistence and efficacy. This work was conducted in collaboration with Dr. Robert Behle (USDA-ARS, retired) and was published in the Journal of Fungi. Wu mentioned that future research may be directed towards controlled environment applications, where fungal spores would be more protected from adverse environmental conditions, thus likely achieving better control.

Pasco Avery suggested using drone application technology to spray the fungus; Wu responded that drone application would not enhance spore delivery to the lower leaf surface. Pasco also mentioned that chemicals such as gossypol in cotton plants might inhibit fungal activities. Jermaine Perier suggested rotating crops. Eric Clifton asked if the application time would affect fungal persistence. Wu replied that one field trial was conducted in the late afternoon (6-7 pm), but the efficacy only lasted for about one week. In subsequent tests, spores were applied in mid-morning (9-10 am) to test fungal persistence.

Abigail Kropf (Iowa State University): In greenhouse applications with entomopathogenic nematodes (EPN), persistence was evaluated by baiting with wax worm larvae. Julie Graesch brought up indoor drone applications to reduce labor input.

NEW PROJECT REVIEW AND PLANNING

Orchard Systems

Lorenzo Rossi (University of Florida): The diversity of microorganisms, especially entomopathogenic fungi, was assessed in cover crops in different Florida citrus groves at various times of the year. This project is being conducted in collaboration with Pasco Avery.

Additionally, the establishment of endophytic fungi (*Beauveria bassiana* and *Cordyceps javanica*) was evaluated in citrus rootstocks. Positive results were observed in the greenhouse but need confirmation in the field. Methods of inoculation included leaf spray, soil drenching, and seed soaking. Among them, leaf spray was the most successful, with a 20% recovery in leaves but localized in leaves and stems only. Soil drenching was not effective, as most spores stayed near the soil surface. Seed treatment did not yield positive results. Future work will be directed towards grafted citrus plants.

Suzanne Wainwright mentioned new products in the market for mixing *B. bassiana* or *Metarhizium brunneum* granules in the soil profile to improve conidial distribution. This led to a discussion about black market microbial products. Julie Graesch mentioned that some entomopathogenic fungi (EPF) products from other countries were labeled as biostimulants to be exempted from registration. Jinbo Wang (USDA-APHIS) talked about the Plant Protection Act and emphasized that both the EPA and USDA have regulatory policies on microbial products.

Colin Wong (USDA-ARS, Byron, GA) (working with Shapiro-Ilan's group): Reports on the control of flat-headed borers with EPN and on the control of hickory sharkworm with viruses. Shapiro-Ilan's lab worked on a method of nematode mass production for growers to produce their own nematodes. Waxworm larvae were inoculated by placing a few EPN-infected larvae in a box of healthy larvae, i.e., nematodes emerge from the dead insect and infect healthy hosts. The infected insect cadavers were placed on a mesh above hydrogels, and the emerging nematodes were then harvested from the gel. Suzanne Wainwright mentioned that a bad odor might be produced by dead insects during the nematode production and because of that some growers have stopped rearing their own nematodes.

Kyle Slusher (USDA-ARS, Byron, GA) (working with Shapiro-Ilan's group): Reports on the control of ambrosia beetles with EPN.

Pasco Avery asked which parts of tree were attacked by ambrosia beetles; Slusher replied it was the tree trunk. The possibility of spraying trunks with EPF was discussed. Julie Graesch mentioned stopab.org and the ambrosia beetle grant. Ann Hajek asked if EPN were applied before or post infestation of ambrosia beetles; Slusher answered that it was post infestation application. Albrecht Koppenhöfer asked what would gel (nematode formulation) do to the ambrosia beetle adults as gels might create physical barriers to prevent adults from entering the tree trunk. Jimmy Klick brought up the ambrosia beetle problems in China, where EPF products were sprayed with water or adjuvants. Avery added that EPF products Met 52 (now formulated as Lalgard M52 OD), PFR-97™ 20% WDG (PFR-97), and BotaniGard® ES (BotaniGard) sprayed with water under the tree canopy persisted for 3 weeks.

Brian Lovett (USDA-ARS): The EPF products (BotaniGard and PFR-97) were evaluated for ambrosia beetle control in apple orchards. In laboratory bioassays, low fungal concentrations were ineffective and high concentrations killed insects. Pasco Avery asked what symbionts the ambrosia beetles carried.

Pasco Avery (University of Florida): Compatibility studies were conducted for PFR-97 mixed with various ag chemicals, no difference at 6 h. Table of compatible agrochemicals with EPF-based products was produced and handed out to interested people and groups at the Citrus Show in Ft. Pierce, FL. Avery also evaluated the potential of PFR-97 incidental drift from 10^2, 10^3 10^5 and10^6 spores ml^{-1} affecting the air potato beetle used for biological control of air potato vines, and found that larvae were not affected by exposure to *C. javanica* spores contained in the product PFR-97. A prior compatibility study using adult air potato beetles also found that they were not affected after exposure to PFR-97 at the various concentrations listed above compared to the control (no spray). Results have been published in the Florida Entomologist journal.

