

**United States Department of Agriculture
Project Initiation**

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Project Title

Sunflowers as treatment and preventative for bumble and honey bee pathogens

Project Details

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Program Code [A5170] New Frontiers in Pollinator Health: From Research to Application	

Project Director

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Recipient Organization

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Performing Department

Biology

Non Technical Summary

Pollinators provide billions of dollars in crop pollination annually, and parasites have been implicated in declines of many bee species. Nutrition, including diet quality as well as quantity, is an important factor in bee health. We found that sunflower pollen dramatically reduced Crithidia infection in bumble bees and Nosema infection in honey bees, suggesting the potential for sunflower pollen supplements and plantings as easily-implemented approaches to improving bee health. Our research goals are to evaluate the benefits and costs of sunflower pollen supplements on bee colonies, assess the impacts of sunflower pollen supplements on pathogen resistance in the field, and determine the degree to which sunflower plantings affect parasite infection in bees. Each objective will include bumble and honey bees, and honey bee objectives will take advantage of Bee Informed Partnership data and directly include beekeepers in assessment. In addition to directly engaging beekeepers in our research, extension goals include hiring a Pollinator Extension Educator who will use a new state apiary, engaging an Advisory Board representing diverse stakeholders,

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and conducting a wide range of outreach activities to reach local, regional and national audiences. Our proposal is directly relevant to Priority 1, "Development and evaluation of strategies to reduce pollinator declines caused by factors such as habitat changes or loss, nutritional imbalances, pathogens..." This research will open up new mechanisms to manage bee health via diet manipulation and foraging options, using simple approaches that are accessible and applicable to a diversity of stakeholders.

Animal Health Component

Animal Health Component: 0%

Research Effort Categories

Basic	25%
Applied	75%
Developmental	0%

Classification

Knowledge Area (KA)	Subject of Investigation (SOI)	Field of Science (FOS)	Percent
211	0120	1070	100%

Knowledge Area

[211] Insects, Mites, and Other Arthropods Affecting Plants

Subject Of Investigation

[0120] Land

Field Of Science

[1070] Ecology

Keywords

Apis mellifera, Nosema, Bee Informed Partnership, Bombus impatiens, Crithidia bombi, bee parasites

Goals / Objectives

Research objectives and predictions. We will address the following linked objectives, ranging from lab to field experiments, to assess the effects of sunflower on bee health and disease: Objective 1. Evaluate the benefits and costs of supplementing bumble and honey bee colonies with sunflower pollen on bee disease and health. We will use laboratory experiments to test how sunflower vs. mixed wildflower pollen supplements affect bee disease and health. Experiments will be performed on commercial and wild bumble bees, and honey bees. Obj. 1A: Bombus (Year 1; Adler, Irwin). We will use commercial colonies of B. impatiens, and colonies of B. impatiens, B. griseocollis, and B. bimaculatus produced from wild-caught queens. Using commercial colonies of B. impatiens is of direct relevance to industry stakeholders, testing how pollen diet affects parasite infection and bee health in managed bees. By extending our experiments to multiple wild bumble bee species, we will assess the degree to which sunflower pollen affects disease and health in wild pollinators important for crop pollination. Obj 1B: Apis (May-Nov 2017; Evans). We will test

