APPENDIX D - SAES-422

**Format for Multistate Research Activity**

**Accomplishments Report**

***Note:*** *This report is submitted each year of an activity’s duration and is due 60 calendar days following the annual meeting. The SAES-422 is submitted electronically by AAs into NIMSS. Annual Reports for MRF projects are available to NIFA through NIMSS.*

# Project/Activity Number: NC1173

**Project/Activity Title:** Sustainable Solutions to Problems Affecting Bee Health

**Period Covered:** 01/01/2020 to 12/31/2020

**Date of This Report:** 02/01/21

**Annual Meeting Date(s):** 01/09/2021

**Participants:**

**Table

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**Brief Summary of Annual NC1173 Multi-State Project Meeting**

Minutes taken by Michelle Flenniken (Montana State University), Judy Wu-Smart (University of Nebraska, and Margarita Lopez-Uribe (Penn State)

The NC1173 business meeting was conducted as part of the 2021 American Bee Research Conference (ABRC) with the American Association of Professional Apiculturists (AAPA) meeting via Zoom due to the SARS-CoV-2 pandemic. The ABRC was held for two days (Jan 7-8, 2021) and serves as the scientific program for the NC1173 multi-state group. An agenda for the ABRC meeting is online (https://aapa.[cyberbee](https://aapa.cyberbee.net/wp-content/uploads/2021/01/2021_ABRC_Schedule_Final-downloaded-Jan5_2021-1.pdf).net/wp-content/uploads/2021/01/2021\_ABRC\_Schedule\_Final-downloaded-Jan5\_2021-1.pdf), was submitted in conjunction with this report, and proceedings will be published in the coming months.

The business meeting was called to order at 10:00 AM by chairperson Dr. Michelle Flenniken from Montana State University. Attendance was recorded via zoom login information. Dr. Michelle Flenniken reviewed the current status of the multi-state project. She reported that there are currently 37 members listed in NIMMS (27 of whom were in attendance) representing 26 institutions. Dr. Michelle Flenniken reminded members to submit their publications, activities, and milestones, so that these efforts may be reported in the 2021 NC1173 report due within 60 days of the mee ting.

Dr. Erica Kistner-Thomas provided an update on USDA-NIFA-AFRI opportunities. She reported that since their move to Kansas City, Missouri, they have hired many new people after a dramatic loss in staff (i.e., ~ 80%). The ~128 new hires included 18 new national program leaders, including herself, Megan O'Rouke may also be another good contact for this group. In 2020 there were 61 applications submitted to the Pollinator Health Program and funded 11 projects. The 2021 budget is similar therefore she expects a similar number of projects will be funded. Dr. Erica Kistner-Thomas encouraged new faculty members who have not yet received a USDA-funded research grant and with less than 5-years of experience to apply for the “new-investigator” opportunity, with a funding level of $300,000 for up to 2-years. In 2021, she noted that the maximum requires for “standard USDA grants was raised to $750,000 and the deadline for submission is May 27, 2021 at 5 pm EST (see: <https://nifa.usda.gov/funding-opportunity/agriculture-and-food-research-initiative-foundational-applied-science-program>). Members can contact her directly with questions (i.e., Dr. Erica Kistner-Thomas, National Program Leader, National Institute of Food and Agriculture, Institute of Food Production and Sustainability, Email: [ekistnerthomas@usda.gov](mailto:ekistnerthomas@usda.gov)).

Dr. Michelle Flenniken reported that the previous NC1173 report, which was submitted by Dr. Judy Wu-Smart, was submitted in 2020 and Project Director William (Bill Barker) highlighted components of that report. Specifically, the success of NC1173 members work together to garner additional funding for research (i.e., FFAR funding awarded for a collaborative effort by Auburn University, University of Nebraska, University of Georgia, and investigators in Spain); IPM research efforts at Iowa State University, University of Nebraska, and Montana State University; and landscape level research, including the BeeScape website developed by investigators at Penn State (https://beescape.org/). He encouraged the team to focus our report to highlight synergistic efforts, including highlighting publications and grants co-authored by NC1173 members across institutions. In addition, he remarked that he liked the use of “bold text” to highlight NC1173 co-authors. Dr. William (Bill) Barker suggested that NC1173 members read the Sand County Almanac by Aldo Leopold and Braving Sweetgrass by Robin Wall Kimmerer and acknowledged that most land grant universities are situated on historical tribal lands. He encouraged members to work with Native Americans / indigenous people, new/beginning farmers, and veterans in our research and outreach projects, as well as consider focusing on large-scale regenerative systems and/or the development of those systems. He noted that Dr. Christina Hamilton, the other NC1173 Administrative Advisor could not join us this year.

Dr. Flenniken reiterated a decision from the 2020 meeting, next meeting will be held in with the American Bee Research Conference, which will be held in conjunction with American Honey Producers Association (AHPA) Meeting, which will be in Baton Rouge, Louisiana at the Crown Plaza Hotel, November 30-December 4, 2021; the specific date and time will be finalized closer to the meeting time. The 2023 NC1173 meeting will be held with the ABRC meeting in conjunction with the American Beekeeping Federation (ABF) in January 2023. Dr. William (Bill) Barker will confirm that the future meeting plans are in line with USDA guidelines, but given the importance of NC1173 members meeting with stakeholder groups, and flexibility due to the pandemic that it is likely that our future meeting plans will be approved. Information on future meetings is provided on the AAPA website (<https://aapa.cyberbee.net/abrc-2021/>).

The floor was opened for discussion of other business. Dr. Judy Wu-Smart asked the group about their interest in a larger-scale effort to further research problems of pesticide contamination, particularly large-scale contamination as a result of processing chemically treated seeds (as she described during her ABRC/NC1173) presentation. Dr. Juliana Rangel suggested that Dr. Judy Wu-Smart should consider leading an USDA-NIFA-AFRI CAPS Project grant on this topic. Dr. Michelle Flenniken also commented on the importance of this research topic that has important real-world impact for bees and other pollinators.

Dr. Margarita Lopez Uribe announced that she will send an email to see if those who submitted ABRC abstracts would like to publish them in Bee Culture and that authors may opt out of the publication of their abstracts at that time.