Research has indicated that rainfall is a major deterrent for the persistence and subsequent field efficacy of EPF-based products over time. Therefore, research studies using a rain fast product were conducted to evaluate the persistence of *C. javanica* spores over time to adhere to the leaf surface of citrus plants using a rain simulator. Rain fast adjuvant product Ampersand® (used as food additive and OMRI labeled) was able to hold spores on the leaf surface even after major rain events. Julie Graesch asked if Ampersand would affect insects picking up spores on the sprayed surface; Avery said it had not been investigated yet.

Ann E. Hajek (Cornell University) An epizootic caused by fungal pathogens occurred among *Halyomorpha halys*, brown marmorated stink bugs, while they were overwintering, with infections also occurring after overwintering. We report that one of the two pathogens responsible was *Colletotrichum fioriniae* (Marcelino & Gouli) Pennycook, a species well known as a plant pathogen and endophyte and which has only previously been reported naturally infecting elongate hemlock scales, *Fiorinia externa*. To prove pathogenicity, *H. halys* adults challenged with conidia died from infections and the fungus subsequently produced conidia externally on cadavers.

The microsporidian, *Nosema maddoxi* Becnel, Solter, Hajek, Huang, Sanscrainte & Estep, infects brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), populations in North America and Asia and causes decreased fitness in infected insects. This host overwinters as adults, often in aggregations in sheltered locations, and variable levels of mortality occur over the winter. We investigated pathogen prevalence in *H. halys* adults before, during, and after overwintering. Population level studies resulted in detection of *N. maddoxi* in *H. halys* in 6 new US states, but no difference in levels of infection by *N. maddoxi* in autumn versus the following spring. *Halyomorpha halys* that self-aggregated for overwintering in shelters deployed in the field were maintained under simulated winter conditions (4°C) for 5 months during the 2021–2022 winter and early spring, resulting in 34.6 ± 4.8% mortality. Over the 2020–2021 and 2021–2022 winters, 13.4 ± 3.5% of surviving *H. halys* in shelters were infected with *N. maddoxi*, while *N. maddoxi* infections were found in 33.4 ± 10.8% of moribund and dead *H. halys* that accumulated in shelters. A second pathogen, *Colletotrichum fioriniae* Marcelino & Gouli, not previously reported infecting *H. halys*, was found among 46.7 ± 7.8% of the *H. halys* that died while overwintering, but levels of infection decreased after overwintering. These 2 pathogens occurred as co-infections in 11.1 ± 5.9% of the fungal-infected insects that died while overwintering. Increasing levels of *N. maddoxi* infection caused epizootics among *H. halys* reared in greenhouse cages after overwintering.

The prevalence of the microsporidium *Nosema maddoxi* in populations of brown marmorated stink bug (*Halyomorpha halys*) from three sites in Guria and eight sites in Samegrelo, Georgia was described. Investigations were conducted on adults collected in fall and spring from fall 2020 to spring 2022. The prevalence of infection differed by both region and season. Total infection of *N. maddoxi* in adults from the Guria region was significantly higher than the Samegrelo region. Infection levels in fall were higher than in spring in the Guria region, but inconsistent over seasons in the Samegrelo region. Infection levels did not differ by sex. We hypothesize that higher population densities and more adult aggregation in Guria compared with Samegrelo helps to explain the higher infection levels in Guria.

Hajek also mentioned the Insect Pathology Short Course has been offered every other year at Cornell University and can only take 24 students each time.

Edwin Lewis (University of Idaho): Larvae of hard pearl (?) were difficult to control with commercial EPN species. EPF was used to target adults. A trapping method was developed by employing fungal pathogens that did not kill insects rapidly in pheromone traps attracting males, so that infected adults were be able to move and transfer the pathogen to their mate. The larval stage lasted 3-4 years, and adults did not feed.

Pasco Avery asked where eggs were laid; Lewis answered that it was around the soil surface and larvae burrow down after hatching. Color had no impact on the trap that took effects by pheromone.

