

# Nutrition Management for European Foulbrood (EFB) Recovery in Honey Bees

## Final report for GNC18-268

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Grant Recipient: Michigan State University

Region: North Central

State: Michigan

Graduate Student:

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Faculty Advisor:

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Dr.

## Project Information

### Summary:

Michigan is the second most diverse agricultural state, supported by commercial beekeepers who provide crop pollination. However diseases such as European foulbrood (EFB) threaten the wellbeing honey bee colonies and the beekeeping industry in Michigan and other regions. EFB is a bacterial disease that affects the larvae, often killing them, which can ultimately lead to colony mortality. EFB can also negatively affect colony size, which has economic implications for beekeepers through depressed pollination contract prices and honey production. While the link between EFB and nutrition has not been well established in a field-realistic setting, understanding it is an essential step in making management recommendations for beekeepers. Therefore, this study will evaluate the relationship between EFB recovery rates and nutrition to determine best management practices for mitigating these effects. To do so, it will analyze the effect of supplemental feeding management and probiotic use, as compared to traditional in-hive management on EFB recovery speed. This will be achieved by tracking the recovery rates of infected hives under six different in-hive treatments, including a control (untreated), antibiotics (traditional), antibiotics and a plant-based supplement, plant-based supplement, pollen-based supplement, and probiotics. This research will be conducted in Michigan during the summer feeding period immediately following blueberry pollination. Results will provide beekeepers with best management practices to optimize colony recovery and advice on the necessity of supplemental feeding. To evaluate the adoption potential of the management suggestions made through extension to beekeepers, I will administer surveys to assess change in knowledge, awareness and attitudes. Additionally, I will assess intended change in management as a result of extension. Finding treatment alternatives to antibiotics

could be more environmentally sustainable and affordable for beekeepers. Furthermore, because the FDA began mandating veterinary prescriptions for bee antibiotics in 2017, this is a particularly relevant issue. Additionally, disease pressure on the beekeeping industry will have broader implications on national pollination services and the food system. Not could EFB cause social and economic tension between growers and beekeepers, as decreased pollination availability increases demand and price, but it could also affect food quality and price for society as a whole.

#### Project Objectives:

I will communicate with beekeepers through meetings, articles, extension, and email newsletters to increase knowledge related to EFB treatment, including A) resources to accurately diagnose EFB, B) EFB management costs (economic and environmental), and C) nutrition and supplemental feeding effects on EFB. This information is expected to facilitate changes in skills and attitudes for nutrition-based management, initiating two main action outcomes. First, beekeepers will more accurately field-diagnose EFB, decreasing unnecessary, costly management. Second, beekeepers will practice evidence-based in-hive management practices to treat EFB.

Understanding the economic and social inter-dependence of the beekeeper-grower relationship is essential to maintaining reliable stocks of colonies and stable pollination contract services and prices. Through extension presentations and resources, blueberry growers will gain a better appreciation for the economic and environmental consequences of their support of honey bees. Growers adopting stress-reduction practices may reduce the incidence and/or severity of EFB. Supportive practices include night spraying, planting flowering strips, and carefully selecting hive drop sites. As a result of this awareness, growers may alter their practices to support the beekeeping industry on which they rely.

## Cooperators

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## Research

#### Materials and methods:

We tested colony recovery speed from EFB in groups of colonies under six different in-hive treatments, (1) antibiotics (Terramycin), (2) antibiotics (Terramycin) with a

