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: 01/01/2021 to 12/31/2021

Date of This Report: 02/15/22

Annual Meeting Date(s): 01/13/2021- 01/14/2021

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Brief Summary of Annual NC1173 Multi-State Project Meeting

Minutes taken by Michelle Flenniken (Montana State University) and Margarita Lopez-Uribe (Penn State)

The NC1173 business meeting was conducted as part of the 2022 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 13-14, 2022) and serves as the scientific program for the NC1173 multi-state group.
An agenda for the ABRC meeting is online (https://aapa.cyberbee.net/abrc-2021/), 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 EST 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. The new Project Director / Administrative Advisor, Dr. Brian McCornack (Kansas State University), was introduced to the group and he briefly introduced himself to our team. Michelle reported that there are currently 40 members listed in NIMMS (14 of whom were in attendance) representing 27 institutions. In addition, Tom Welsh, Margaret Couvillon, Jennifer Tsuruda, Autumn Smart, and Esmaeil Amiri, who is a new Asst. Prof. at Mississippi State University joined the meeting too. 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 meeting. She also encouraged members to give specific impactful one-or-two-line examples of NC1173 successes for USDA Program Directors. Dr. Judy Wu-Smart reminded the group highlight joint multi-state and/or multi-institution achievements.

Dr. Michelle Flenniken reported that the previous NC1173 report was submitted in February 2021. Next year Dr. Brian McCornack will highlight components from the 2020 and 2021 reports at the NC1173 business meeting. Dr. Christina Hamilton, the other NC1173 Administrative Advisor did not join us this year.

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%). Dr. Kistner-Thomas was a new program leader last year, and this year other additional Entomology staff include Dr. Vijay Nandula, Lead NPL for Crop Protection and Pest Management, and Mr. Logan Appenfeller a newly hired Program Specialist. In 2021 there were 60 applications submitted to the Pollinator Health Program and funded 10 projects, including one from Dr. Lopez-Uribe was funded. The FY2021 budget included $1.95 billion dollars to NIFA, a $10 million increase in AFRI and $3 million increase to SARE. The majority of pollinator research funding has been allocated to honey bee research. This year, she saw some more bumble bee grants coming in as well. The 2022 budget has not yet been approved, but she expects it to be similar to this year and therefore a similar number of projects will be funded. Priority areas for current administration include studies that incorporate climate change and its impact on pollinators and agriculture and studies that advance racial justice, equity, and opportunity. NIFA’s pollinator research priorities include: pest and pathogen management (including *Varroa* IPM), bee nutrition, bee genetics and breeding, abiotic stressors including climate change and pesticides, and the role of the microbiome on bee health. Funds are also available to support pollinator extension, education, and conferences. There is funding for approximately two conference grants per year ($50,000 max each).

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. She also encouraged new/young investigators, including postdocs, (as well as others) to volunteer to serve on grant review panels (https://prs.nifa.usda.gov/prs/volunteerPrep.do). She noted that the maximum requires for standard USDA grants is $750,000 and the deadline for submission is August 25 at 5 pm EST (see: https://nifa.usda.gov/sites/default/files/rfa/FY-2021-2022-AFRI-Foundational-and-Applied-Science-RFA-Final-07172020.pdf). 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: erica.kistnerthomas@usda.gov). A Question-and-Answer session followed Dr. Kistner-Thomas’s presentation and highlights from that informative session included the following: (a) a committee of stakeholders from SCRI decides what is funded via that program (see USDA-NAREE for a list of members), although you can submit two projects (e.g., a seed grant and full grant as PI) it is not encouraged (consider being a PI on one grant and a Co-PI on another).

Dr. Flenniken reiterated a decision that the next NC1173 meeting will be held with the American Bee Research Conference, which is the scientific component of the NC1173 meeting, in conjunction with the American Bee Federation (ABF) (https://www.abfnet.org/) in Jacksonville, Florida, as well as have a virtual/online option to encourage participation. In addition, Dr. Flenniken noted that she and Dr. Lopez-Uribe will strive to send out notices for ABRC and NC1173 to entire membership prior to the ABRC deadline, as well as send out virtual meeting links to entire NC1173 membership (i.e., including those that do not register / attend ABRC), this was inadvertently not done this year. Future meetings will strive to provide opportunities to interact with the American Honey Producers Association (AHPA), Canadian Association of Professional Apiculturists (CAPA), Apiary Inspectors of America (AIA) and each other. It was also noted that we should discuss ways to try to have more interaction with native/wild bee experts that do not always attend ABRC. We encourage all NC1173 members to attend ABRC, particularly when a virtual option is available. Increased notice regarding the meeting will help facilitate that. Information on future meetings is provided on the AAPA website (https://aapa.cyberbee.net/).