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the effect of four pollen treatments (no pollen, wildflower pollen, 2:1 wildflower:sunflower pollen, and 1:2 wildflower:sunflower pollen) on adult worker bees from each of three healthy colonies (i.e., capped and uncapped brood with normal brood pattern, bee bread and honey stores) of *A. mellifera* in which both Deformed wing virus and the trypanosomatid parasite *Lotmaria passim* have been identified. Objective 2. Assess the degree to which sunflower pollen supplements protect against parasite infection in field colonies. We will supplement field colonies of bumble and honey bees with sunflower vs. wildflower pollen or protein supplements, working with commercial beekeepers for honey bees. Colonies will be allowed to freely forage for nectar and additional pollen, naturally exposing them to parasites and viruses. We will measure the time-course of parasite and viral acquisition, the extent of parasite load, colony foraging and productivity. We predict that supplementing colonies with sunflower pollen will slow and reduce parasite acquisition, and, using our optimized ratios from Obj 1, that sunflower pollen will improve colony performance compared to wildflower pollen. Obj 2A: *Bombus* (Summer Year 2, Adler, Irwin, McFrederick). We will deploy 40 colonies each (n=20/treatment/location) of commercial bumble bees (*Bombus impatiens*) in the Northeastern US (South Deerfield Station, 250 acres, Amherst, MA) and southeastern US (Lake Wheeler Road Field Laboratory, 1500 acres, Raleigh, NC). Obj 2B: *Apis* (Years 1 and 2; Adler and Evans with BIP, Inc.). We will take advantage of the BIP network of commercial beekeepers and Tech Team standardized disease assessments (<https://beeinformed.org/programs/tech-teams/>) to test whether pollen supplements increase disease resistance and colony health in commercial honey bee operations. In Year 1, we will conduct a dose trial to ascertain the dose with the greatest medicinal benefit and colony performance. This trial will be conducted using 32 colonies within a single commercial apiary; there will be four treatment levels (0, 25%, 50% or 75% sunflower mixed with MegaBee (megabee.com), a standard commercial honey bee protein supplement. In Year 2, we will conduct a similar but expanded field trial comparing the selected optimal supplement dose (e.g., 50% sunflower), a negative control of no supplement, and a positive control of 100% MegaBee. This full trial will be conducted at three commercial apiaries, with three treatments and three pallets (12 colonies)/treatment/apiary, for a total of 36 colonies in each apiary. As an add-on study in Year 2, we will conduct a similar assessment with Massachusetts beekeepers, restricting inclusion to commercial beekeepers with at least 5 years of experience and at least 40 colonies, to test efficacy in multiple locations. Objective 3. Document whether growing sunflower on farms affects pathogen acquisition and colony productivity. We will use multiple approaches to address this objective. Obj 3A (Year 1, Baylis): We will take advantage of longer-term data from the Bee Informed Project (BIP) to assess how sunflower acreage around honey bee colonies correlates with pathogen loads. We predict that increasing acreage of sunflower will reduce pathogen acquisition and increase colony health, consistent with our pilot data (Obj 3), but the effect of sunflower acreage on colony health may be non-linear, with the largest health benefits at intermediate acreage and greater costs at large-scale sunflower mono-cultures. Obj 3B (Year 3, Adler, Irwin & McFrederick): We will experimentally plant sunflower on farms at varying acreage and measure pathogen acquisition and colony productivity for bumble and honey bee colonies in North Carolina. This approach will provide precise control over sunflower variety, acreage and cultivation. In Massachusetts, we will place bumble and honey bee colonies at a mixture of small-scale organic and conventional farms with varied sunflower acreage, and measure pathogen acquisition and colony production. This allows us to move from experimental to real-world farms and connect with end users. Extension Objectives and Integration. Because our proposal may provide simple, cost-effective solutions to reduce bee disease without the use of chemicals, we have an unparalleled opportunity to combine research and extension efforts to test effects while also communicating with end users. Directly including industry and beekeepers in our research is one of several extension objectives. Because we propose to mitigate bee diseases through the use of sunflower pollen supplements and plantings in pollinator habitat, there is the potential for engagement and outreach with a wide range of stakeholders, including beekeepers, the commercial bumble bee industry, vegetable and fruit crop growers and the general public interested in pollinator-friendly plantings to preserve pollination services. Although not required, we will create an Advisory Board representing these interests to solicit input on objectives and communicate results via regular meetings. Furthermore, in Massachusetts there is an unprecedented opportunity for extension impact due to the recent creation of a state apiary (MA Dept. of Agricultural Resources, or MDAR) on the UMass campus and the lack of a Pollinator Extension Educator at UMass. The state has a very high proportion of small-scale farms that utilize extension services, and currently the nearest pollinator extension educator is at Cornell University. Hiring a Pollinator Extension Educator who can take advantage of the new

