

**Northeastern IPM Center – IPM Partnership Grants – 2009 – Proposal
Statement of Work (SNPs and Minigrants) or Project Description (other Project Types)**

PD: Dr. Douglas W. Dingman

Project Title: Testing the Effectiveness of a Sanitizing Agent for Suppression of American Foulbrood in Beehives

Introduction/General Background:

Honeybee colony health has become an important issue as a result of the alarming reports on honeybee colony losses caused by colony collapse disorder. Many factors can contribute to a loss in colony health. Some factors are easily observed, while other factors negatively impact colony health “behind the scenes.” Although these unseen factors generally do not cause catastrophic harm to the colony, they serve to suppress the health of the colony, keep the colony struggling for vigor, and predispose it to other biotic or abiotic factors.

American foulbrood (AFB), caused by the endospore-forming bacterium *Paenibacillus larvae*, is considered an easily observed factor that can devastate colony health and result in the complete loss of the colony. Many beekeepers routinely use terramycin as a prophylactic antibiotic treatment to prevent AFB. Lack of an observable symptom of the disease is considered successful prevention. However, AFB can propagate within a hive at the level of an unseen factor (i.e., a sub-clinical infection).

In California, a 2003 survey of 570 hives demonstrated that 33.3% of the hives contained *P. larvae* spores. Also, this survey showed that 69-80% of Midwestern and Western U. S. beekeepers had at least one colony containing *P. larvae* spores (Eischen, *et al.*, 2005). A survey conducted in Connecticut, started October 2007 and continued in 2008, identified 5 out of 51 apiaries (9.8%) as containing hives with observable AFB symptoms. However, testing for sub-clinical AFB by microbiological culturing identified 19 additional apiaries with infections. This finding (47% of Connecticut apiaries positive for *P. larvae* spores) is in agreement with the 2003 California survey.

These findings suggest that the overall vigor of many honeybee colonies is suppressed due to stress caused by a continual low level of infection. Additionally, because switching of hive bodies and frames between colonies (within the same apiary or between different apiaries) is common beekeeping practice, disease spread and the potential for AFB outbreaks is significant. Current antibiotic therapy appears to be mitigating this disease, at present. However, a 2005 report on *P. larvae* isolates from Midwestern and Western U.S. beehives demonstrated that 27% of the isolates were resistant to terramycin (Cox, *et al.*, 2005). Continued prophylactic use of terramycin will likely increase the percentage of resistance and require a change to the next antibiotic, tylosin. Because *P. larvae* is present in such a large number of beehives, the loss of AFB control through development of antibiotic resistances by *P. larvae* would devastate beekeeping. Therefore, continued suppression of AFB infections for bee colony health is important and affective sanitation regimes are a key to this strategy.

The mandatory method for control of diagnosed cases of AFB in Connecticut is destruction by burning. This policy extends throughout the United States and to many other countries. The loss of equipment and bees plus the necessary replacement expenses make such control measures extremely costly. Some equipment can be salvaged via scorching the inside of hive boxes and bottom boards or via boiling equipment in lye (Brødsgaard and Hansen, 1999). In addition, irradiation can sterilize contaminated equipment. Still, a sanitation protocol that is

effective, easy, and convenient to perform routinely will be of significant benefit to beekeepers. Routine sanitation will improve colony health by suppressing or removing sub-clinical AFB infections, will lower or eliminate the use of antibiotics, and will provide beekeepers an easy way to salvage spore-contaminated equipment.

Previous Findings on Efficacy of Sanitizing Agent:

Laboratory investigations, using a hydrogen peroxide/peroxyacetic acid liquid sanitizer, have shown the sanitizer to effectively inactivate *P. larvae* spores in solution (unpublished data; Fig. 1). The sanitizer was also demonstrated effective at spore inactivation using simulated beehive conditions (beeswax coated wooden strips)(data not shown). These findings indicated that this sanitizer will most likely be successful at decontaminating spore-laden beekeeping equipment. However, field trials using AFB contaminated beehives are necessary to verify practicable use of this agent and to develop a standard protocol for routine usage on beehives and other beekeeping equipment.