The floor was opened for discussion of other business. Through consensus it was decided that Dr. Michelle Flenniken will serve as NC1173 chair for two more years (i.e., through the February 2023 reporting deadline) and she will lead and be responsible for submitting the 2021 and 2022 NC1173 annual reports. For the 2022 report (in January 2023) she will get more assistance from Dr. Margarita Lopez-Uribe. Dr. Priyadarshini Chakrabarti Basu is joining the team now as the Vice-Vice Chair, so that we can better transfer duties over a longer time period and to facilitate organization and progress for renewing our NC1173 funding approval. The current funding ends in 2024, therefore this team with assistance from Dr. Judy Wu-Smart, will need to submit a renewal in 2023. Dr. Judy Wu-Smart led the efforts for our current NC1173 funding and therefore she has experience to share and she encouraged streamlining objectives to make facilitate easier and accurate reporting (i.e., since several topics intersect). The NC1173 renewal team will include: Dr. Margarita Lopez Uribe (who will be Chair in 2023), Dr. Priyadarshini Chakrabarti Basu (who will be Vice-Chair in 2023) with assistance from Dr. Michelle Flenniken (outgoing Chair in 2023) and Dr. Judy Wu-Smart (previous Chair). We will meet as a subcommittee to outline this process early with the goal of managing this effort in conjunction with our other roles. The floor was opened for discussion of other business, but in the absence of additional discussion Dr. Flenniken adjourned the meeting at 10:57 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 seasonal 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). Williams (Auburn),Cook and Evans (USDA), and Delaplane (Georgia) collaborated to evaluate a new oxalic acid based-treatment, 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. Oxalic acid vaporization is approved for use and the UF Team (Jack, Ellis) were involved in determining the doses required to control *Varroa* mite infestation, and Sagili (OSU) and is testing the efficacy of the recommended label dose for *Varroa* management in the northwestern US. These efforts, in conjunction with research aimed at identifying novel compounds with miticide properties, or novel ways to deliver existing miticides (Williams, Auburn; Cook, USDA) are important to limiting honey bee colony losses due to mite infestation. Toward the development of alternative mite limiting treatments, the Sheppard Lab (WSU) continued to breed a novel strain of Metarhizium fungus through multiple host generations for improvement as a biological control agent against *Varroa* mites and conducted outdoor studies comparing Metarhizium to oxalic acid treatment. Their results showed that the Metarhizum exhibited comparable control to this existing mite treatment, and therefore they submitted prepatent application. Notably, these chemical centered efforts are aligned with continued investigation of hygienic behavior (e.g., removal of mite infested brood) (Spivak, UM) and mite-biting behavior (Li-Barlay, OSU; Harpur, Purdue) and bee breeding programs centered around these traits (Objective 2), as well as promotion of integrated pest management programs (IPM) that promote monitoring for mites and treating when necessary. All of these efforts are needed to mitigate mite-associated honey bee colony losses.

NC1173 members are 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 transmission between different bee species within 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. Likewise, NC1173 members from Penn State are examining inter-specific pathogen transmission between managed and wild bees with insect communities (López-Uribe, Hines, Grozinger). For example, López-Uribe (PSU) investigated prevalence in titers of honey bee viruses in wild bees in cucurbit systems and found that DWV is highly prevalent in wild bees but at very low titers (Jones et al 2021). Hines (PSU) investigated differential microsporidian infection levels by bee species and by habitat. In addition, López-Uribe (PSU) also found that DWV titers tend to be higher in feral than managed colonies, and similarly, immune gene expression is significantly upregulated in feral colonies (Hinshaw et al 2021). The team is also involved in virus discovery and monitoring (Lopez-Uribe PSU; Grozinger PSU) and demonstrated that facilitated viral transmission through injection (mimicking transmission by *Varroa*) shifted viral populations between DWV-A and -B and resulted in selection of specific viral genotypes (Grozinger PSU). In addition, several NC1173 members are involved in virus discovery and monitoring (Flenniken MT, State; Schroeder UMN; Lopez-Uribe PSU; Grozinger PSU; Tarpy, NCSU).

NC1173 members are investigating honey bee antiviral defense mechanisms and the impact of putative immune stimulants, which are provided via supplemental feeding, on the outcome of infections. Honey bee antiviral responses include activation of canonical immune pathways (e.g., Toll, JAK/Stat), the heat shock response, and dsRNA-triggered mechanisms in limiting virus infections (e.g., RNAi and non-sequence specific dsRNA-triggered immune responses) (Flenniken MT State).The influence of nutrition (specifically phytosterols, protein and lipid ratios) on the outcome of pathogenic infections is an active research are in several NC1173 labs (i.e., Sagili OSU; Rangel, TAMU; Grozinger, PSU).

Likewise several labs are examining the impact of putative immune stimulants that may reduce virus infection (i.e., thyme oil, propolis extracts, and fungal extracts) (Flenniken, MT State; Spivak, UM, and Sheppard, WSU).The Flenniken lab utilized laboratory-based studies to determine that honey bees infected with a panel of viruses including DWV and two model viruses (i.e., Flock House virus and Sindbis virus) that were fed 0.16 ppb thyme oil in sucrose syrup exhibited greater expression of key immune genes and reduced virus abundance (Parekh et al 2021). In contrast, in the same study bees that were fed the antifungal agent used to treat nosemosis (fumagillin) or sublethal doses of an insecticide (clothianidin) had reduced immune gene expression and higher viral loads compared to fed sucrose only diets. Together these results indicate that chemical stimulants and stressors impact the outcome of virus infection and immune gene expression in honey bees. This result was not unexpected, but it is important to quantitatively assess in order to potentially develop and promote management strategies aimed at boosting honey bee immune systems and reducing pathogenic infections. Along those lines, the UF team (Ellis) investigated how propolis extracts can be used to prevent and treat *Vairimorpha* (formerly *Nosema*) *ceranae* infection in honey bees and the Sheppard Lab (WSU) advanced testing of polypore mushroom extracts for use as a honey bee feed additive. In addition, the Sheppard lab carried out nutritional analysis of fungal extracts indicated that they contain a suite of minerals and other constituents that are similar to honey and pollen. This team completed an AAFCO requested longevity study comparing fungal extracts to honey and sugar syrup. WSU has coordinated with AAFCO and the FDA to seek a smooth and quick route to registration of the mushroom extract as a honey bee feed additive. Behavioral responses including the relationship between hygienic behavior and virus transmission are also under continued investigation (Spivak, UM). Likewise, the Rangel Lab (TAMU) is also examining the effects of 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 of viruses and other pathogens from honey bee queens to their offspring (Rueppell & Tarpy, NCSU), and the horizontal transmission of viruses from workers to queens (Lee, Spivak, Schroeder, UM; Tarpy, NCSU). Further, Oregon State University team (Sagili and Melathopoulos) is examined the factors involved in high prevalence and intensity of European Foulbrood disease in honey bee colonies pollinating early season specialty crops such as blueberries.