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apiary will provide a state hub for pollinator extension and leadership on extension objectives. This new hire will conduct workshops, create and maintain a website, interface with the public and, with all members of the leadership team, participate in a wide range of outreach activities. Finally, our involvement with BIP provides a national platform for disseminating results, as well as through eXtension.org posts. Thus, our Extension Objectives are to (1) directly involve beekeepers in assessing the efficacy of sunflower pollen supplements and plantings for honey bee health, (2) create an Advisory Board that engages with diverse stakeholders in multiple regions, (3) hire a Pollinator Extension Educator for Massachusetts, and (4) develop a wide range of regional and national outreach activities.

Project Methods

Obj. 1A: *Bombus* (Year 1; Adler, Irwin). We will use commercial colonies of *B. impatiens*, and colonies of *B. impatiens*, *B. griseocollis*, and *B. bimaculatus* produced from wild-caught queens. For commercial *B. impatiens*, we will use 20 colonies per treatment (3 pollen treatments x 2 parasite treatments), resulting in 120 colonies. Because of the challenges of raising colonies from wild bees, we will reduce sample size to 5-10 colonies per treatment per species in wild bees. Parasite treatment. When colonies have 10 workers, five workers per colony will be removed, inoculated with *Crithidia*, and returned to their colonies. We will perform a sham inoculation for colonies in the uninfected treatment. Pollen diet treatment. We will start pollen treatments after inoculation. We will give the colonies up to 20 g of pollen every two days, and will record the weight of pollen used/consumed. The experiment will be run for up to 80 days or until the colonies only produce sexuals (new queens and males). Response variables: Parasite infection, food consumption and colony performance. We will assess the time course of within-colony infection every week. At termination, we will assess *Crithidia* infection using gut samples from 30% of the living workers using microscopy. We will record consumption of sucrose solution weekly and pollen every two days. To assess colony performance, we will record losses (worker mortality, discarded larvae, and oophagy) and weekly colony weight. Any sexuals produced will be counted and removed. When the experiment is terminated, we will freeze the colonies, count the workers, additional sexuals and queen brood clumps, and number of larvae and eggs. Obj 1B: *Apis* (May-Nov 2017; Evans). We will test the effect of four pollen treatments on adult worker bees from each of three healthy colonies of *A. mellifera* in which both Deformed wing virus and the trypanosomatid parasite *Lotmaria passim* have been identified. Newly emerged bees from each colony will be separated into 16 cups, with 4 cups per treatment and 40 bees per cup (3 colonies x 16 cups/colony = 48 total cups, or 12 cups/treatment). All cups will receive ad lib sugar water via a plastic feeder (Evans et al. 2009). Cups will be maintained for three days, at which point half the cups (two of the four cups in each colony x treatment combination) will be used to assess mortality and infection in a field colony, and the other half will be maintained in the lab, with daily measures of mortality, at which point surviving bees will be frozen for genetic analyses. Obj 2. Assess the degree to which sunflower pollen supplements protects against parasite infection in field colonies (Year 2). Obj 2A: *Bombus* (Summer Year 2, Adler, Irwin, McFrederick). We will deploy 40 colonies each (n=20 /treatment/location) of commercial bumble bees (*Bombus impatiens*) in the Northeastern US (South Deerfield Station, 250 acres, Amherst, MA) and southeastern US (Lake Wheeler Road Field Laboratory, 1500 acres, Raleigh, NC). Colonies will be initially controlled for number of workers and brood. Colonies will be randomly assigned to sunflower or wildflower pollen supplement treatments. Pollen diet treatments. We expect to feed *Bombus* colonies up to 150 g of pollen weekly. Colonies will not be given supplemental sugar solution, to motivate foraging. Parasite screening. Five workers will be sampled per colony every other week for 12 weeks to assess infection by pathogens, including *Crithidia*, *Nosema* spp., *Apicystis bombi*, iflaviruses and dicistroviruses. Colony foraging. We will use radio frequency ID tags to quantify colony foraging rates. Once per week for 12 weeks, we will observe colonies for entering workers, recording whether they are carrying corbicular pollen loads. For up to 10 bees per colony per collection period, we will collect one corbicular pollen load and identify pollen to species. Colony health. We will estimate productivity weekly by weighing colonies, and will visually inspect the colonies and count and remove any males or queens under red light at night. We will terminate the colonies at 12 weeks. We will freeze the colonies and count final worker production, the production of queen cells and brood in each colony. Obj 2B: *Apis* (Years 1 and 2; Adler and Evans with BIP, Inc.). We will take advantage of the BIP network of commercial beekeepers and Tech Team standardized disease assessments to test whether pollen supplements increase disease resistance and colony health in commercial honey bee operations. In Year 1, we will conduct a dose trial using 32 colonies within a single commercial apiary; there will be four