Dr. Juliana Rangel asked about the minutes of the 2020 meeting and the election of current officers and Dr. Michelle Flenniken provided the minutes and let the group know that in 2020 she was elected as Chair of NC1173 and Dr. Margarita Lopez-Uribe (Penn State) was elected as vice-chair for 2020-2022. The floor was opened for discussion of other business, but in the absence of additional discussion Dr. Flenniken adjourned the meeting at 10:40 am (EST).

**NC1173 Objectives**

1. To evaluate the role, causative mechanisms, and interaction effects of biotic stressors

(i.e., parasitic mites, pests, and pathogens) and abiotic stressors (i.e., exposure to pesticides, poor habitat and nutrition, management practices) on the survival, health and productivity of honey bee colonies as well as within pollinator communities.

2. To facilitate the development of honey bee stock selection, maintenance and production programs that promote genetic diversity and incorporate traits conferring resistance to parasites and pathogens.

3. To develop and recommend "best management practices" for beekeepers, growers, land managers and homeowners to promote health of honey bees and pollinator communities.

**NC1173 Accomplishments:**

**Objective 1a: (Biotic Stressors: Pests & Pathogens)**

The *Varroa destructor* mite is one of the deadliest honey bee pathogens currently facing the US beekeeping industry. *Varroa destructor* is an ectoparasitic mite that feeds on honey bees and decimates colony populations resulting in colony death. *Varroa* mites also transmit viruses within and between colonies. High mite infestation coupled with high levels of viruses, including deformed wing virus (DWV), are often associated with overwinter losses of honey bee colonies. NC1173 members are addressing the *Varroa destructor* mite challenge by developing novel chemical and biological control options for management (Johnson, OSU**;** Ellis, UF; Shepard, WSU), determining the efficacy of commonly used miticides (Ellis, UF), and examining management practices, including the use of screened bottom boards, that reduce mite populations (Huang, MI State). The Connecticut Agricultural Experiment Station team (Eitzer, Stoner, Cowles are collaborating with Williams (Auburn) and Cook and Evans (USDA) to evaluate Aluen CAP for *Varroa* mite management. This product is considered organic and will likely be compatible with use during nectar flow. It provides a 42-day continuous exposure of oxalic acid through honey bee interaction with cellulose matrix strips left in the hive draped over brood frames. Aluen CAP is produced by Cooperativo de Trabajo Abicola Pampero (Buenos Aires, Argentina). Both U.S. data and a registration partner will be needed in order to obtain a U.S. EPA registration. These efforts in conjunction with continued investigation of hygienic behavior (Spivak, UM) are aimed at mitigating mite-associated colony losses.

NC1173 members are also examining the impact of pathogens on colony health and longevity (almonds-Flenniken, MT State; samples from 2015 US National Honey Bee Disease Survey – Grozinger, PSU). These efforts take into account the relative role of a variety of abiotic and biotic factors, including landscape, chemical exposure, and inter-bee taxa pathogen transmission with insect communities (Wu-Smart, UNL, Hines, Grozinger, PSU; Flenniken MT State; Tarpy, NCSU; Rangel, TAMU). For example, Hines (PSU) investigated differential microsporidian infection levels by bee species and by habitat and Flenniken (MT State) examined relative prevalence and abundance of a virus in sympatric mining bees and honey bees. As another example, Rangel and collaborators found that ants living in and around honey bee colonies indeed serve as mechanical vectors of several honey bee associated viruses. In addition, the influence of nutrition (specifically phytosterols and protein) on the outcome of pathogenic infections is another area of active research (in orchards-Ramesh OSU, in laboratory-based experiments, Flenniken, MT State, and in honey bees infected with *Nosema* or DWV, Rangel, TAMU).

NC1173 members are also investigating honey bee antiviral defense mechanisms including the heat shock response and dsRNA-triggered virus restriction (Flenniken MT State), and the transcriptional and epigenomic responses that regulate honey bee gene expression in response to IAPV infection (Li-Byarlay, Central State and Tarpy NCSU); Rangel, TAMU. Examining the impact of putative immune stimulants that may reduce virus infection (i.e., fungal extracts and thyme oil) (Sheppard, WSU; Flenniken, MT State), and the role of propolis in promoting colony health (Spivak, UM). The team is also involved in virus discovery and monitoring (Flenniken MT, State; Schroeder UMN; Lopez-Uribe PSU; Grozinger PSU; Tarpy, NCSU). Behavioral responses including hygienic behavior, as well as the potential of social fever, are also under continued investigation (Spivak, UM). Members are also looking at self-removal behavior of workers in response to *Varroa* parasitization and/or other stressors (Rangel, TAMU). Members of the NC State Apiculture Program investigated vertical transmission viruses and other pathogens from honey bee queens to their offspring (Rueppell & Tarpy, NCSU), the transcriptome and epigenome dynamics of honey bees in response to virus infection (Rueppell & Tarpy, NCSU; Li-Byarlay, CSU), and the horizontal transmission of viruses from workers to queens (Lee, Spivak, Schroeder, UM; Tarpy, NCSU).

**Short outcomes, Objective 1a:** The strong association between *Varroa destructor*, deformed wing virus (DWV), and high overwintering colony losses (OCL) of honey bees is well established. Research indicates that increasing floral diversity in pollinator habitats reduces pathogen levels (Hines, Grozinger, PSU). Managed bee populations in the US have a greater diversity of viruses than previously realized (Grozinger, PSU) and virus infections at the colony level are dynamic, and thus longitudinal studies that precisely control sampling date are important to understand the impact of pathogens on honey bees at the colony level (Flenniken, MT State). At the molecular level, IAPV-infection causes epigenetic changes (e.g., DNA methylation) in pupae, which impacts gene expression in adult honey bees(Li-Byarlay CSU), and that the heat shock stress response pathway is an important antiviral defense response to a model virus (Flenniken, MT State). Investigators at WSU are breeding a novel strain of Metarhizium fungus for improvement as a biological control agent against *Varroa* mites (Sheppard, WSU). The team conducted outdoor studies comparing Metarhizium to oxalic acid, a commonly utilized mite treatment, and showed that the Metarhizum exhibited comparable mite control (Sheppard, WSU). Additional testing (i.e., nutritional analysis) of polypore mushroom extracts for potential use as a honey bee feed additive were carried out and indicated that the extracts have a mineral composition similar to honey and pollen and an AAFCO requested longevity study was completed (Sheppard, WSU). WSU has coordinated with AAFCO and the FDA to seek a smooth and quick route to registration as a honey bee feed additive.