plant-based pollen patty (Bee-Pro), (3) a plant-based pollen patty alone (Bee-Pro), (4) a 15% pollen-based patty (Global Patties), (5) probiotics (SuperDFM), and (6) a no-treatment control in which the colony was opened but nothing was added each time. Antibiotics are the conventional form of treatment for European foulbrood. There are multiple potential limitations associated with using antibiotics. Antibiotic use in food producing animals, including honey bees, now requires a veterinary feed directive for (FDA, HSS 2015). Antibiotic use also limits when honey may be harvested (Bargańska et al. 2011). Finally, while it has antibiotic resistance has not developed for EFB (Waite et al. 2003), it has developed in another larval bacterial disease (Evans 2003). As more sustainable alternatives, we tested protein supplements, including a plant-based and pollen-based patty, which we hypothesized would enhance recovery speed by reducing nutrition stress (Bailey 1961) and boosting immunity (Alaux Cédric et al. 2010). Based on beekeeper feedback we also tested probiotics. Probiotics have been shown to increase larval immune response (Evans and Lopez 2004) and decrease larval pathogenic bacterial levels (Daisley et al. 2019).

Each treatment was replicated in 10 colonies. These replicates were split between four different apiaries. Two apiaries which each contained 72 colonies were managed by one beekeeper and had four treatment replicates each. These apiaries were in Allegan and Kalamazoo Counties, Michigan, approximately 11.5 km from each other. Approximately 40 km south in Cass and St. Joseph Counties, Michigan were the other two apiaries which were approximately 38 km from each other. These other two apiaries were managed by a different beekeeper and each contained approximately 30 colonies. Each of these apiaries had a single replicate of each treatment. Both collaborating commercial beekeepers pollinate on high bush blueberry (*Vaccinium corymbosum*) in May, then moved bees into holding yards from June through July 2019. We worked with these commercial beekeepers because in recent years commercial beekeepers across North America have reported high incidence of EFB in colonies which service blueberry pollination contracts (Dufour et al. 2020). In our system in 2019 we observed infection in over half of commercial colonies coming off blueberry pollination (Milbrath, unpublished).

Upon colonies arriving in holding yards, each colony was inspected for visually detectable signs of EFB. Signs of EFB included 3<sup>rd</sup>-5<sup>th</sup> instar larvae which showed corkscrewing, yellowing, melting, exposed trachea, holes in the cappings and scaling (Bailey 1961, Forsgren 2010). Colonies were rated on an ordinal scale based upon number of cells exhibiting signs of disease. Colonies with greater than 100 diseased cells received a 3, 11-100 diseased cells received a 2, 1-10 diseased cells received a 1, and no diseased cells received a 0. This ranking system was chosen to be consistent with research being conducted by other researchers across North America. Diagnosis of infected colonies was confirmed by sending sampled larvae to the USDA-ARS Beltsville Laboratory, where the presence of *Melissococcus plutonius* was confirmed by with light microscopy. Colonies were also inspected for potentially confounding effect including the absence of a laying queen and high *Varroa destructor* mite levels. *Varroa* abundance was estimated using an alcohol wash (Fries et al., 1991). All enrolled colonies had an EFB severity rating of at least 2. Within apiary, colonies were blocked by severity into each of the six treatments. Treatments were applied per label. Antibiotics were applied every four to five days for a total of three treatments. Probiotics were applied once, per label, except for in the single-treatment yards, where they were applied upon enrollment, then again five days later, due to a miscommunication of methods. All patty-supplemented colonies were maintained at 0.9 kg per colony at each visit.

Colony inspections occurred every two weeks from enrollment to six weeks post-enrollment to track recovery speed. At each inspection, EFB infection severity was recorded as well as colony survival and queen status. At enrollment and at the six-week follow up, number of frames of adult bees were also recorded (Nasr et al., 1990). At enrollment before treatment, and at the first post-treatment inspection (four-weeks post enrollment), a sample of six nurse bees was obtained by shaking a frame of brood vigorously and scooping bees which remained on the frame. Nurse bees were immediately put on dry ice, then transferred to  $-80^{\circ}\text{C}$  until processing. Nurse bee heads were weighed, and fat body size was quantified using the ether wash method, as described in Wilson-Rich 2008 (David et al., 1975; Doums et al., 2002; Wilson-Rich et al., 2008). Nurse bee condition can serve as early indication of colony health and help to elucidate the mechanism through which different treatments are affecting colonies (Forsgren et al. 2005; Roetschi et al. 2008).