The Huang Lab (MSU) used cell invasion bioassays on a modified four-well arena, we showed that *V. destructor* significantly preferred to invade the worker and drone larvae of *A. mellifera* rather than *A. cerana*, suggesting that the new host is much more attractive to the parasite than the original one. Using gas chromatography-mass spectrometry (GC-MS), they revealed significant differences between the cuticular hydrocarbon (CHC) profiles of worker and drone larvae of the two bee hosts. *A. mellifera* worker and drone larvae were found to express significantly higher amounts of methyl-alkanes, while *A. cerana* larvae produced higher amounts of alkenes. Cell invasion bioassays with glass dummies showed that the mites preferred the glass dummies coated with the CHCs of *A. mellifera* worker or drone larvae, which indicates a role of larval CHCs in mediating the preferential cell invasion of *Varroa*.

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; Flenniken, MT State). 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, the heat shock stress response pathway is an important antiviral defense response to a model virus and chemical stimulants (0.16 ppb thyme oil) boost immune resonses and reduce virus infection levels, whereas chemical stressors have the opposite effects (Flenniken, MT State). Investigators at WSU are breeding a novel strain of *Metarhizium* fungus for improvement as a biological control agent against *Varroa* (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). The UF Team (Ellis) and colleagues demonstrated the feeding honey bees propolis extracts can reduce *V. ceranae* spore counts and, in some cases, help prevent infection in adult honey bees. They (Jack, Ellis) also determined that the labeled rate for oxalic acid vaporization is insufficient to control *Varroa* in managed honey bee colonies. They found that the dose needed for effective *Varroa* control is higher than that labeled for use. This recent finding may inform a label change for oxalic acid.

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). Ongoing studies of pathogen dynamics of wild and managed bees in cucurbit systems (López-Uribe PSU) will shed light on the role of managed pollinators as reservoirs of pathogens that can be transmitted to wild bees. López-Uribe was awarded a USDA-NIFA Pollinator Health grant to work on the interactions between biotic and abiotic stressors under climate change. Via a meta-analysis, Williams (Auburn) found that antagonistic interactions between pesticides and parasites appear to be overlooked by the scientific community. The UF Team (Jack, Ellis) tested new compounds for efficacy against *Varroa* and safety for honey bees. They found a promising new treatment for *Varroa* that they plan to move into Phase 2 testing in future project reporting periods. Furthermore, they continue to screen for additional compounds that can be used to control the mite.

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, NC1173 members are examining the levels of pesticide residues in pollen from honey bees and floral resources in relation to the pesticides applied by growers in Northeastern pollinator-dependent crops (Eitzer, Stoner, CAES; Averill, U Mass), 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; Johnson, Ohio State), 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. As described above, the Flenniken lab carried out caged based studies to quantify the impact of a beekeeper applied fungicide (fumagillin) and sublethal doses of clothianidin on virus infection and suppression of immune gene expression. 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, are working to assess the potential toxicity of pesticides and spray adjuvants to various honey bee life stages and characterize risks associated with exposure to these compounds (Jack and Ellis, UF; Johnson, Ohio State). Williams (Auburn) demonstrated negative effects of the next-generation pesticide, flupyradifurone, on honey bee behavior and survival, while his other work revealed effects of neonicotinoids on bumble bee, but not orchard bee, physiology. Winfree (Rutgers) has published results 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).

Knowledge of pesticide stressors on pollinators has been incorporated into crop integrated pest management programs to reduce pollinator exposure to pesticides at critical times of the crop production season. Integrated Pest and Pollinator Management systematizes this practice and should be considered in all insect-pollinated crops (Biddinger and Rajotte, PSU, Joshi UArk). Penn State NC1173 Grozinger and Patch members evaluated the attraction of pollinators to 20 ornamental perennial plant species and cultivars and used network theory to identify the most important species (Erickson et al 2021). They demonstrated that successional forests and clearings in forests support more floral diversity and more pollinators (Mathis et al 2021 and Lee et al 2021). Moreover, the protein:lipid ratio of pollen-based diets significantly influences resilience to pesticide exposure (Crone et al 2021). Grozinger and Patch (PSU) also investigated the economic value of pollination services in the US and demonstrated that the economic value dependent on pollination service totals 34.0 billion USD in 2012, considerably higher than previous estimates. PSU NC1173 members also evaluated the impacts of land use, habitat, weather and climate on wild bee communities in the mid-Atlantic states and on honey bee winter survival in Pennsylvania and demonstrated that weather conditions in previous seasons were primary drivers of wild bee diversity and abundance and honey bee winter survival (Kammerer et al 2021 and Calovi et al 2021). 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).