**Outputs Objective 1a:**

NC1173 published numerous peer-reviewed publications related to pest and pathogen stressors, which are listed at the end of this report; many of them are open-access. A USDA APHIS PPQ grant was secured to continue investigation into landscape factors driving wild bee pathogen levels in another region (North Carolina), which, together with Pennsylvania data, will help refine best management practices for promoting healthy bees (Hines, Grozinger, PSU). Williams (Auburn) found that the parasitic mite *Varroa destructor*, but not neonicotinoids, negatively affected worker food glands in honey bees. Conversely, drones appeared more sensitive to neonicotinoids, as those exposed to the insecticides exhibited reduced sperm quality. By performing a meta-analysis, antagonistic interactions between pesticides and parasites appear to be overlooked by the scientific community. They also identified alternative food sources for small hive beetle in the SE United States.

**Objective 1b: (Abiotic Stressors: Pesticides, Forage Availability, Nutrition)**

Major abiotic stressors contributing to honey bee health decline include pesticide exposure and malnutrition. NC1173 members are addressing these factors through studies examining the pesticide residue levels found in bee forage (floral nectar and pollen) in ornamental plants treated with systemic insecticides (Eitzer, Stoner, and Cowles, The Connecticut Agricultural Experiment Station), examining the pesticide residue levels found in bee forage in urban areas (Rangel, TAMU; Huang, MI State; Ellis, UF), examining the role existing tree lines play as drift barriers to reduce off-target contamination from neonicotinoid-laden dust released during corn planting into forbs growing near corn fields (Wu-Smart, UNL), examining which plants bees are utilizing in natural landscapes and in open spaces near agricultural crops (Kim, Speisman, KSU; Wu-Smart, UNL), and the microbial (bacterial and fungal) communities in bee forage (Danforth, Cornell) to better understand the nutritional requirements of managed and wild bees (*Osmia cornifrons* (*Megachilidae*)) and the role fungicides play on these microbes. Further, some of these issues with pesticide exposure, malnutrition, and or pollination deficits/limitations are being examined in specific cropping systems (apples-Danforth, Cornell; black cherry-Hoover & Grozinger, PSU; corn/soybeans-Wu-Smart, UNL). In addition, Ellis and team are working to assess the potential toxicity of pesticides to various honey bee life stages and characterize risks associated with exposure to these compounds (Ellis, UF). Winfree (Rutgers) published the results of a nation-wide study showing that many crops, and especially spring-blooming fruit crops, are pollination-limited in the USA (Reilly et al 2020). Ongoing work includes identification of the main flowering plant species used as pollen sources by spring-flying native bee species such as *Andrena* and *Osmia*, which are important pollinators of spring tree fruit (Winfree, Rutgers).

Evaluating the nutritional needs of bees to improve planting schemes (Grozinger, Patch, PSU). NC1173 members evaluated the protein and lipid ratios of pollen of 82 plant species and three bee species, and demonstrated a broad range of ratios, which seem to correspond with different preferences for the different bee species (Vaudo et al 2020). Using DNA barcoding, they found in urban landscapes, honey bees foraging preferences shift throughout the growing season from trees, to weedy plants, to ornamental plant species in the fall (Sponsler et al, 2020a, b). They found significant variation in visitation patterns of pollinators to different annual ornamental plant species and cultivars, and found a significant effect of season, year, and site (Erickson et al, 2020).

Developing a National Insecticide Toxic Load Map (Grozinger, PSU). We published a spatial map of pesticide use patterns across the United States (Douglas et al 2020). Total toxic load of applied insecticides has increased nationally over the last 20 years, with some counties showing increases of over 100-fold. This map allows stakeholders and policymakers to identify sites to focus alternative management or conservation efforts. Funding was provided by the USDA-NIFA-AFRI, the USDA FFAR, the National Socio-Environmental Synthesis Center.

Evaluating the role of landscape and climate on wild bee health (Grozinger, PSU). To support large-scale studies of the effects of land use, habitat, weather and climate on wild bee species, the PSU team organized, validated, and shared an analysis-ready version of one of the few existing long-term monitoring datasets for wild bees in the United States (Kammerer et al 2020). This work was supported by funding from the USDA-NIFA Postdoctoral training program and the USDA FFAR. In addition, the PSU team secured funding to develop resources to improve pollen diagnostic abilities by developing a streamlined metabarcoding pipeline (Grozinger) and pollen image library (Boyle) (Funding from PA Dept of Agriculture). Efforts by Eitzer, Stoner, Cowles (Conn) include the establishment of high-yielding floral resources plots. These plots will be used to support honey bee genetic improvement programs, and also to enhance landscapes to support a broad diversity of pollinators. They plan to obtain quantitative data on honey yield from hives using these plots which could spur their acceptance in fixed-land honey crops. To date 10 of 12 planned species have been established on these plots. Methods to establish these plants are described in this video: <https://www.youtube.com/watch?v=rzyEyNf5CaU> .

NC1173 members in the NC State Apiculture Program investigated the effects of abiotic stressors (temperature and pesticides) on honey bee queens and particularly their reproductive quality (Pettis, Tarpy). They determined the thermal limits to sperm survival in queen spermathecae (sperm storage organs) as well the proteomic changes in queens as a consequence of temperature and pesticide exposures.

The team at Michigan State University (Z. Huang Lab) studied the effect of neonictinoids on honey bee mortality at different temperatures and found the toxicity of both imidacloprid and thiamethoxam were more toxic to winter bees (higher mortality) at 25°C compared to 35°C. They also evaluated longevity of caged worker bees after they were fed with different pollen substitutes. They found that commercial pollen substitutes show large variations in sustaining honey bee longevity.