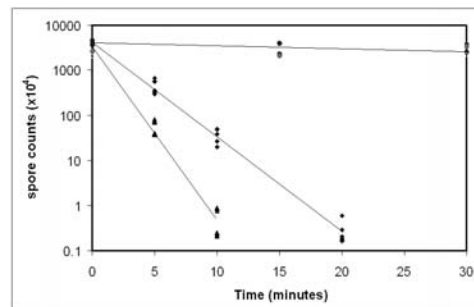


Fig. 1. Endospore inactivation profile. Spore counts following exposure to water (control, ●) or diluted sanitizing agent (1:1000, ◆; 1:500, ▲).

Objective:

This investigation will ascertain the efficacy of a liquid sanitizing agent to lower *P. larvae* spore levels in adult bees taken from colonies with AFB. Specific goals are:

- Establish a base-line level of *P. larvae* spores in hives infected with AFB.
- Remove bees and beeswax from infected hives, sanitize contaminated hive bodies and frames, replace adult bees into sanitized hives, and monitor for *P. larvae* spore levels.
- If sanitation proves effective, modify experimental design into a standard protocol that beekeepers can routinely use to sanitize beehives.

Project Description:

Assessment of *P. larvae* spore levels in AFB infected beehives. Three or four Connecticut beekeepers with sub-clinical AFB in their beehives will be recruited for participation in this study. For each beekeeper, infected beehives will be monitored biweekly for *P. larvae* spore levels for one month. Spore levels will be measured in adult honeybees collected from the brood chamber. Maceration of bees in sterile water, enrichment for bacterial spores by heating, and dilution onto selective media (Alippi, 1995; Lindström and Fries, 2005) will be used to obtain a spore level per bee in each hive.

Sanitation of beehives. Honeybee queens in each hive will be identified and caged for removal. All adult bees, removed from beehives using the shake method (Hansen and Brødsgaard, 2003), will be held in screened boxes for two days to rid adult bees of spores. Each empty beehive will be disassembled and wax will be removed from surfaces of frames and hive bodies by scraping. All beehive parts will be soaked in sanitizer for 20 min, rinsed, and allowed to air dry. For controls, three beehives will remain untreated throughout the experiment and three hives will undergo the sanitation process without being soaked in sanitizer.

Introduction of bees to cleansed beehives. Beehives will be assembled in according to original parts and the shaken bees will be introduced into their original hives. Adult honeybees will be monitored for *P. larvae* spores before addition and after addition to cleansed hives. Spore levels for all colonies will be measured bimonthly until late fall and in early spring of the following year. Comparison of pre-treatment and post-treatment spore levels over time will dictate the efficacy of this sanitizing agent and protocol to cleanse beehives of AFB.

Develop method for beekeepers. Using commercial and hobbyist beekeeper input, the sanitation protocol will be modified to best integrate into beekeeper practices. Several new beekeepers will be recruited to test the modified protocol. Observations during, and oral feedback from the beekeepers after, sanitation of beehives will be used to improve the protocol. This outreach and co-participation is vital for developing a community-based protocol that will be workable and result in a high level of compliance.

Overview of Project:

Funding Request. The need for a sanitizing protocol to improve colony health is immediate. These funds are needed to purchase supplies necessary for investigating the effectiveness of a sanitizing agent and to partly cover technician salary and wages for time spent assisting this research.

Time Line. This study will require a full beekeeping season beginning in April. Two months are needed to recruit beekeepers and to monitor spore levels. To complete hive sanitation procedures and replace the bees will require another two months. Monitoring of spore levels, post-treatment will cover the rest of the season and into next year.

Impact. This investigation has a high prospect for success and the impact to the beekeeping community (local, national, and worldwide) will be significant and immediate. To promote this influence, research findings will be published in a scientific journal (focused on honeybee research) and as summarized notes in beekeeping trade journals. Additionally, oral presentations will be given to beekeeper groups. Development of this sanitizing protocol will change beekeeping practices, save beekeepers unnecessary expenses, help suppress AFB with less use of antibiotics, and help to improve long-term colony health.

References

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