<u>Honey bee exposure to pesticides in urban environments</u> (Rangel, TAMU; Huang, MI State; Ellis, UF) These NC1173 members conducted a nationwide study focused on characterizing honey bee exposure to pesticides in nectar and pollen collected in urban settings. Furthermore, they performed a risk assessment using the US EPA's BeeREX model when oral toxicity values were available for compounds discovered in the pesticide screen. The screen identified 17 different pesticides in nectar and 60 in pollen. Most of the samples (~73%) contained no pesticide residues. Using BeeREX, the team demonstrated that four insecticides showed a potential acute risk to honey bees (imidacloprid, chlorpyrifos and esfenvalerate in nectar, and deltamethrin in nectar and pollen). Nevertheless, exposure of honey bees to pesticides via nectar and pollen was low in the sampled urban areas.

The Spiesman lab at Kansas State University conducted wild bee visitation surveys in tallgrass prairie landscapes across eastern Kansas. Our goals are to understand how climate variability affects variability in interaction network structure. Sample processing is underway, and results will be known in August 2022.

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 . Of the species planted, the hairy mountain mint and narrow-leaved mountain mints have had the best combination of being the most attractive (and presumably having the highest yield of nectar) and easiest to establish in solid stands. Species that were unsatisfactory have been lemon balm (mostly attractive to wool carder bees) and *Scrophularia marylandica*, which does not appear to be especially attractive.

Building the first pollen nutrition database for bee pollinators in North America (Ramesh Sagili, OSU and Priya Chakrabarti Basu, MSU): The research team was recently funded with a $500,000 grant from USDA-AFRI to collect pollen from 100 major bee-pollinated plants across North America and analyze the nutritional content of their pollens. The project team has partnered with NRCS, USDA and USGS, in additional to large pool of citizen scientists in USA and Canada for pollen collections. Pollen lipids, proteins, phytosterols and amino acids will be analyzed and the database will be publicly available for researchers, policy makers, citizens and stakeholders. The results will also be published in peer-reviewed journals. Currently in addition to the PI and co-PI, there are two graduate students working in this project.

NC1173 members from Oregon State (Sagili Honey Bee Lab) and Mississippi State (Chakrabarti Honey Bee Lab) are also working on investigating the role of varying concentrations of 24-methylenehcolesterol (an important micronutrient) on honey bee colony health and physiology. In addition, they are also investigating the impacts of sterol biosynthesis inhibitory fungicides on honey bees and plant pollen sterol nutritional quality.

The MSU Bee Lab (PD Chakrabarti) is newly established and is currently recruiting graduate students to work on projects related to pesticide toxicity, nutrition, climate change and interaction of multiple stressors on bee pollinators. PD Chakrabarti is currently building collaborations with stakeholders across Mississippi and is also in the process of establishing a research apiary on main campus at MSU.

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 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 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.

Chronic and persistent bee losses occurring in Mead NE uncovered an improper method of disposing pesticide treated crop seeds through ethanol production. The disposal practice resulted in high concentrations of pesticide-laden waste byproducts which were land applied as soil conditioners without farmers' knowledge of pesticide contamination loads. Soil samples taken 2+ years after land application of contaminated waste byproducts (also known as distiller's grains or wetcake) exhibited high pesticide loads, including systemic pesticides, such as neonicotinoid insecticides (clothianidin (average 7579 ppb; high 24,615 ppb; n=6) and thiamethoxam (average 3569 ppb; high 12,241 ppb; n=6)) and several strobin- and azole- type fungicides (fluoxastrobin (average 5770; high 16,650; n=6) and tebuconazole (average 16,555; high 38,595; n=6). Pesticide residues at these levels would be harmful to bees particularly ground nesting bees as well as when pesticide residues are translocated into non-target pollinator-friendly plants. The UNL Bee Lab recruited the Mead Pollution Research team (~13+ University of Nebraska Medical Center, University of Nebraska-Lincoln, & Creighton University researchers) and secured gap funds to support field sampling of vegetation, soil, and water in 2021. Received NC1173 Multistate project funds ($150,000; 3-yrs) to begin the One Health Student Internship with Dr. Liz Van Wormer (co-PI). This program seeks to mentor a cohort of undergraduates to work collaboratively across disciplines and along researchers seeking to collect environmental and ecological samples and conduct investigations into potential impacts caused by AltEn pollution. UNL Bee Lab sampled wildflowers, trapped pollen, and in-hive food stores to investigate the potential route(s) of pesticide exposure for honey bee colonies impacted by AltEn pollution. Pesticide analyses of plants and in-hive samples are being conducted by Drs. Michelle Hladik (USGS-CA) and Dan Snow (NE Water Science Lab). Protein/lipid analyses of field collected plants, in-hive pollen stores, and brood jelly will be conducted by NC1173 member Dr. Chakrabarti Basu (Mississippi State University) to assess potential degradation of food quality. In addition to collecting environmental samples for pesticide testing, sentinel hives and observation hives were placed on site to assess impact to colony development, age-polyethism or division of behavioral hive tasks, and queen rearing capabilities.

In addition, The Danforth lab (Cornell) conducted a series of experiments to determine how widely-used fungicides (Difenoconazole and Captan) 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.

The Johnson lab (Ohio State) explored the effects of bloom-time pesticide applications made to almonds on honey bee worker adults and larval queens and workers. They assessed the toxicity of insecticide-fungicide combinations and the effect of spray adjuvants, alone and in combination with pesticide tank-mix partners on honey bee survival.

The Huang Lab (MSU) continued to examine transportation stress on honey bees. They determined gene expression in bees that were "transported" (shaken in a lab for 6 days) and control (not shaken). They are interested in determining whether transported bees would down regulate their detoxification genes because a previous unpublished study showed shaken bees were more sensitive to pesticides. They determined the gene expression of bees in both shaken and unshaken bees for two P450 genes, c305, C6BE, GST (glutathione S-transferase), and defensin (a gene encoding an antimicrobial peptide) and two reference genes (actin and GADPH). Contrary to their prediction, they did not find reduced gene expression in the transported group (and one gene (i.e., c305) exhibited increased expression). They are continuing to explore this line of investigation.