The Connecticut Agricultural Experiment Station team (Eitzer, Stoner, Cowles) collected trapped pollen from honey bee colonies in different environments (ornamental plant nurseries and botanical gardens), and are analyzing the plant sources of the pollen and the pesticide residues found in the pollen. The plant sources were analyzed using palynology (identification of acetolyzed pollen using light microscopy) and by DNA metabarcoding (contracting with researchers at the University of Maine and Ohio State University, respectively). The pesticide residues are extracted using a version of the QuEChERS protocol and then analyzed using liquid chromatography/mass spectrometry (contracting with researchers at Cornell University and the US Department of Agriculture) and gas chromatography and mass spectrometry (US Department of Agriculture). They are comparing the results of the two methods of plant identification and relating the plant sources of the pollen to the pesticide residues found. The Connecticut team is collaborating with Webster at Kentucky State University to measure queen pheromone levels using a new liquid chromatography/high resolution mass spectrometry rather than a derivatization-based GC/MS procedure (Eitzer, Stoner, Cowles).

The UNL Bee Lab reported challenges with abnormally high bee losses at a large 93,000 acres research property (Wu-Smart, UNL). Colonies losses were consistent with 0% survivability over multiple years and bees showed signs of acute mortality potentially due to pesticide exposure. In 2019, UNL shifted research focuses to specifically investigate and examine these bee losses using the “Dead Bee Trap Monitoring Study” and this year they identified a potential connection between observed bee losses to pesticide contamination problems originating from an ethanol plant just north of the property. As a result of our research, we have discovered a novel practice of “disposing” surplus pesticide treated seed through ethanol processing that produces solid and liquid byproduct waste highly contaminated with pesticide residues. Furthermore, these byproducts were distributed a s soil conditioners to local farmers. The NE Department of Ag (NDA) and EPA estimate when “applied at 20 tons per acre, with a clothianidin concentration of 427,000 ppb, would result in 17.08 lbs. of active clothianidin applied per acre which is 85 times the maximum annual field load allowed by a typical register pesticide label.” The highest level documented in the NDA reports was 556,000 ppb clothianidin detected in distiller’s grain collected from the harvester. The report also noted the ethanol plant manager stated that the collected sample “was ready for land application”. In response to this issue, UNL formed a working group consisting of researchers, regulatory officials, stakeholders, and legal experts to help understand the regulatory, legal, and research challenges and help identify research priorities, funding opportunities, immediate action steps, and long-term goals. The working group consists of over twenty professionals, non-profit partners, legal experts, students and researchers including three NC1173 members (Wu-Smart (UNL), Spivak (UMN), Johnson (OSU). Drs. Michelle Hladik (USGS-California) and Dan Snow (UNL Water Science Lab) have been assisting with pesticide analyses of water, soil, vegetation, and hive samples collected on site. Given the complexity of both the research and policy challenges, UNL will hire someone to coordinate discussions among state agencies, researchers, external partners, and stakeholders and to specifically help us coordinate targeted research with One Health collaborators in spring 2021 (through stakeholder funding via Project Apis m./The National Honey Board funds (~$60K for one year)). Efforts will prepare the working group to gather preliminary data and identify research priorities for future USDA/EPA funding. UNL is also working with officials from NE Department of Ag, NE Department of Environment & Energy, and US EPA to report our bee loss data and better understand the regulatory process and enforcement oversight for the contaminated liquid effluent and solid byproducts generated during this ethanol process. Below is a Guardian article recently published regarding this local issue that has national implications.

<https://amp.theguardian.com/us-news/2021/jan/10/mead-nebraska-ethanol-plant-pollution-danger>

In addition, The Danforth lab (Cornell) conducted a series of experiments to determine how a widely-used fungicide (cyprodinil) impacts larval development in a common, easily-manipulated, mason bee (*Osmia cornifrons*; *Megachilidae).* They developed two experiments to specifically determine how fungicides impact the normal progression of larval development and whether the fungicidal treatments impacted the microbial community of the pollen provisions upon which these larvae are feeding.

In addition, the TAMU bee lab (Rangel, Texas A&M University) wrapped up a project looking at the effects of pesticide exposure on honey bee queen and drone reproductive health. They looked at whether exposure of queen and drone larvae to wax contaminated with field relevant concentrations of miticides and agro-chemicals affected sperm count and viability, size, queen egg-laying capacity, chemical composition of queen mandibular gland pheromones, and ovariole size.

**Short outcomes, Objective 1b:**

The PSU team, (1) identified plant species that are preferred by honey bees for foraging in urban areas, (2) demonstrated that ornamental plant species can serve as important forage resources for managed and wild bees, but there is considerable variation among cultivars (Grozinger, Patch). (3) demonstrated that landscape toxic load has increased dramatically over the past 20 years, particularly in regions of the US that are dominated by field crops that use neonicotinoid seed treatments, and (4) provided an analysis-ready, 14 year data set of wild bee populations in mid-Atlantic (Grozinger). Likewise, the research team including Rangel (TAMU), Huang (MI State) and Ellis (UF) analyzed the pesticides present in nectar and pollen samples in urban and suburban areas across the U.S. Williams (Auburn, AL) and team observed that drones are possibly more susceptible to abiotic stressors like neonicotinoids compared to unexposed drones. This may be explained by the haploid susceptibility hypothesis. Lastly The insights gleaned from the studies carried out at Oregon State University on honey bee nutrition have advanced the understanding of sterol metabolism and regulation in honey bees and will assist in formulation of a more complete artificial diet for honey bees (Sagili).

**Outputs, Objective 1b:** NC1173 members (Grozinger & Patch -PSU) developed an insecticide toxic load index, which integrates data from multiple government databases to provide a measure of the total toxicity of all the pesticides applied to a particular crop in a particular state (Douglas et al 2020). Development of this index allowed to examine patterns in insecticide use and toxicity over the last ~20 years, and demonstrated a significant increase in toxic load, due primarily to neonicotinoid seed treatments. This index has been incorporated into our Beescape portal (see Obj3 below) and is being used in large scale analyses of bee health.