Shaohui Wu (The Ohio State University): In the previous work in Shapiro-Ilan's lab, Wu explored formulations for EPN and EPF. In searching for novel formulations of EPNs, a liquid starch (Trueliving Heavy Spray Starch) and white kaolin clay provided excellent UV protection of the infective juvenile nematodes. Kaolin clay had been used in orchards in pest protection but had not been used for nematode application; it was found that 5% white kaolin clay had the best protection for sprayable applications. The liquid starch had been traditionally used for shirts after laundry, and this was the first time that its agricultural use had been explored; 50% concentration gave full protection of nematodes from UV radiation. However, in soil applications outdoors, Barricade and hydrogel performed better than liquid starch and white kaolin clay, probably because of the anti-desiccation effects involved. The work was in collaboration with Dr. George Mbata's lab at Fort Valley State University and was published in the journal Biological Control. Also, in collaboration with Drs. Dana Ment and Guy Mechrez from Volcani Center, ARO in Israel, Wu evaluated the UV protection of novel nanoparticle formulations for both EPN and EPF. For EPN, SiO_2 based nanoparticle oil-in-water pickering emulsions were ineffective, while TiO_2 based nanoparticles protected nematodes from UV radiation, published in the Journal of Invertebrate. Pathology. Meanwhile, for EPF formulations, TiO_2 nanoparticles were effective in protecting *M. brunneum* conidia, but SiO_2 nanoparticles had no anti-UV effects. In addition, Wu also looked at the persistence of a novel capsule formulation of the EPN *Sternernema feltiae* ENO2 strain (Nemaplus® Depot) under laboratory and field conditions. In pecan orchards, when nematode products were applied in their recommended methods (traditional aqueous application via soil surface drenching; capsules mixed with soil in 2-inch depth of ditch), capsules persisted longer than aqueous applications. However, when both products were applied in soil ditch, they had similar field persistence, probably because nematodes were better protected by soil or weather conditions (frequent rainfalls) were favorable to nematode activities.

Jimmy Klick asked about the persistence of EPN in foliar spray and was told that the work was going to be conducted in collaboration with Dr. Mbata's lab. Klick also asked about the cost of hydrogel; Wu shared that costs of various formulations were mentioned in the paper (Wu et al., Biological Control, 2023).

NEW PROJECT REVIEW AND PLANNING

<u>Small Fruits and Vegetables</u>

Julien Levy (Texas A&M University): Research focused on the interactions occurring between Solanaceae crops and Bactericera *cockerelli* (the tomato / potato psyllid). The potato psyllid is the vector of '*Candidatus* Liberibacter solanacearum' (*C*Lso), a phloem-limited pathogen associated with multiple economically important diseases of solanaceous crops. In the USA, the main crops affected by this pathogen are potatoes and tomatoes, causing "zebra chip" and "permanente del tomato", respectively. In this project, we focused on the use of alternative strategies to control insect populations and in particular their effect on the transmission of the pathogen. To reduce pesticide use, bioinsecticides such as entomopathogenic fungi are becoming increasingly attractive to control pests The purpose of this experiment is to determine if exposure to entomopathogenic fungi such as *Beauveria bassiana* (GHA) and

Phialemonium inflatum TAMU490, will affect the ability of psyllids to transmit *C*Lso bacteria to tomato plants.

This research was conducted by undergraduate students' part of the Aggie Research Program.

Test of two entomopathogenic fungus *B. bassiana* strain GHA, and *P. inflatum* TAMU490.
In the first semester the student learned to culture each fungus and prepare the fungal suspension. They learned how to grow and maintain insect colonies, collect insects, and sex tomato psyllids. They also practiced applying the fungus to the plant and to the psyllids. We conducted a pilot experiment by infesting both treated (sprayed with a 10^7 spore /ml of fungus) and un-treated plants with adult tomato psyllids. One week prior to infestation plants were sprayed twice (and thereafter spraying continued twice a week). Plants were infested by placing 4 insects on one single leaf in a mesh bag, after one-week insects were removed from the mesh bag and mortality was measured. Three weeks post infestation the plant infection by *C*Lso was measured by PCR. In this pilot experiment we did not notice any significant differences between non-treated plants and plants treated by spraying them with a fungus suspension.

We are planning to repeat this experiment with undergraduate students in 2025. A second experiment will test if entomopathogen-treated plants influence the insect host choice.

Jermaine Perier (USDA-ARS): reported on the compatibility of EPN with chemicals, IGR/adjuvant.
Suzanne Wainwright commented that change of chemistry in soil might affect EPN activities, as it was reported that EPN were found to be ineffective to kill fungus gnats, and temperature might have also played a role. Julie Graesch commented on foliar application of EPN. Edwin Lewis added that studies were conducted for smaller infective juveniles (IJs) to be produced in larger quantities. Jimmy Klick asked about breeding of EPN for more persistent and virulent strains. Julie Graesch brought up that between *in-vivo* and *in-vitro* production of EPNs, growers mostly used *in-vitro* produced EPNs, and nematodes carried in a sponge had relatively shorter shelf life. Lewis added that Shapiro-Ilan did some studies on *in-vivo* and *in-vitro* production of EPNs.

Jimmy Klick (Driscoll's): Nematode products (Nemasys® from BASF) were used to control seed corn maggots in strawberry nurseries with 2 billion IJs per acre. Before planting, plants were soaked with nematodes, and nematodes persisted at least one month post planting. Imidacloprid was used in combination with EPN for control of the pest.

Direct application of the fungus *Simplicillium* sp. had no effect in controlling cyclamen mites. Control of chilli thrips was explored using azadirachtin, *B. bassiana* and *C. javanica*. A strain of *B. bassiana* isolated from grubs was used as a biopesticide in organic strawberry production in

Mexico; Brian Lovett asked if the *B. bassiana* strain was identified. Control of ambrosia beetles in blueberry production with EPF was explored in China.