Short outcomes, Objective 1b:
The PSU team, (1) demonstrated that ornamental plant species can serve as important forage resources for managed and wild bees, but there is considerable variation among cultivars (2) early successional forests and clearing significantly improve pollinator abundance in forest systems (3) identified the optimal protein:lipid ratios of honey bee diets to support resilience to pesticides (4) found that weather in previous seasons strongly influenced wild bee abundance and diversity and honey bee colony winter survival (5) showed that economic value of insect pollination services to agricultural systems in the US is considerably higher than previous calculated
Likewise, research teams 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. The resulting manuscript has been accepted and will be published during the next project reporting period. Williams (Auburn, AL) and team observed that antagonistic interactions between parasites and pesticides are common in honey bees. Stoner (CAES, in collaboration with US EPA, U Maine, UMD) has found major gaps in the ability of commonly used DNA metabarcoding methods to detect, much less quantify, pollen collected by honey bees from maize and buckwheat in mixed pollen samples. This calls into question much research relying solely on metabarcoding for pollen analysis without cross-referencing to microscopic palynology. 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 and Chakrabarti). Tests of tank-mix pesticide combinations have identified a subset of spray adjuvants that are both toxic at field-relevant concentrations and were made more toxic when mixed with pesticides (Johnson).

Outputs, Objective 1b: NC1173 member (Grozinger, PSU) worked with collaborator Anthony Robinson to evaluate the utility of the Beescape portal for beekeepers and identified strategies to improve the usability and value of the portal. Given the severity of the systemic pesticide pollution issue occurring in NE and unclear alternatives for proper safe disposal of treated crop seeds, NC1173 member Wu-Smart has engaged with community leaders, legislators, and advocates which has led to legislative actions at the local level: NE bill LB507 The **Ethanol Development Act**, "*in which the use of treated seed corn in the production of agricultural ethyl alcohol shall be prohibited if such use results in the generation of a byproduct that is deemed unsafe for livestock consumption or land application",* (testimony Feb 3, 2021); NE bill LB634 which seeks to provide a cause of action for unsafe disposal of treated seed (testimony March 10, 2021); and MN bill HF766 a type of "extended producer responsibility" bill introduced by MN Representative Rick Hansen (District 52A) that seeks better product stewardship and accountability from producers (testimony March 22, 2021). In these testimonies, information was provided that highlighted critical needs to examine and regulate treated seed practices to better control and manage systemic pesticide pollution. Conducted interviews and discussions with NJ Assemblyman Clinton Calabrese regarding NJ Bill A2070 which prohibits most outdoor non-agricultural uses of neonicotinoids (passed Jan 2022).

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 -CAES; 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, in 2021 WSU handed off the production of i. i. New World Carniolan i. i. breeder queens to commercial queen producer. Starting in 2021, NWC breeder queens supplied to the queen production industry came from queen producer partners (rather than WSU). WSU continued to 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, the WSU team 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 2021, they 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.

Spivak and UM team initiated a new bee breeding program in 2019 to understand mechanisms of resistance to *Varroa* mites and diseases. They evaluate treated-parent colonies and untreated-daughter colonies for wintering survivorship and for three behavioral traits that help bees defend against pathogens and parasites: high propolis collection (quantified using propolis traps), rapid hygienic behavior (quantified using the freeze-killed brood assay), and low *Varroa* mite population growth over the season (comparing the proportion of mites in worker brood over time). Untreated colonies that survive winter are used as breeding stock the following summer. Parent colonies of the survivors are evaluated and sampled for future evaluation of 'omic pattern associated with resistance.

The Huang Lab (MSU) identified three genes for mushroom body large-type Kenyon cell-specific protein-1 (*Mblk-1)* encodes a putative transcription factor and is expressed preferentially in the large-type Kenyon cells of honey bee mushroom body. *Mblk-1* is thought to be involved in brain function by regulating transcription of its target genes, however its function in the honey bees is obscure. They showed that *Mblk-1* had significantly higher expression in the brains of forager bees relative to nurse bees regardless of their age. Inhibition of *Mblk-1* decreased sucrose responsiveness in foragers. Finally, we determined that *Mblk-1* indirectly targets the mRNA of gustatory receptors. These findings suggest that *Mblk-1* plays important roles in regulating honey bee division of labor.

Short outcomes, Objective 2:
Purdue University (Harpur) breeding program with mite resistant traits has been successfully made available in ten US States. The Purdue team is currently exploring integrating genomic selection and genetics into breeding decisions and they have developed international collaborations (Gregor Gorjanc; Rosalind Institute) to begin developing theory and empirical data to incorporate genomics into bee breeding. Harpur is funded by the Eva Crane Trust) to explore the genetic diversity of honey bees across the United States to understand baseline levels of genetic diversity. 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. 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. Grozinger and Rasgon are developing tools for genetic manipulation/transformation of bees (Penn 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. Genomic studies by Patch and collaborators demonstrated that honey bees likely originated in Asia and identified genomic “hotspots” for supporting adaptive radiation in subspecies. With funding from the Pennsylvania Department of Ag, López-Uribe and Harpur are sequencing the genomes of feral colonies and several genetic stocks to estimate levels of Africanization in colonies in the northeast of the US. With funding from an NSF-CAREER award, López-Uribe is investigating the evolutionary responses of bees’ sensory systems to the domestication of flowers in crops. Spivak (UM) is funded by USDA AFRI (2018-67013-2753) to conduct a multi-trait breeding program for mite resistance (described above). NC1173 members (Ellis, UF) are working with collaborators to develop a stock certification program for honey bees in Puerto Rico.