**Objective 2: (Genetics, Breeding, & Diversity)**

Breeding mite and disease resistant traits in honey bee stock and diversifying honey bee genetics and selection efforts are more sustainable solutions to address the pest and pathogen issues in honey bees and is a long-term goal for NC1173 members. Efforts include studies to examine how establishing high-yield nectar crops support high densities of honey bee colonies required for mating yards in honey bee genetic improvement programs (Eitzer, Stoner, Cowles -Connecticut Ag Station; Harpur Purdue University) and breeding and selection of high grooming/mite biting behavior in Ohio feral colonies (Li-Byarlay, CSU). Sheppard and the WSU team continued selection and maintenance of the New World Carniolan strain of honey bee, including inputs of cryopreserved *A. m. carnica* germplasm from Slovenia origin. They produced and supplied instrumentally inseminated (i. i.) “breeder” queens to a number of commercial queen producers of open-mated NWC “production” queens. Together with industry partners, WSU initiated a plan to hand off the production of i. i. New World Carniolan i. i. breeder queens to commercial queen producers in 2021. Starting in 2021, NWC breeder queens supplied to the queen production industry will come from queen producer partners (rather than WSU) and WSU will be responsible for maintenance of the genetic stocks and infusion of *A. m. carnica* genetics from our repository of cryopreserved Old-World semen. In addition, WSU continued selection and distribution of a Caucasian strain of honey bees derived from Old-World origins. Using cryopreserved semen from collections of *A. m. caucasica* germplasm made over the past decade in the country of Georgia. WSU continues its effort to reintroduce this strain of honey bee to US beekeepers. In 2020 we supplied i.i. breeder queens to a number of commercial queen producers, who produced open-mated daughters for sale throughout the US. This strain is especially interesting to beekeepers from colder climates within the US (Sheppard, WSU). Keith Delaplane’s group (U Georgia) along with Debbie Delaney’s (U Delaware) completed a 2-year NIFA CPPM-funded project examining interactions of VSH-selected drone semen with polyandry (queens inseminated with 9 or 54 males). Colony mite levels increased in wild-type lines as one moved from polyandry=9 to =54, but decreased in VSH lines from polyandry=9 to =54, consistent with published theory that hyperpolyandry evolves under the influence of rare, beneficial alleles. Colony survival rates were highest in queens/colonies inseminated with VSH semen at the 54-drone polyandry rate. Additionally, polyandry, irrespective of semen source, significantly increased colony bee populations and comb construction rates. These results support the practice of more intentionally integrating high mating numbers with targeted trait selection efforts. Delaplane and Delaney also partnered with Geoff Williams (Auburn) in a FFAR-funded project to examine the use of inter-colony brood mixing as a beekeeper-friendly proxy method for obtaining colony benefits of hyperpolyandry.

**Short outcomes, Objective 2:** Purdue University (Hapur) breeding program with mite resistant traits has been successfully made available in seven US States. Harpur has secured funding (Eva Crane Trust) to explore the genetic diversity of honey bees across the United States to understand baseline levels of genetic diversity. In addition, Harpur and Lopez-Uribe secured a USDA AFRI (USDA AFRI 102265) to determine how different honey bee genetic lines perform in the hands of beekeepers. Selection and breeding for high grooming and mite biting behavior from Ohio feral colonies, evaluation queen development (48-hr queen cells) and egg laying rate of different honeybee stocks, work with 30 Ohio beekeepers to collect different wild colonies, and collaborate with Purdue University on mite biters as satellite breeding site via instrumental insemination (Li-Byarlay CSU). NC1173 members (Ellis, UF) are working with collaborators to develop a stock certification program for honey bees in Puerto Rico. Harpur (Purdue) and Lopez-Uribe (PSU) received a USDA-CARE grant to compare the performance of different honey bee stocks in different regions of the MidAtlantic. The project involves the participation of 30 beekeepers in IN and PA. PSU NC1173 members Grozinger and Rasgon are developing tools for genetic manipulation/transformation of bees (State), which will facilitate the identification and validation of genetic markers for bee health and behavior; this project is funded by the NSF-EDGE program. Grozinger (PSU) worked with Nino (UCD) on a project that demonstrated that seminal fluids can trigger post-mating changes in honey bees queens when injected into the abdominal cavity, suggesting these target receptors are outside of the reproductive tract, as is the case in *Drosophila* (Grozinger, PSU, and Nino, UCD).

NC1173 members at NC State have investigated how different honey bee stocks (genotypes) can respond differentially to a suite of pesticide exposure (Rinkevich, Tarpy, NCSU). These findings highlight the need to control for not just environmental context but genetic background as well when conducting toxicological and physiological assessments of honey bees.

**Outputs, Objective 2:** NC1173 members worked directly with over numerous beekeepers and nearly through extension programs and courses, which were mostly virtual in 2020. CSU provided extension talks to 620 beekeepers and general public including four extension webinars at CSU, plenary talk at two regional agricultural and beekeeping conference, one scientific publication on mite biting behavior and mite-resistance in review (Li-Byarlay, CSU). Sagili and team at OSU organized a virtual conference for Western beekeepers. Flenniken and other NC1173 Members gave presentations to the Bee Inform ed Tech Transfer team.

**Objective 3: (Management)**

Management practices to maintain healthy honey bees and landscapes that support pollinators are in high demand and recommendations continue to evolve with new research. Therefore, NC1173 members strive to engage with stakeholders to better provide the most up-to-date, science-based recommendations to beekeepers, pesticide applicators, farmers, homeowners and policy makers. Recommendations include how to better manage pests and pathogens in honey bees, enhancing landscapes for pollinators, and options to reduce exposure or mitigate effects of pesticides. NC1173 members conducted studies to identify the most attractive and nutritionally beneficial species of plants in urban (Sponser et al 2020, Erickson et al 2020) and agricultural or semi-natural settings (Treanore et al 2019, Russo et al 2020). NC1173 members (McLaughlin, Hoover and Grozinger PSU) conducted studies in Spring 2020 to define the pollinator communities for black cherry, a key timber species in northeastern forests suffering from declining regeneration, and the environmental factors that influence pollinator abundance and diversity. Using a combination of passive and active sampling techniques, pollinators were collected from flowering black cherry trees in the Allegheny National Forest and in State College, PA. PSU team members analyzed pollen macronutrients from 82 plant species and collected pollen from three bee species to demonstrate that there is considerable variation in the protein:lipid ratios, thus potentially allowing us to select plants that provide optimal nutrition for different bee species (Grozinger, Patch, Hines).

**Short outcomes, Objective 3:** NC1173 members (López-Uribe and Underwood PSU) have worked with a group of 30 beekeepers to develop the protocol for best management practices for beekeepers that have different philosophies towards chemical treatments (Underwood et al 2019). NC1173 members (McLaughlin, Hoover and Grozinger PSU), found that key pollinators of black cherry, which flower in May and early June depending on location, appear to be early spring bees from the family *Andrenidae,* but the overall pollinator community also included bees from the families *Megachilidae* and *Halictidae*, flies, and beetles. Fruit was also collected from each tree as it dropped in the early and late fall onto the forest floor surrounding our experimental trees, and ripened fruit was tested for seed viability. Analyses are in progress to determine which variables are predictive of pollinator community morphotypes and viable seed production, which include several biotic and abiotic factors such as the presence of key pollinator communities observed directly interacting with black cherry flowers and metrics of tree health.