All statistical analyses were completed in R-studio version 3.5.2 (R Core Team, 2018). To determine differences in treatment group infection severity, analysis of variance (ANOVA) using general cumulative link models (CLM) was done using the ordinal package (Christensen 2019), followed by Tukey's pair-wise comparisons using the emmeans package (Lenth et al. 2020). Within each round, treatments were compared for EFB status, with apiary, nested within beekeeper as independent variables. Treatment differences in colony growth in frames of adult bees was analyzed using ANOVA on a linear model, with treatment and apiary nested within beekeeper as independent variables (R Core Team 2020). To determine differences among treatments in nurse bee head and fat body weight change pre- and post-treatment, we likewise used ANOVA with treatment and colony nested within apiary, nested within beekeeper as independent variables.

## Research results and discussion:

Initial EFB severity was not different among treatments upon enrollment, due to appropriate blocking ( $\chi^2_5=4.22$ ,  $p=0.52$ ). Two-weeks post enrollment, after all treatment applications, colonies which were treated with antibiotics, including both the antibiotic only treatment and the antibiotic with soy-patty treatment, had greater recovery than those which were not treated with antibiotics ( $\chi^2_5=25.38$ ,  $p<0.01$ ). Among the non-antibiotic treated colonies, 69% had a severity rating of 2 or 3, and only 5% had completely recovered, while 75% of the antibiotic treated colonies had completely recovered. By four weeks post-enrollment, which was two-weeks after treatment had concluded, 64% of all colonies showing no signs of disease. There were no treatment differences among the colonies ( $\chi^2_4=6.49$ ,  $p=0.17$ ), but the the antibiotic-only treatment could not be statistically compared because all colonies recovered. One-month post-enrollment there were once again treatment differences ( $\chi^2_5=14.22$ ,  $p=0.01$ ). All colonies besides those in the pollen-based patties treatment had a rating between 0 and 1. In contrast, 60% of the pollen-based patty group had a severity rating of 2, and had significantly more severe signs of EFB as a group than either the control ( $\beta=3.57$ ,  $z=2.98$ ,  $p=0.03$ ) or antibiotic-only ( $\beta=3.46$ ,  $z=3.00$ ,  $p=0.03$ ) treatment groups.

There was no difference in colony growth between the different treatments ( $F_{5,49}=1.86$ ,  $p=0.12$ ).

Nurse bee fat body size was not different among treatments pre-treatment ( $F_{5,43}=0.75$ ,  $p=0.59$ ) or post-treatment ( $F_{5,49}=0.99$ ,  $p=0.43$ ). Nurse bee head weight was also not different among treatments pre-treatment ( $F_{5,38}=0.62$ ,  $p=0.68$ ) or post-

treatment ( $F_{5,31}=0.77$ ,  $p=0.58$ ).

Post treatment course, colonies treated with antibiotics recovered more quickly than treatment alternatives. This finding demonstrates the continued effectiveness of antibiotics. While colonies in other treatments eventually showed similar recovery to antibiotic-treated colonies, it is unclear from this short window how long the colonies were sick before intervention, and if colonies would maintain recovery in the long term. Indeed, pollen-patty fed colonies experienced a resurgence of EFB severity. These findings do not suggest that supplemental feeding or probiotics should be used as treatment alternatives. They are costly implements that our data do not support as effective treatment options. The mechanism associated with recovery in these colonies is also unclear, given there were no differences in nurse bee physiology. There was large variability among the colonies within treatments, therefore additional studies, with larger sample size are still needed to investigate alternative treatments for EFB.