Outputs, Objective 2:
NC1173 members worked directly with over numerous beekeepers and nearly through extension programs and courses, which were mostly virtual in 2021. Penn State offered a winter webinar series that was attended by at least 3,000 beekeepers from all over the US. Harpur (Purdue) provides Varroa-resistant stock (Indiana Mite biters) to beekeepers across 10 States, and researchers (Li-Byarlay, CSU; Lopez-Uribe PSU).

Central State University provided extension talks to 600+ beekeepers and general public including extension webinars at CSU, talks at national bee conferences, one scientific publication on mite biting behavior and mite-resistance published in Frontiers in Ecology and Evolution (Smith et al, CSU). Flenniken, Sagili, and other NC1173 Members gave presentations to the Bee Informed Tech Transfer team and numerous beekeeping organizations (e.g., California State Beekeepers Association, Montana State Beekeepers Association, Southwest Ohio Beekeeping Club (with over 200 attendees online)). Spivak lab delivered over 50 talks to over 5000 beekeepers locally, nationally and internationally.

Dr. Walter S. Sheppard (WSU) completed the Video - Honey Bee Breeding and Practical Selection Methods, which has received more than 8.2K views, including national and international audiences. The video was awarded a Bronze Award for Educational Video from the Association of Natural Resource Extension Professionals and has been shown in numerous classrooms. It is also featured in a Podcast discussion, Beekeeping Today Podcast, presented by Bee Culture Magazine. Links to the video and podcast are available on line: Video on Selection Methods and Honey Bee Breeding, YouTube: https://youtu.be/R8-9DgXcrfI and Vimeo: https://vimeo.com/380776410.

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(especially concerning the Varroa mite (Williams, Auburn), enhancing landscapes for pollinators, and options to reduce exposure or mitigate effects of pesticides. NC1173 members gave presentations to numerous stakeholder groups (highlighted in other section), and Harpur (Purdue) created a digital seminar series for beekeepers called "Winter Cluster" and provided training to bee breeders on Instrumental Insemination and Queen Rearing.

Through a USDA-OREI funded project, López-Uribe and Underwood successfully developed a sustainable and economically profitable protocol to incorporate organic management practices into beekeeping. NC1173 members conducted studies to identify the most attractive and nutritionally beneficial species of plants in urban (Erickson et al 2021) and forest settings (Mathis et al 2021, Lee et al 2021). 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).

Central State University in Ohio held workshops in a combination of online and in person for improve diversity of queen bees by distributing 48-hour queen cells for local beekeepers. CSU plans to do future trainings on workshops to Ohio beekeepers on how to do grafting and queen rearing for genetic diversity and breeding.

Potter et al. (University of Kentucky) completed and published a multi-year study of alternative lawns consisting of dwarf varieties of white clover (*Trifolium repens*) in pure stands or intermixed with turfgrass. Dwarf clovers have been selected for small leaf size and low growth habit, allowing them to tolerate low mowing heights and blend better with lawn grasses. The study showed that the dwarf clovers augment nitrogen and resist white grubs, reducing need for fertilizer and insecticide inputs, and support both honey bees and diverse assemblages of wild bees similar to those that visit conventional white clover.

The CAES team (Stoner, Zarrillo) initiated studies on the pollinator community of chestnut, *Castanea* spp., working with volunteers from the American Chestnut Foundation and utilizing the diverse collection of chestnut species, inter-species crosses, and cultivars, a product of over 100 years of chestnut breeding efforts, at our experimental farm.

The Huang Lab (MSU) examined mechanisms of floral attraction to honey bees and determined the nectar production of two varieties of *Bidens*, *Portulaca*, and *Tagetes*. One that was highly attractive to honey bees and one not in each plant. They found significantly higher nectar production in the more attractive variety in *Bidens* and *Portulaca*, but we failed to obtain measurable nectar in either variety of *Tagetes*.

The Spivak Lab (UM) concluded a study on the establishment and benefit of "Bee Lawns," which was funded by the Minnesota Environmental and Natural Resources Trust Fund and determined that there is increased interest in enhancing areas dedicated to lawns using flowering species to support pollinators. They intentionally introduced low-growing flowers to turfgrass lawns to promote bee diversity and reduce inputs, while maintaining the traditional aesthetics and recreational uses associated with lawns. They found that Kentucky bluegrass and hard fescue are promising turf companion grasses for future forb/turf inter-seeding. Of the eight forbs tested, *Trifolium repens L*., *Prunella vulgaris ssp. lanceolata*, *Thymus serpyllum auct. non L*., and *Astragalus crassicarpus Nutt*. were most promising. In the bee lawns established in Minneapolis parks, they found 56 species of bees on *T. repens*, with *A. mellifera* as the most common species observed. Florally enhanced lawns supported more diverse bee communities than lawns with just *T. repens*, and the bee communities supported by florally enhanced lawns were significantly different from the bee communities supported by lawns containing just *T. repens*. This study has generated a huge interest across the state of Minnesota, even leading to a legislative initiative called "Lawns to Legumes" program that offers a combination of workshops, coaching, planting guides and cost-share funding for installing pollinator-friendly native plantings in residential lawns in Minnesota.