Through collaborations, the NC State team has developed better understanding of how queen shipment, handling, and introductions are influenced by externals factors, especially temperature, and the development of predictive physiological signals that may be indicative of queen failure, which is a primary concern in apiculture management (Pettis, Tarpy, NC State). They also started a new collaborative paradigm on the variation in egg size as a function of hive size and other management factors (Rueppell, Tarpy, NC State). Williams (Auburn, AL) performed a citizen science experiment, and documented the negative effects of the COVID-19 pandemic on bee research and extension activities. As expected, there were significant disruptions in spring 2020, including travel associated with scientific communication and networking, as well as field work. Sagili (OSU) synthesized a Survey-derived best beekeeping management practices to improve colony health and reduce mortality. These studies related to honey bee colony management provide additional tools/practical methods for beekeepers to enhance colony health and survival.

**Outputs, Objective 3:** Wild Honey Bees in Community Environments – Identification, Biology, and Reducing RisksShaku Nair, Dawn H. Gouge, Ayman Mostafa ,Shujuan Li, Kai Umeda, Hongmei Li-Byarlay, 2020, <https://extension.arizona.edu/pubs/wild-honey-bees-community-environments-%E2%80%93-identification-biology-reducing-risks>

Penn State Extension offered weekly two webinars series in 2020. During the 75-minute live webinars, there is an interactive Q&A session. The webinars reached over 8,000 participants from all states in the United States, and 16 countries around the world. Topics for the webinars included several aspects of bee diversity, bee management, and stressors related to bee decline.

Lopez-Uribe and Underwood have developed a highly effective protocol for managing honey bee colonies using organic approaches. The details of this approach has already been disseminated through webinars, talks to beekeeping clubs, and there is an upcoming extension publication with the details of this protocol.

PA Pollinator Protection Plan (led by Boyle, includes all faculty members. We worked together with a team of 36 individuals representing 28 state- and national-organizations and stakeholder groups to develop the Pennsylvania Pollinator Protection Plan (P4). Dr. Natalie Boyle organizes meetings of the P4 group every 4 months.

Beescape (Grozinger). In April 2019, we launched an online tool called “Beescape” (beescape.org), which allows users (across the continental US) to explore landscape quality (forage quality, nesting habitat quality for wild bees, for bees at their selected sites. Using wintering colony survival data from the Pennsylvania State Beekeepers Association Survey (Calovi et al, 2021). we developed a new decision support tool, BeeWinterWise.

**K-12 students.** With Penn State’s Center for Science and the Schools, we received funding from the USDA-PD-STEP program to develop programming targeting k-12 teachers from underserved rural and urban communities. We created lesson plans and other content that are available online (Grozinger, Patch, Boyle; Penn State’s Center for Pollinator Research).

With faculty in Penn State’s Learning, Design and Technology Program we developed a 1 hour workshop for families with pre-K through middle school children for use in rural libraries and museums (Grozinger).

We have conducted several school visits, and presentations as part of the PSU “Nature Explorers” summer camp (elementary school) and the Pennsylvania School of Excellence in Agricultural Sciences high school summer program (Hines).

Patch (PSU) is leading the development of a >4 acre Pollinators and Bird Garden at the Arboretum at PSU, which will feature 400 plant types and serve as a venue to showcase research on plant-pollinator interactions and conservation. The garden will open in spring 2021 and is expected to welcome 100K visitors each year.

**Impacts**

**Oregon State University (Sagili).** The Oregon Master Beekeeper Program serves the needs of both backyard and commercial beekeepers in Oregon and Idaho. This is the first program in the USA with unique hands on training (mentor-mentee type). This program has received an overwhelming response since its inception in 2011. Currently there are more than 1,700 registered participants and a long wait list. The program has educated and trained commercial beekeepers in the state that pollinate about 90% of the crops, as well as extension agents, beginner beekeepers, farmers, Oregon Department of Agriculture and USDA field personnel (NRCS). Four other states in the USA have sought our assistance for starting their new MB programs based on our model. Oregon Master Beekeeper Program was cited a unique Master Beekeeper Program in the country (Western Apicultural Society Journal, August 2019, American Bee Journal 2020).

**University of Nebraska-Lincoln (Wu-Smart)**. In 2020, UNL Bee Lab provided 6 beekeeping workshops across four major Nebraska cities. These workshops included 5 introductory level classes (Year 1 & Year 2 Beekeeping) to 673 new or aspiring beekeepers to educate about basic honey bee management and IPM strategies for honey bee pests/diseases. Some Jan-Mar courses were offered in-person, but all others were quickly converted to virtual programs for Covid-19 safety. Current membership in the Great Plains Master Beekeepers (GPMB)Training Program (website: <https://gpmb.unl.edu/>) is now 840 participants and 51% are women (Demographics: 409 Male, 429 Female, 689 Caucasian, 17 African American, 9 Tribal members, 46 Latinx, 22 Other, 156 Military. Through GPMB, we have recruited new partnering beekeeping groups and certified the first 9 “master” and 2 “journeymen” level beekeepers. Certified Masters may now proctor field exams in their own representative states for GPMB (Project Funded through USDA BRFD # 2018-70017-28546). In 2020, UNL Bee Lab and GPMB course participants included beekeepers across 8 states (NE, KS, IA, CO, SD, WY, and MO). From pre- and post-course evaluations, 98.5% of participants (390/396) responded they would recommend our beekeeping courses. Furthermore, 65.6% of participants responded they intend to expand their business because of our value-added course offerings. At the beginning of 2020, about 67% responded they treat for mites and at the end of the year we saw an increase (70% of respondents) in participants who now implement integrated pest management approaches for mites. When asked “What changes in your beekeeping operation will you enact after this class?” several responded they intend to implement these practices. (Example responses: *“I will take all the information presented within this module to my own experiences. One thing that stood out to me that I plan on doing is making sure to treat hives for mites even if we do not visibly see them.* (Year1)*”;“I will have a better assessment on how to inspect hives which would allow me to better help and increase the hive's condition.*(Year2)*”; “Definitely add the Dead Bee Traps! Will definitely start using your Inspection sheets. Also, I'm glad to hear that you support the sugar roll method (*Varroa IPM short course*)”; “Love the hands on training so far. Speakers always make the students feel comfortable enough to ask any question no matter the level of beekeeper (*Year 2*).”* **In 2021, UNL will continue to expand extension training**  **and focus on program sustainability.** UNL has already started working with external partners from Michigan State University to establish a targeted program for military veterans (“Heros to Hives”) in Nebraska and are seeking continuation funding for GPMB efforts.