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treatment levels (0, 25%, 50% or 75% sunflower mixed with MegaBee (megabee.com), a standard commercial honey bee protein supplement. Treatments will be assigned randomly, with two pallets assigned to each treatment. Treatments will be applied in early fall, after the last summer honey is removed. Standard BIP colony assessments will be performed 4 times over the study. These measurements include colony size (frames of bees), brood quality (quantitative ranking), overt bee disease (present/absence of Chalkbrood, sac brood, European foulbrood, and American foulbrood), queen status, and lab assessments of Varroa mites (mites/100 bees) and Nosema spores (million spores/bee). In Year 2, we will conduct a similar but expanded field trial comparing the selected optimal supplement dose, a negative control of no supplement, and a positive control of 100% MegaBee. This full trial will be conducted at three commercial apiaries, with three treatments and three pallets (12 colonies)/treatment/apiary, for a total of 36 colonies in each apiary. Sampling for colony performance (colony size and brood quality), visible parasites and Nosema will be conducted as in the dose study.

Obj 3. Document whether growing sunflower on farms affects pathogen acquisition and colony productivity.

Obj 3A: Apis (Year 1; Baylis). We propose to use the multi-year national survey of honey bee health collected by BIP for USDA-APHIS from 2009-2016, combined with crop data from National Agricultural Statistics Service, and rainfall and temperature forecast survey from the National Oceanic and Atmospheric Administration. Geocoded data on honey bee health outcomes will be matched with data on the acreage of sunflowers grown within the honey bee foraging radius of these apiaries. We will also extract temperature and precipitation data associated with the month prior to the date the sample was collected.

Obj 3B: Bombus and Apis (Summer Year 3; Adler, Irwin, McFrederick). We will deploy commercial colonies of bumble bees (*B. impatiens*) and honey bees at commercial farms (MA) and agricultural stations (NC) to test how varying acreage of sunflower affects pathogen acquisition and colony health. In MA, we will use 22 commercial farms in Hampshire County that are a mix of conventional and organic small-scale farms. In NC we will experimentally plant sunflower along an acreage gradient, using 17 experimental agricultural stations. At each farm in each region, we will deploy three *B. impatiens* and three *Apis* colonies ($n = 66$ colonies in MA and 51 colonies in NC per species). We will place colonies into the field in early to mid June, when workers are naturally starting to forage. Colonies will be initially controlled for number of workers and brood and will be tested to confirm disease absence using PCR, as in Obj 2.

Floral resources. We will estimate floral resources at each farm every other week. We will also use GIS to quantify landscape types (farm, suburban, forest, old field, other) around each farm up to 10 km from the center point, and will estimate floral resources in each landscape type. We will assess Colony foraging, Parasitism status and Colony health every other week as in the methods for Obj 2, using molecular analysis for *Bombus* and visual assessments for *Apis*.