Short outcomes, Objective 3:
With funding from the PA Department of Ag, López-Uribe is leading a statewide bee monitoring project through participatory science with Master Gardeners.
Williams (Auburn, AL) performed a citizen science experiment that examined effectiveness of different beekeeper learning types concerning *Varroa*, and performed several experiments to identify novel active ingredients or management actions against the mite. 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:
PA Bee Monitoring Program: López-Uribe is working in collaboration with Penn State Extension and Master Gardeners on a statewide bee monitoring project. The goals of the project are to increase knowledge of bee diversity across Pennsylvania and to collect long-term data to detect changes in abundance and species composition over time.

Harpur (Purdue) - The cluster brought in beekeepers, researchers, and extension specialists from around the world and hosted over 300 people. We focused on topics related to *Varroa* treatment, winterizing colonies, Fall garden management for bees, and youth beekeeping. Our Instrumental Insemination and Queen Rearing course trained 30 people from across the US. We are expanding these courses by providing them in a digital format in 2022.

Potter (University of Kentucky) delivered virtual lectures on "Bees, Pesticides, and Politics: Challenges and Opportunities Sustainable Urban Landscapes" at major 2021 conferences and venues attended by thousands of stakeholders (e.g., American Hort national webinar, Conn. Native Plants and Pollinators Conf., Illinois Green Industry Conf., Alabama "Raising Trees" webinar series, Michigan Green Industry Assoc., NC State Master Gardeners/Extension Agents, Ohio State Univ. Landscape and Turf webinar, KY Pollinator Stakeholder group, Green & Growing Atlanta, and others).

Stoner (CAES) provides technical assistance to the Pollinator Pathway network (https://www.pollinator-pathway.org/), which organizes diverse stakeholders (including environmental organizations, land trusts, garden clubs, municipal governments and other community groups) to encourage planting native plants attractive to pollinators and reducing or eliminating pesticide use. This organization, which started in southwest CT, now stretches throughout New England, New Jersey, eastern New York and Pennsylvania, and down the East Coast to Maryland. Over 70 towns (out of 169 total) in Connecticut alone now have active Pollinator Pathway programs.

Impacts

NC1173 Highlights Identified by Members and Teams

Penn State University (Lopez-Uribe, Grozinger, Underwood,

PA Pollinator Protection Plan (led by Boyle, includes all faculty members. We worked 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.

PA Digital Pollen Library. With support from the Pennsylvania Department of Agriculture and Penn State's Institute for Computational and Data Science: Research Innovations with Scientists and Engineers team, we are developing a sortable, online digital database of flowering plants and their pollen grains. Beekeepers, land managers, educators and gardeners can utilize this reference as a planning and informational tool to identify the nutritional needs of pollinators in ongoing palynology studies (Boyle, Grozinger).

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).
With faculty from Penn State's Center for Immersive Technologies and Center for Science and the Schools we developed a virtual 3D bee internal and external anatomy tool and a bee foraging ecology agent-based model game (Patch and 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 led the development of a >4 acre Pollinator and Bird Garden at the Arboretum at PSU, which features >400 plant types and serve as a venue to showcase research on plant-pollinator interactions and conservation. The garden opened in summer 2021 and is expected to welcome 100K visitors each year.
<u>PA Bee Monitoring Program</u>: López-Uribe is working in collaboration with Penn State Extension and Master Gardeners on a statewide bee monitoring project. The goals of the project are to increase knowledge of bee diversity across Pennsylvania and to collect long-term data to detect changes in abundance and species composition over time.

Purdue (Harpur)
Collaborations within the program. Outreach to over 1000 beekeepers around the word through extension programs including two extensions courses on bee breeding. Additionally, the annual Bee Field Day included 150 beekeepers from across Indiana. Their Insemination course brought in 30 beekeepers from across the US and trained them to create sustainable breeding programs. Their queen rearing course trained beekeepers to locally produce their own breeding queens and create sustainable operations. Each of these extension programs were evaluated with surveys.
For example, 300 people attended the Winter Cluster program and 90% of attendees reported that they would incorporate what they learned in their management practices

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). As of October 2021, the regional Great Plains Master Beekeeping (GPMB) Training Program has 996 registered members representing 3 countries (US, India, Belgium) and 20 states. More noteworthy is the demographic distribution and equal representation among women and men interested in beekeeping which is unique among agriculture focused programs. (Male: 484; Female: 500; *Hispanic/Latino: 19, *Black: 16, *Asian: 7, *Spanish: 3, *Other: 25, *Hawaiian: 3, *Native American: 7, *Caucasian: 915; Military: 214).
We brainstormed with industry partners and assessed long-term needs and options for servicing

Midwest beekeepers of all experience level and types of operations from migratory commercial beekeepers to hobbyists. The GPMB program received additional support from USDA-NIFA Beginning Farmer Rancher Development Program ($496,890* June 2021) *to encourage experiential learning using guided training kits and open apiaries.* The increased quality of engagement seeks to promote professional and economic growth for participating beekeeping farmers as well as strengthen the relationship between local associations and the beekeepers. The following five collaborating beekeeping associations will receive funding support to establish open apiaries and offer in-field training for their association members, which may reach ~1,800 Midwest beekeepers: Omaha Bee Club (~106 members), Nebraska Beekeepers Association (~175 members), the Northeast Kansas Beekeepers Association (~600 members), Kansas Honey Producers Association (formerly The Kansas State Beekeepers Association) (~290 members); and the Missouri State Beekeepers Association (~625 members). And the Center for Rural Affairs will continue to help provide targeted educational materials and exploratory beekeeping courses for Latinx and Tribal communities in Nebraska. The GPMB program invites guest speakers including numerous NC1173 members and or their students to speak at training conferences. In 2021, NC1173 members or students involved with GPMB included Couvillon, Lopez-Uribe, Rangel, Tarpy, Sheppard, Williams, Underwood, Sagili. GPMB also provides an easy outlet for distributing information and resources produced by extension educators and researchers promoting best management practices. Therefore, GPMB members are also involved with working groups such the IPM4Bees and Midwest MP3 groups as new collaborations with the National “Heros to Hives” Program hosted through the Michigan Food & Farming Systems to establish a targeted program for military veterans.