**2020 Virtual Programs:** In response to cancelled in-person training UNL developed, organized, and delivered several virtual learning alternatives. The most notable have been included below:

*(1) GPMB Virtual Fun Day:* In 2020, all partnering beekeeping associations had to cancel their annual summer bee fun day events so GPMB responded by delivering a Virtual Bee Fun Day webinar to give our partner organizations and GPMB members an educational outlet during the Covid-19 quarantine. Over two days (June 13-14, 2020) we provided ~16 hours of lectures, field demonstrations, and maker’s workshop classes. We had 681 registered participants, and 23 speakers from research & academic institutions (n=7), extension professionals (n=5), non-profit organizations (n=1), graduate students (n=2), and local professional beekeepers (n=8). Of the 23 speakers, ***10 are NC1173 members and or active AAPA members***. With no less than 190 attending at any given time throughout the day, the turnout was quite large, and feedback was very positive regarding the breadth of topics covered from basic hive management to how honey bee artificial insemination works. ([See Program Schedule](https://unl.box.com/s/vm6u281xm6rpfoney1lbb75qdw4a2z5e)). Due to Covid-19, we did not charge for this program, but suggested participants may contribute $10 donations/per person to sustain future GPMB efforts. We received 105 gifts ranging from $2-$200 and raised a total of $3,233 ($3,103.68 after 4% fees). Participants were given the option to also donate to their local beekeeping associations, so partners received some revenue as well. For more information: <https://gpmb.unl.edu/>

*(2) Virtual Hap-Bee Hour Chats:* We collaborated with Randall Cass from Iowa State University Extension to offer weekly virtual open discussions. Hap-Bee Hour was offered weekly from April through November (30+ sessions; every Friday 5-6 pm via Zoom) and now offered monthly. The informal virtual gathering allowed beekeepers to share photos, videos, and discuss problems and we prepared seasonal tips to help beekeepers keep up with hive management needs. There were ~20-40 participants from NE, IA, KS, MO and CA and recordings of the Hap-Bee Hour chats are archived and made available online.

*(3)* *Girls Scouts of NE “The Good, the Bad, and the Ugly”:*This 3-day program (targeting 4-12th graders) was developed in collaboration with the Girls Scouts, Kimmel Orchard, UNL Entomology (**J. Wu-Smart** & L. Lynch O’Brien), NE State Arboretum, and the NE Depart. of Agriculture was delivered in July and August 2020. Each participant received teaching kits with live pollinator plants, bee nest materials, aquatic insect and invasive insect monitoring tools and resources to complement virtual lessons ([Program link](https://unl.box.com/s/467ie4bzkeawlxho95bbr5kq19ya32z3)). We reached 55 students from across 18 states (34% from Nebraska; (18% from west coast CA, OR), (18% from east coast MD, NC, RI, VA), and (5.5% from south FL, TX)) as well as representation from 9 additional states (23.6% from CT, GA, IL, MO, NJ, NY, OH, PA, SC) (Partially funded through the US EPA Environmental Education Grant #123268).

**Central State University (Li-Byarlay)** received USDA-SARE grant from North Central Region to investigate the possibility of using 48-hr queen cells to help beekeepers to diversify honeybee genetics in their own apiaries and increase their colonies collections with mite-resistance trait such as high grooming and mite biting. Working with extension, CSU bee lab provided four online webinars on sustainable beekeeping by using swarm traps, queen development, decreasing viral infections and temperature control.

***Summary table and list of publications by topic reported by NC1173 committee members for 2020. NC1173 authors are indicated in bold.***

|  |  |
| --- | --- |
| **Publications by topic** | **2020** |
| Obj 1a: Biotic (Pests & pathogens) | 21 |
| Obj 1b: Abiotic (Pesticides, nutrition, landscapes) | 31 |
| Obj 2: Genetics, Breeding, Diversity | 9 |
| Obj 3: Management | 10 |
| Other Publications | 4 |
| **Total** | **75** |
| Publications with >1 NC1173 authors | 17 |

**NC1173 Member Publications (01/01/2020 to 12/31/2020)**

\*Papers applicable to multiple objectives are only reported once.

**Metz BN**, **Wu-Smart J**, Simone-Finstrom M. **2020**. Proceedings of the 2020 American Bee Research Conference. Insects 11(6):362.

Simone-Finstrom, M., **Nino, E., Flenniken, M**., **Wu-Smart, J.** **2020**. Proceedings of the 2019 American

Bee Research Conference. Insects 11(2):88.

**Objective 1a: Biotic Stressors (Pests & pathogens)**

Faurot-Daniels#, C., I., Glenny#, W.,Daughenbaugh, K.F., McMenamin, A.J., Burkle, L., and **Flenniken, M.L.**, Longitudinal monitoring of honey bee colonies reveals dynamic nature of virus abundance and indicates a negative impact of Lake Sinai virus 2 on colony health, (**2020)***,* #equal co-authorship, *PLoS ONE, doi: 10.1371/journal.pone.0237544. eCollection 2020*

McMenamin, A.J., Daughenbaugh, and **Flenniken, M.L**, The Heat Shock Response in the Western Honey Bee (*Apis mellifera*) is Antiviral, (2020), *Viruses*, 12, 245; doi:10.3390/v12020245.

Amiri, E., J. J. Herman, M. K. Strand, **D. R. Tarpy**, and **O. Rueppell**. (2020). Egg transcriptome profile responds to maternal virus infection in honey bees, *Apis mellifera*. *Infection, Genetics and Evolution*, **85**: 104558.