NEW PROJECT REVIEW AND PLANNING

Urban and natural landscapes, rangelands, and nurseries

Albrecht M. Koppenhöfer (Rutgers University): The annual bluegrass weevil (ABW) is a major insect pest of golf courses in eastern North America with widespread and broad-spectrum insecticide resistance. Previously, we had shown that entomopathogenic nematodes (EPNs) can provide good control of ABW larvae on golf course fairways. Due to the availability of numerous insecticides that are easier to use and mostly cheaper than EPNs, it is unlikely that golf courses will widely adopt EPN use. But golf courses that want to delay insecticide resistance or already have resistant ABW populations may consider using EPNs as an alternative to standard synthetic insecticides.

We tested the effect of insecticide resistance in ABW on the efficacy EPNs in greenhouse and field experiments. In a first greenhouse experiment we found that the efficacies of the EPNs *Steinernema carpocapsae*, *Steinernema feltiae*, and *Heterorhabditis bacteriophora* were only 9-19% lower against moderately resistant ABWs (resistance ratio (RR50) to the pyrethroid bifenthrin: 55x) than against highly susceptible ABWs (RR50: 2x). In a second greenhouse experiment, the efficacy of *S. carpocapsae* was 30-34% lower against highly (95x) and extremely (343x) resistant ABW than against highly susceptible (1-2x) ABWs. In field experiments conducted twice with ABW populations having resistance levels to bifenthrin of 55x, 95x, and 343x, *S. carpocapsae* provided similar control against the 55x and 95x populations, but 16-20% control lower against the 343x population.

In the latter field experiment, we also tested a not previously against ABW tested EPN species, *Steinernema riobrave*, that had provided excellent control against several other weevil species. However, against ABW *S. riobrave* was significantly less effective than *S. carpocapsae*, but its efficacy tended to increase with insecticide resistance level, albeit not statistically significantly so. And *S. riobrave* was as effective as *S. carpocapsae* against the 343x resistant ABW population.

Our findings suggest that EPNs can be used for ABW control and for insecticide resistance management against insecticide resistant ABW population, albeit their efficacy may be somewhat reduced against extremely resistant populations.

Ann E. Hajek (Cornell University)

Spotted Lanternflies. To evaluate the genetic diversity of *Beauveria* spp. infecting spotted lanternflies (SLF), in 2018- 2020 we collected SLF and nearby non-target insects killed by *Beauveria* spp. from 18 field sites in southeastern Pennsylvania. We identified 159 *Beauveria* isolates from SLF and six isolates from non-targets. Five isolates of *B. bassiana* and one isolate of *B. brongniartii* were identified from the non-targets. Based on sequence data from the nuclear B locus (Bloc) intergenic region, all the isolates from SLF were identified as *B. bassiana*, but there were 20 different strains within this species, grouped into two clades. Three *B. bassiana* strains (A, B, and L) were found in most field sites and were the most prevalent. Representative isolates for these three strains were used in laboratory bioassays and were compared to a commercialized *B. bassiana* strain (GHA). Strain B was inferior to A, L, and GHA against nymphs; strains A and L had greater efficacy than B and GHA against adults. We also quantified conidial production on SLF cadavers.

The Hajek lab has been studying entomopathogenic fungi killing spotted lanternflies (SLF; *Lycorma delicatula*), previously predominantly focusing on *Batkoa major* and *Beauveria bassiana*. During studies of the epizootiology of this system two additional species of entomopathogenic fungi killing SLF were collected in 2018 and 2020 and identified. Therefore, when three more probably entomopathogens were isolated from SLF adults in 2021, we decided to sample forested sites containing tree of heaven throughout 2022, especially targeting nymphal stages (June through July and early August). These studies generally included Koch's postulates to confirm which fungi were pathogens. We have documented 19 species of entomopathogenic fungi killing spotted lanternflies. All are in the Order Hypocreales (Ascomycota) but are from numerous families. During the 2022 season little rain fell during the nymphal stages; most infection occurred among adults when there was more rainfall. However, lack of infection among nymphs is consistent with our previous studies years without a mid-summer drought, and the long-lived adults appear to be more susceptible as they age. Many of the 19 fungal species were not abundant and the most common pathogen was *B. bassiana*.