### **Participation Summary**

**3** Farmers participating in research

## Educational & Outreach Activities

**1** Curricula, factsheets or educational tools

**1** Online trainings

**4** Webinars / talks / presentations

### **PARTICIPATION SUMMARY:**

**200** Farmers

**100** Ag professionals participated

Education/outreach description:

To date, an educational curriculum has been developed on European foulbrood disease background, in-hive identification, diagnosis support, prevention and sustainable treatment options, based upon the findings of the NC-SARE grant. This curriculum has been presented to the Nebraska Beekeepers' Association through a webinar, which included 30 beekeeper attendees. In addition, this curriculum was presented to Michigan beekeepers at the Michigan Beekeeper's Association annual spring conference and to the Southeastern Michigan Beekeepers Association through an online webinar. These club meetings primarily reach hobbyist and beginner beekeepers.

Additionally, findings from this project have been presented to growers and commercial beekeepers at the Great Lakes EXPO in Grand Rapids, MI at the blueberry session. This is a grower-focused show, with 3,600+ attendees. Findings were also presented to bee researchers and beekeepers (commercial, sideliners and hobbyists) at the joint American Bee Research Conference and American Beekeeping Federation Annual Conference in Schaumburg, IL. These data were also presented at an entomology departmental seminar in Pennsylvania State

University.

Considerations from this research have been included in the Blueberry Pollinator Stewardship Plan, which provides best management suggestions to blueberry growers on how to support healthy pollinators on their farms. I also plan to write an article for the American Bee Journal on these research findings as well as a journal article for a scientific audience.

## Project Outcomes

**2** Farmers reporting change in knowledge, attitudes, skills and/or awareness

**1** Farmers changed or adopted a practice

**3** New working collaborations

Project outcomes:

My project has found support for and communicated the importance of judicious use of antibiotics for honey bee disease management. Based upon my research findings, I offer tools for accurate diagnosis and advocate for evidence-based methods to treat cases of European foulbrood. Responsible antibiotic use contributes to sustainability by preventing antibiotic resistance, which has not yet developed in European foulbrood (Waite et al. 2003), preventing antibiotic residues in hive food products (Bargańska et al. 2011), and preventing negative health consequences to adult bees (Li et al. 2019). Healthier adult bee populations and harvestable honey also provides economic benefits to beekeepers in the form of income. Likewise, using evidence-based, effective treatments saves beekeepers money by removing the need for investment in ineffective in-hive products as treatments. European foulbrood is a spatially- distributed disease (Belloy et al. 2007), so providing beekeepers with the tools for effective treatment can also provide environmental benefits by decreasing inter-colony transmission. Responsible beekeeping in turn provides social benefits between neighboring beekeepers. Additional social benefits include enhancing mutual understanding between beekeepers and the blueberry growers with whom they contract. Honey bee health stressors, such as European foulbrood, may decrease colony supply and increase beekeeper inputs, forcing beekeepers to charge more for pollination services. This would lead to negative economic consequences for growers. Therefore, both groups are stakeholders in sustainable treatment of European foulbrood. My project supports beekeeper decision through field evidence and communicates these findings to both beekeepers and growers, bolstering future sustainability including economic, environmental and social benefits. Indeed, personal communications and feedback from beekeepers have indicated their new knowledge, skills and intended action changes as a result of my outreach for this project.

Knowledge Gained:

This experience has increased my interest in sustainable agriculture research in order to find more sustainable alternatives to conventional methods. Growers and beekeepers have been very interested in this research due to its applications. Collaborating with and answering questions that serve beekeepers and growers has given me a sense of fulfillment, knowing my research will have direct implications for people and the environment. These collaborations also offer valuable insights

and meaningful feedback, which have enhanced my own knowledge, experience and skills. In particular, through this project I gained better communication skills by having the opportunity to communicate research to a new audience. My awareness of the importance of pairing education with sustainability research has increased as a result of this grant experience. It has made me seek more outreach opportunities and consider implications of my other research. Overall, this experience has enhanced my knowledge of treatment-free beekeeping, reinforced my attitude and awareness of the importance of researching sustainable agriculture practices, and given me new skills in sustainable agriculture research communication.

Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the U.S. Department of Agriculture or SARE.



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