**Li-Byarlay**, H., H. Boncristiani, G. Howell, M. K. Strand, **D. R. Tarpy**, and **O. Rueppell**. (2020). Transcriptome and epigenome dynamics of honey bees in response to lethal virus infection. *Frontiers in Genetics*, **11**: 566320.

Kevill, J.L., **K. Lee**, **M. Goblirsch**, E. McDermott, **D. R. Tarpy**, **M. Spivak**, and **D. C. Schroeder**. (2020). The pathogen profiles of queen honey bees does not reflect those of their colonies workers. *Insects*, **11**: 382.

Amiri, E., C., M. K. Strand, **D. R. Tarpy**, and **O. Rueppell**. (2020). Honey bee queens and virus infections. *Viruses*, **12**: 232. doi:10.3390/v12030322.

**López-Uribe MM,** Ricigliano V, Simone-Finstrom MD (2020) Defining Pollinator Health: Understanding bee ecological, genetic and physiological factors at the individual, colony and population levels. *Annual Review of Animal Bioscience*. doi:10.1146/annurev-animal-020518-115045

McNeil, D.J., E. McCormick, A. Heimann, M. Kammerer, M. Douglas, S.C. Goslee, **C.M. Grozinger**, and

**H. M. Hines.** 2020.Bumble bees in landscapes with abundant floral resources have lower pathogen loads. *Nature Scientific Reports*.

Ray, A.M., Lopez, D.L., Martinez, J.F., Galbraith, D.A., Rose, R., vanEngelsdorp, D., Rosa, C., Evans, J.D., and **C.M. Grozinger**. (2020) “Distribution of recently identified bee-infecting viruses in managed honey bee (*Apis mellifera*) populations in the United States” *Apidologie* DOI: 10.1007/s13592-020-00757-2.

Liu, F.\*, X. Xu, Y. Zhang, **Z.Y. Huang** \*, H. Zhao. 2020. A meta-analysis shows that screen bottom boards can significantly reduce *Varroa destructor* population. Insects, 11(9): 624. doi: [10.3390/insects11090624](https://dx.doi.org/10.3390%2Finsects11090624).

Chapter Two - Current trends in the oxidative stress and ageing of social hymenopterans

H Li-Byarlay, XL Cleare, Advances in Insect Physiology 59, 43-69, 2020.

Jennifer O. Han, Nicholas L. Naeger, **Brandon K. Hopkins**, David Sumerlin, Paul E. Stamets, Lori M. Carris, and **Walter S. Sheppard**. .  Directed evolution of Metarhizium fungus improves its biocontrol efficacy against Varroa mites in honey bee colonies. Submitted to Current Biology

Brandon K. Hopkins, Jason Long, and **Walter S. Sheppard**. Comparison of indoor (refrigerated) vs outdoor winter storage of commercial honey bee (Apis mellifera) colonies in the Western US. Submitted to J. Econ. Entomol.

Jack, C.J., van Santen, E., Ellis, J.D. 2020. Evaluating the efficacy of oxalic acid vaporization and brood interruption in controlling the honey bee pest *Varroa destructor* (Acari: Varroidae). Journal of Economic Entomology, 113(2): 582-588. <https://doi.org/10.1093/jee/toz358>.

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**Spivak M**, Danka RG. 2020. Perspectives on hygienic behavior in *Apis mellifera* and other social insects. *Apidologie* DOI: 10.1007/s13592-020-00784-z

Goblirsch M, Warner JF, Sommerfeldt BA, **Spivak M**. 2020. Social fever or general immune response? Revisiting an example of social immunity in honey bees. *Insects* 11: 528 doi:10.3390/insects11080528

**Spivak M**, Cariveau DP. 2020. Flowers as parasite transmission hubs. *Nat Ecol Evol*. <https://doi.org/10.1038/s41559-020-1200-z>

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Saelao P, Borba RS, Ricigliano V, **Spivak M**, Simone-Finstrom M. 2020. Honeybee microbiome is stabilized in the presence of propolis. *Biology Letters* 16: 202003. doi.org/10.1098/rsbl.2020.0003

Iwanowicz DD, **Wu-Smart, JY**, Olgun T, **Smart, AH,** Otto, CR, Lopez, D, **Evans, JE**, Cornman R. **2020**. An updated genetic marker for detection of Lake Sinai Virus and metagenetic applications. Peerj 8:e9424. DOI: 10.7717/peerj.9424.

Olgun T, Everhart SE, Anderson T, **Wu-Smart J.** **2020.** Comparative analysis of viruses in four bee species collected from agricultural, urban, and natural landscapes. PLoS ONE 15(6): e0234431.

**Objective 1b: Abiotic Stressors (Pesticides, nutrition, landscapes)**

**Vaudo AD,** Biddinger DJ, Sickel W, Keller A, **López-Uribe MM**. (2020) Phylogenetic pollen preferences facilitate naturalization and pollination services of introduced bees in new habitats. *Royal Society Open Science* 7(7):200225 doi/10.1098/rsos.200225

Phan N, Joshi N, Rajotte E, **López-Uribe MM**, Zhu F, Biddinger DJ. (2020) A new ingestion bioassay protocol for assessing pesticide toxicity to the adult Japanese orchard bee (*Osmia cornifrons*) *Scientific Reports.* <https://doi.org/10.1038/s41598-020-66118-2>

Villalona, E., B.D. Ezray, E. Laveaga, A.A. Agrawal, J.G. Ali, and **H.M. Hines**. 2020. The role of toxic nectar secondary compounds in driving differential bumble bee preferences for milkweed flowers. *Oecologia*.

Vaudo, A. D., J. F. Tooker, **H. M. Patch**, D. J. Biddinger, M. Coccia, M. K. Crone, M. Fiely, J. S. Francis

**H. M. Hines**, M. Hodges, S. W. Jackson, D. Michez, J. Mu, L. Russo, M. Safari, E. D. Treanore, M.

Vanderplanck, E. Yip, A. S. Leonard, and **C. M. Grozinger**. 2020. Pollen protein:lipid macronutrient ratios

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**Objective 2: Genetics, Breeding, Diversity**

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**Objective 3: Management**

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**Other Publications**

Daniel Potter and students in his lab posted two national webinars with study guides on the GROW Plant Health Exchange website’s Pollinator Hub (formerly Plant Management Network): <https://www.planthealthexchange.org/Pages/default.aspx>:

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