Batkoa major caused high levels of infection in SLF in 2017 and questions were addressed about the general biology of this poorly known fungal pathogen, using *Galleria mellonella* larvae exposed to conidial showers. Death of *G. mellonella* followed a diurnal cycle with most larvae dying within 4 h before or after the end of photophase. Time for initiation of rhizoid emergence also followed a diurnal rhythm and, on average occurred 3.6 h after host death. While *B. major* sometimes began producing rhizoids to attach cadavers to substrates while *G. mellonella* were alive (but moribund), often hosts were dead before rhizoids began emerging. On average, conidial discharge began 18.6 h after host death and was greater 4–8 h before the end of photophase, compared with 4–8 h after scotophase began. At 20 ◦C under high humidity, initiation of conidial discharge was 95% complete within 24 h after host death. To evaluate *B. major* activity by temperature, we tested percent conidial germination over 24 h from 5 to 35 ◦C. When showered onto water agar, all primary conidia produced secondary conidia. For 20 and 25 ◦C, at 3 h after showering ≥89% of primaries had produced and discharged secondaries and from 10 to 30 ◦C, secondaries were produced by over 75% of primary conidia within 12 h. When cover slips were placed over primary conidia to force production of germ tubes, germination was much slower, with >85% germination from 20 to 30 ◦C only by 24 h. *Batkoa major* therefore times host death and initiation of conidial discharge for night-time hours and conidial germination occurs within 24 h over a broad temperature range (10–30 ◦C).

With Dr. Stefan Jaronski and Jason Bielski, we asked to what extent contact with *B. bassiana* on different body parts impacted infection. To investigate contact via walking on spores, trunks of tree of heaven (TOH) were sprayed with BioCeres-WP (containing *Beauveria bassiana* strain ANT-03). Trees in the vicinity sprayed with water were controls. Spotted lanternflies from nearby trees were then caged over the sprayed areas for 3 days. SLF were then collected, transported to quarantine, and reared on TOH for 21 d. On the 21st day, any SLF that had not died were frozen, after which these were placed at high humidity to promote fungal outgrowth if they had been infected. Data are presently being analyzed but *B. bassiana* killed almost all the SLF on sprayed trees and among the survivors, *B. bassiana* was present within their bodies. Infection among controls was minimal. We then applied *B. bassiana* GHA to wings versus abdomens of SLF adults; the question was when spraying *B. bassiana* on adults on a tree trunk, would the wings covering their bodies act as a shield that the fungus would not be able to infect. About 50% of SLF with abdominal inoculations became infected although about 25% of wing inoculations also became infected. Data are presently being analyzed further.

***Sirex* Woodwasps.** Parasitic nematodes and hymenopteran parasitoids have been introduced and used extensively to control invasive Eurasian *Sirex noctilio* woodwasps in pine plantations in the Southern Hemisphere where no members of this community are native. *Sirex noctilio* has more recently invaded North America where *Sirex*-associated communities are native. *Sirex noctilio* and its parasitic nematode, *Deladenus siricidicola*, plus six native hymenopteran woodwasp parasitoids in New York and Pennsylvania, were sampled from 204 pines in 2011–2019. *Sirex noctilio* had become the most common woodwasp in this region and the native parasitoids associated with the native woodwasps had expanded their host ranges to use this invader. We investigated the distributions of these species among occupied trees and the interactions between *S. noctilio* and natural enemies as well as among the natural enemies. *Sirex noctilio* were strongly aggregated, with a few of the occupied trees hosting hundreds of woodwasps. Nematode parasitism was positively associated with *S. noctilio* density, and negatively associated with the density of rhyssine parasitoids. Parasitism by the parasitoid *Ibalia leucospoides* was positively associated with host (*S. noctilio*) density, while parasitism by the rhyssine parasitoids was negatively associated with density of *S. noctilio*. Thus, most *S. noctilio* come from a few attacked trees in a forest, and *S. noctilio* from those high-density trees experienced high parasitism by both the invasive nematode and the most abundant native parasitoid, *I. l. ensiger*. There is little evidence for direct competition between the nematodes and parasitoids. The negative association occurring between rhyssine parasitoids and *I. l. ensiger* suggests rhyssines may suffer from competition with *I. l. ensiger* which parasitize the host at an earlier life stage. In addition to direct competition with the native woodwasp *S. nigricornis* for suitable larval habitat within weakened trees, the large *S. noctilio* population increases the parasitoid and nematode populations, which may increase parasitism of *S. nigricornis*.

Dimorphic nematodes in the genus *Deladenus* have been used or are being considered for use in biological control of the invasive Eurasian woodwasp, *Sirex noctilio*, which threatens pine (*Pinus* spp.) trees. *Deladenus* species that are parasitic on *Sirex* can kill woodwasp eggs and occupy these same eggs for their own dispersal. These nematodes also have mycophagous phases that feed on the white rot fungal symbionts of *Sirex*, *Amylostereum* species. The mycophagous stage of the Hungarian strain of *Deladenus siricidicola* developed for control of *S. noctilio* in
Australia feeds exclusively on the species *A. areolatum*. The mycophagous stage of a North American *Deladenus* species being evaluated for biological control, *D. proximus*, feeds on either *A. chailletii,* or *A. areolatum. Amylostereum* species and strains associated with *Sirex* have differential impacts on survival and growth of these nematodes. We investigated whether differences in species and strains of *Amylostereum* infuence the numbers of *Deladenus* juveniles adjacent to cultures, as this would impact potential parasitism of *Sirex*. Fungal species or strain did not influence persistence of juveniles in the fungal vicinity although retention could be influenced by the fungal strain consumed by parents. Investigating *D. proximus*, we tested whether the most common invasive strain of *A. areolatum* associated with *S. noctilio* in North America (IGS D) impacted nematode growth, compared with the common native *Amylostereum chailletii*. *Deladenus proximus* increased very slowly when feeding on *A. areolatum* IGS D, compared with *A. chailletii*; when provided *A. areolatum* IGS D, 55 eggs were produced after 4 weeks compared with 8.1×10^4 eggs after 2 weeks when *A. chailletii* was provided. In summary, behavior of *Deladenus* juveniles resulted in no or low avoidance of *Amylostereum* species.