Midwest Managed Pollinator Protection Plan (MP3) led by Michigan State University Megan Milbrath, Ana Heck seeks to help states develop pollinator managed plans and navigate through challenges related to plan implementation. This work is supported by the USDA National Institute of Food and Agriculture, Crop Protection and Pest Management Program through the North Central IPM Center (2018-70006-28883).

The IPM4Bees Midwest Working Group is supported through the North Central IPM Center (USDA-NIFA #2018-70006-28883) and in collaboration with Drs. Judy Wu-Smat (UNL) Matthew O’Neal (ISU) and Extension Educator Randall Cass (ISU). The working group aims to foster learning, collaboration, and information/resource sharing among researchers, extension professionals, and other stakeholders that work in honey bees, native bees, and bee-related integrated pest management (IPM). The Midwest IPM4Bees received additional funding to continue efforts and recruited Dr. Megan Milbrath and Extension Educator Ana Heck from Michigan State University to help coordinate 2021-2023 activities.

Central State University (Li-Byarlay) received USDA-SARE grant from North Central Region 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 by learning skills in queen rearing and grafting. Working with extension in 2021, CSU bee lab provided four online webinars on sustainable beekeeping by using swarm traps, queen development, decreasing viral infections and temperature control.

Auburn University (Williams) coordinated a virtual monthly beekeeping seminar series – At Home Beekeeping – alongside apicultural specialists and scientists from the eastern United States

(Ellis & Jack, UF; Delaplane & Berry, Georgia; Tarpy, North Carolina State; Webster, Kentucky State; Tsuruda, Tennessee; Chakrabarti Basu, Mississippi State; Healy, Louisiana State; Simone-Finstrom & Lau, USDA-ARS; Rangel, TAMU. That program has been hugely popular, attended by over 2,300 participants during the reporting period. Overall, 89% and 69% of participants indicated that they plan to implement learned practices and that attendance will save them money moving forward, respectively.

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

Publications by topic	**2021**
Obj 1a: Biotic (Pests & pathogens)	25
Obj 1b: Abiotic (Pesticides, nutrition, landscapes)	32
Obj 2: Genetics, Breeding, Diversity	17
Obj 3: Management	14
Other Publications	5
Total	**93**
Publications with >1 NC1173 authors	12

NC1173 Member Publications (01/01/2021 to 12/31/2021)
*Papers applicable to multiple objectives are only reported once.

Wu-Smart J, Kittle S., Couvillon, M., López-Uribe, M. 2021. Proceedings of the 2021 American Bee Research Conference. Bee Culture (March edition) pp 46-58.

Objective 1a: Biotic Stressors (Pests & pathogens)

Naree, S., Benbow, M.E., Suwannapong, G., Ellis, J.D. 2021. Mitigating *Nosema ceranae* infection in western honey bee (*Apis mellifera*) workers using propolis collected from honey bee and stingless bee (*Tetrigona apicalis*) hives. Journal of Invertebrate Pathology, 185: 107666, 8 pgs. https://doi.org/10.1016/j.jip.2021.107666.

Jack, C.J., van Santen, E., Ellis, J.D. 2021. Determining the dose of oxalic acid applied via vaporization needed for the control of the honey bee (*Apis mellifera*) pest *Varroa destructor*. Journal of Apicultural Research, 60(3): 414 – 420. https://doi.org/10.1080/00218839.2021.1877447.

Hinshaw C, Evans KC, Rosa C, López-Uribe MM. (2021) The role of pathogen dynamics and immune gene expression in the survival of feral honey bees. *Frontiers in Ecology and Evolution* 8: 505.

Jones LJ, Ford RP, Schilder RJ, López-Uribe MM. (2021) Honey bee viruses are highly prevalent but at low intensities in wild pollinators of cucurbit agroecosystems. *Journal of Invertebrate Pathology* 185: 107667, Ray, A. M., Davis, S. L., Rasgon, J.L. and C.M. Grozinger. Simulated vector transmission differentially influences dynamics of two viral variants of deformed wing virus in honey bees (Apis mellifera). Journal of General Virology 102(11):001687 https://doi.org/10.1099/jgv.0.001687 (2021).

Williams, M.-K., Cleary, D., Tripodi, A., Szalanski, A. L. (2021). Co-occurrence of Lotmaria passim and Nosema ceranae in honey bees (Apis mellifera L.) from the United States. *Journal of Apicultural Research*. 10.1080/00218839.2021.1960745.

Faber N.R., Meiborg A.B., McFarlane G.R., Gorjanc G., & Harpur B.A. (2021). A gene drive does not spread easily in populations of the honey bee parasite *Varroa destructor*. Apidologie. DOI:10.1007/s13592-021-00891-5.

Wen X, Ma C, Sun M, Wang Y, Xue X, Chen J, Song W, Li-Byarlay H, Luo S, 2021, Pesticide residues in the pollen and nectar of oilseed rape (Brassica napus L.) and their hazards to honey bees. Science of The Total Environment, 786, 2021,147443, https://doi.org/10.1016/j.scitotenv.2021.147443.

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