Anamika Sharma (Florida A&M University): In the USA first decapitating phorid was released in 1997 to manage red imported fire ants. Six species (*Pseudacteon culltellus*, *Pseudacteon curvatus*, *Pseudacteon littoralis*, *Pseudacteon nocens*, *Pseudacteon obtusus*, and *Pseudacteon tricuspis*) of phorid flies have been released in North America since then. Along with phorids, microsporidia *Kneallhazia (=Thelohania) solenopsae* and *Vairimorpha invictae* and entomopathogenic fungus (EPF) *Beauveria bassiana* strain 447 were also considered potential biological control agents in the USA. Currently, we are surveying in north Florida (I-10 corridor) to analyze the establishment and efficacy of six species of phorid flies, two microsporidium and one EPF. Four phorid species were found after surveying 10 sites along the I-10 highway in the Northwest part of Florida from Jacksonville to Pensacola in urban and peri-urban locations. Samples with EPFs were obtained from some of the sites and analysis of samples is under process. Detection of the presence of microsporidium and EPF is under process through microscopy and Polymerase chain reaction.

Small undergraduate projects are being established to test the efficacy of IPM traps for urban insects including termites, fire ants, mosquitoes, and bed bugs. We are combining microbial with insecticides in the baiting system, and combination of microbial with pheromone traps is also being tested.

Jimmy Klick commented on commercial fungal baits.

Navneet Kaur (Oregon State University): Exploring endophyte-mediated resistance response against lepidopteran insect pests in cool-season turfgrass systems. The subterranean sod webworm, also known as cranberry girdler (*Chrysoteuchia topiaria*) is one of the most damaging insect pests in cool-season grass grown for seed crops in Oregon. Chemical control options are limited and require irrigation or rainfall for adequate insecticide incorporation to control *C. topiaria* larvae. Epichloë endophytes associated with cool-season turfgrass species and their mycotoxin profiles are well-documented in offering plant protection against invertebrates; these fungi may offer sustainable pest management tools. Our objectives were to characterize endophyte-mediated resistance to *C. topiaria* in 19 commercially available cultivars of tall fescue, perennial ryegrass, and fine fescue grown for seed in Oregon. Endophyte status (presence and viability) of fungal endophytes and their mycotoxin profiles were measured using polymerase chain reaction (PCR); liquid chromatography-tandem mass spectrometry (LC-MS/MS), respectively. No-choice assays were conducted in the laboratory to measure the impact of endophyte status on *C. topiaria* larvae in two separate no-choice experiments. Our results suggested that increased mortality of *C. topiaria* larvae (R2= 0.8526, experiment 1; R2= 0.6628, experiment 2) in tall fescue cultivars was most influenced by total peramine and ergot alkaloid, and total ergoline concentrations in experiment 1 and 2, respectively. However, no significant effect on insect mortality was found in the perennial ryegrass and fine fescue cultivars included in this study. Overall, these findings suggest a viable grass-endophyte association can be utilized as a sustainable alternative to foliar insecticides for *C. topiaria* management in tall fescue seed crops. A manuscript was submitted to the Journal of Applied Turfgrass Science or Crop Forage and Turfgrass Management in 2023, the decision of the journal is still pending.

The winter cutworm *Noctua pronuba* is another serious lepidopteran pest in Oregon's grass seed production systems, golf courses, sports fields, and lawns. Currently, there are no cultural or biological control options for this pest; therefore, grass seed growers rely on prophylactic insecticide treatments to prevent crop damage. *Noctua pronuba* larvae typically emerge in grass seed fields in early winter when field conditions are too wet for equipment operation, and air temperatures are lower than desired for optimal insecticide efficacy. Utilizing symbiotic fungal endophytes in grass seed crops presents a promising non-chemical remedy due to their earlier discovered plant protection benefits against lepidopteran pests. One grad student, Pear Intasin was hired during Fall 2022 to work on these projects for her MS thesis project. Pear just completed her greenhouse experiments and currently is working on data analyses. Findings from this project will be presented at both scientific and regional grower meetings in near future. The information gained from this project will be used to identify the endophyte profiles for cool-season grass cultivars, which could be used for insect management in both turfgrass and grass seed production systems. We intend to continue our research efforts to understand endophyte insect interactions and build partnership with seed companies to incorporate beneficial endophyte populations for insect pest management in cool-season turfgrass species. Funding for this project was received from Oregon Grass Seed Commissions, Oregon State University's Agricultural Research Foundation, and Western Sustainable Agriculture Research and Extension Grant Program.

Discussions

Stefan Jaronski proposed collaborative review project on biopesticides for crop pest (insect/ arthropod) management.

Theme for next ESA would be discussed via email communication.

Voting was made for the next two-year term:

- **Anamika Sharma** was elected as the Chair
- **Shaohui Wu** as Vice Chair
- **Pasco Avery** and **Eric Clifton** as Member-at-Large
- **Lorenzo Rossi** as Secretary and Treasurer

It was mentioned that joining the S1070 working group as a hatch/multi-state project could get travel fund for attending the annual meeting.

Microbial related publications (research and outreach) from group members (2022-2023):

1. Arnoldi, M., Muschweck, L. Duren, E. B., Avery, P. B., and L. Rossi. 2023. Methods of inoculating citrus rootstock cultivars with *Beauveria bassiana* as an entomopathogenic fungal endophyte. *Proceedings of the Florida State Horticultural Society* 135, 78

2. Bardsley, C.A., Chasteen, K.S., Shapiro-Ilan, D., Bock, C.H., Niemira, B.A., and Govindara, D.K. 2023. Transfer of generic *Escherichia coli* and attenuated *Salmonella enterica Typhimurium* from the soil to the surface of in-shell pecans during harvest. Heliyon (In Press, Accepted 8-30-2023).

3. Chavez, A. V., Duren, E. B., Avery, P. B., Pitino, M., Duncan, R. E., Cruz, L.F., Carrillo, D., Cano, L. M., and R. D. Cave. 2023. Evaluation of spore acquisition, spore production, and host survival time of tea-shot hole borer. *Euwallacea perbrevis*, adults after exposure to four commercial products containing *Beauveria bassiana*. *Insects* 14, 726; doi.org/10.3390/insects14090726.

4. Clifton, E.H., Castrillo, L.A., Jaronski, S.T., Hajek, A.E. 2023. Cryptic diversity and virulence of *Beauveria bassiana* recovered from *Lycorma delicatula* (spotted lanternfly) in eastern Pennsylvania. Frontiers in Insect Science: Focus on Spotted Lanternfly 3: 1127682. https://doi.org/10.3389/finsc.2023.1127682

5. **Dara, S. K.** Role of marketing and outreach for the success of entomopathogenic nematodes. *In* Entomopathogenic nematodes as biological control agents. Eds. D. I. Shapiro-Ilan and E. E. Lewis, CABI. Accepted. (**Book chapter**)

6. **Dara, S. K.** 2023. Biopesticides: market, use, research and education needs, and future. Submitted to West Coast Nut. (**Trade journal article**)

7. **Dara, S. K.** 2023. Entomopathogenic fungi-based biopesticides contribute to more than pest management. CAPCA Adviser 26(6): 48-51. (**Trade journal article**)

8. Geisert, R.W., M.P. Huynh, A.E. Pereira, D.I. Shapiro-Ilan, and B.E. Hibbard. 2023. An improved bioassay for the testing of entomopathogenic nematode virulence to the Western corn rootworm (*Diabrotica virgifera virgifera*): With focus on neonate insect assessments. J. Econ. Entomol. 116: 726–732. https://doi.org/10.1093/jee/toad052.

9. Gonzalez, J.B., Lambert, C.A., Foley, A.M., Hajek, A.E. 2023. First report of *Colletotrichum fioriniae* infections in brown marmorated stink bugs, *Halyomorpha halys*. J. Invertebr. Pathol. 199: 107939. https://doi.org/10.1016/j.jip.2023.107939

10. Hajek, A.E., Brandt, S.N., Gonzalez, J.B., Bergh, J.C. 2023. Entomopathogens infecting brown marmorated stink bugs before, during, and after overwintering. J. Insect Science 23(3): 8; 1-8. DOI: 10.1093/jisesa/iead033

11. Hajek, A.E., Everest, T.A., Clifton, E.H. 2023. Accumulation of fungal pathogens infecting the invasive spotted lanternfly, *Lycorma delicatula*. Insects 14: 912. https://doi.org/10.3390/insects14120912

12. Hajek, A.E., Harris, C.H. 2023. Diurnal patterns and conidial dynamics of *Batkoa major*, a generalist entomophthoralean pathogen. Fungal Ecology 65: 101278. https://doi.org/10.1016/j.funeco.2023.101278

13. Kereselidze, M., Pilarska, D., Guntadze, N., Linde, A., Hajek, A. 2023. Prevalence and distribution of *Nosema maddoxi* infection in *Halyomorpha halys*, an invasive pest of hazelnut orchards in Georgia. Acta Horticulturae 1379.64. 10.17660/ActaHortic.2023.1379.64

14. Koppenhöfer A.M., Kostromytska O.S., Ebssa L. 2022. Efficacy of entomopathogenic nematodes against black cutworms and split applications and syringing to optimize their performance. Golf Course Management, September 2022, 80-84.

15. Leite, L.G., Chacon-Orozcoa, J.G., Shapiro-Ilan, D.I., Baldo, F.B., Cardoso, J.M. 2023. Effects of temperature for optimizing production and storage of *Steinernema rarum* in a novel biphasic process, and efficacy of the nematode against *Sphenophorus levis*. Biological Control 187, 105381. https://doi.org/10.1016/j.biocontrol.2023.105381

16. Li, J., Li, Y., Wei, X. Cui, Y., Gu, X., Li, X., Yoshiga, T., Abd-Elgawad, M.M., Shapiro-Ilan, D., Ruan, W., Rasmann, S. 2022. Direct antagonistic effect of entomopathogenic nematodes and their symbiotic bacteria on root-knot nematodes migration toward tomato roots. Plant and Soil. 484(1-2):1-15. https://doi.org/10.1007/s11104-022-05808-4

17. Morris, E.E., Harris, D.C., Shen, A., Vermeylen, F., Hajek, A.E. 2023. Impact of *Amylostereum* (Basidiomycota: Russulales**)** diversity on *Deladenus* (Nematoda: Neotylenchidae) behavior and fitness. Biological Control 179: 105147. Doi: 10.1016/j.biocontrol.2022.105147

18. Neal, A. S., Avery, P. B., and Cave, R. D. 2023. Survival time, mortality and feeding damage of adult *Myllocerus undecimpustulatus undatus* (Coleoptera: Curculionidae) exposed to biopesticides in laboratory bioassays. *Applied Microbiology* 3(2), 388-399; https://doi.org/10.3390/applmicrobiol3020027.

19. Oliveira-Hofman, C., Steffan, S., Shapiro-Ilan, D. 2023. A sustainable grower-based method for entomopathogenic nematodes production. Journal of Insect Science. 23, 4; 1–5. https://doi.org/10.1093/jisesa/iead025

20. Perier, J.D., Kaplan, F., Lewis, E.E., Alborn, H., Schliekelman, P. Toews, and Shapiro-Ilan, D. 2024. Enhancing entomopathogenic nematode efficacy with pheromones: A field study Targeting the pecan weevil. Journal of Invertebrate Pathology. In Press (Accepted 1-23-24).

21. Rodriguez-Saona, C. and **S. K. Dara.** Entomopathogenic nematodes in berry crops. *In* Entomopathogenic nematodes as biological control agents. Eds. D. I. Shapiro-Ilan and E. E. Lewis, CABI. Accepted. (**Book chapter**)

22. Slusher, E.K., Lewis, E., Stevens, G., Shapiro-Ilan, D. 2024. Movers and shakers: Do nematodes that move more invade more? Journal of Invertebrate Pathology. (IN PRESS, Accepted 1-16-2024).

23. Stevens, G., Erdogan, H., Pimentel, E., Dotson, J., Stevens, A., Shapiro-Ilan, D., Kaplan, F., Schliekelman, P., Lewis, E. 2023. Group joining behaviours in the entomopathogenic nematode *Steinernema glaseri*. Biological Control. In Press (Accepted 3/20/2023).

24. Toledo, P.F.S. Phillips, K., Schmidt, J.M., Bock, C.H., Wong, C., Hudson, W.G., Shapiro-Ilan, D., Wells, L., Acebes-Doria, A.L. 2023. Canopy hedge pruning in pecan production differentially affects groups of arthropod pests and associated natural enemies. Crop Protection (In Press accepted 11/7/23).

25. van Nouhuys, S., Harris, D.C., Hajek, A.E. 2023. Population level interactions between an invasive woodwasp, an invasive nematode and a community of native parasitoids. Neobiota 82: 67-88. doi: 10.3897/neobiota.82.96599

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28. **Wu, S.,** Li, Y., Toews, M.D., Mbata, G., Shapiro-Ilan, D.I. 2023. Novel formulations improve the environmental tolerance of entomopathogenic nematodes. *Biological Control.* 186, 105329. https://doi.org/10.1016/j.biocontrol.2023.105329

29. **Wu, S.,** Toews, M.D., Behle, R.W., Barman, A., Shapiro-Ilan, D.I. 2023. Post-application persistence and field efficacy of a new strain of *Cordyceps javanica* against the sweetpotato whiteflies, *Bemisia tabaci*. *Journal of Fungi. 9*(8), 827. https://doi.org/10.3390/jof9080827

30. **Wu, S.**, Mechrez, G., Ment, D., Toews, M.D., Mani, K.A., Feldbaum, R.A. & Shapiro-Ilan, D.I. 2023. Tolerance of *Steinernema carpocapsae* infective juveniles in novel nanoparticle formulations to ultraviolet radiation. *Journal of Invertebrate Pathology.* 196, 107851. https://doi.org/10.1016/j.jip.2022.107851.