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UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

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PROPOSAL TYPE

For National Research Initiative Competitive Grants Program Proposals Only

[ ] Standard Research Proposal
[ ] Conference
[ ] AREA Award
  [ ] Postdoctoral
  [ ] New Investigator
  Strengthening:
  [ ] Career Enhancement
  [ ] Equipment
  [ ] Seed Grant
  [ ] Standard Strengthening

For Higher Education Program Proposals Only:

Need Area: _____

Discipline: _____

Project Title: **Colony Collapse Disorder Monitoring and Surveillance Sample Collection**

Key Words: Colony collapse Disorder, honey bees, Disease Surveillance, sample collection

(Approximately 250 words) **PROJECT SUMMARY**

In Fall 2006, beekeepers reported extensive losses of colonies across the nation, without recognizable underlying causes. This phenomenon has been named "Colony Collapse Disorder (CCD)". An estimated 23% of the beekeepers in the nation have been affected by this condition, and those affected have lost on average 57% of their colonies between September 2006 and March 2007. These losses threaten the pollination industry and production of commercial honey in the United States.

We received grant monies from the National Honey Board to conduct CCD monitoring and surveillance work; however, at the time of the original NHB grant submission, we believed that honey (which is easily collected and requires no specialized shipping and handling) would be a good indicator of colony pathogen load. Our subsequent work with CCD bees has shown this not to be the case. To properly monitor colonies for viruses and other pathogens, we need to obtain bees that are collected and shipped on dry ice, which requires more frequent site visitation than originally envisioned (or budgeted). In addition, we have partnered with Jeff Pettis at USDA-ARS to test comb sterilization and monitor stresses associated with colony movement; this has resulted in an increased number of tested colonies, from a budgeted 16 to 200 colonies. While USDA-ARS has and continues to contribute monies to this project, we require additional funds both to collect and ship samples from the field. These emergency funds will enable collection of essential samples and aid in finding the cause and prevention of CCD.

NEIPM mini- emergency grant application

Travel funds for Colony Collapse Disorder monitoring and surveillance sample collection.

Co-PI's
Dennis vanEngelsdorp, Senior extension Associate, Penn State University
Diana Cox-Foster, Professor, Department of Entomology, Penn State University

In the fall of 2006, numerous migratory beekeepers reported extensive losses of colonies across the nation, without recognizable underlying causes. This phenomenon was initially termed "fall-dwindle disease" and had since been renamed "Colony Collapse Disorder (CCD)". CCD is a serious threat to the pollination industry and production of commercial honey in the United States.

Members of the CCD working group have been successful in securing $45,832 of funding from the National Honey Board to (see abridged grant attached):

1) Test various comb treatment strategies/ chemotherapy options to prevent CCD.
2) Beta test a multiple commercial-operation, disease-agent surveillance and detection program and establish baseline information on disease incidence as related to stresses associated with commercial operations.

This grant application seeks funds to build on these already secured funds. There are several reasons additional funding is needed and the NEIPM grant monies would enable greater combined success in this research. These two studies in combination should provide essential information to both commercial and sideliner beekeepers and help to improve the overall health of honey bee colonies. Results and recommendations from these studies will be shared with beekeepers through publications and presentations to bee keeping groups. The results of this study could decrease the amount of chemical controls being implemented by bee keepers to control diseases and pathogens. These results should enable greater colony survival and thus benefit fruit and vegetable growers by ensuring more stable pollination services.

At the time of the original NHB grant submission we believed that honey, which is easily collected by beekeepers and requires no specialized shipping and handling, would be a good indicator of colony virus load. Our subsequent work has shown this not to be the case in CCD colonies where old equipment from dead out colonies is being reused (see appendix). In order to properly monitor colonies for viruses and other pathogens, we need to sample bees that are collected and shipped on dry ice, which requires more frequent site visitation than originally envisioned (or budgeted). Additionally, the USDA-ARS honey bee lab in Beltsville Maryland (Jeff Pettis as primary PI) has built a partnership with us and this has resulted in an increased number of colonies being tested for comb sterilization. As a result, the number of colonies included in the study has grown from a budgeted 16 to 200 colonies. While USDA-ARS has contributed and continues to contribute the monies need for colony rental and upkeep, as well as some of the labor

needed for sample analysis, we require additional funds both to collect and ship samples from the field.

Colonies established in equipment from CCD dead outs (either untreated or treated with gamma radiation) have been established, with baseline samples collected for pathogen analysis. These colonies have been placed into three migratory commercial beekeeping operations and will be closely monitored. All aspects of the bee keeping practices will be monitored, including travel, crops being visited (and history of chemical treatments on those crops), chemical treatments of the hives for parasitic mite and small hive beetle controls, splitting of colonies, and supplemental feeding. In addition, climate stresses will also be assessed by monitoring temperature and humidity during travel and open pollination. These parameters will be used in analyses to determine if there is a link with pathogen and parasite incidence in the colonies.

We seek emergency funding to complement existing NHB funding in order to:

1) Increase the number of field visitation to sample colonies
2) Purchase and ship the additional sample materials needed to accommodate the increased size of experimental colonies resulting from collaboration with the USDA-ARS.

Funding request:

Travel needs: $ 5000
Shipping costs: $1400

Title: Treatment and monitoring regimes to insure colony vigor and prevent fall dwindle disease.

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Study Duration: March 2007 until March 2008.

Rationale:

In Fall, 2006, numerous migratory beekeepers reported extensive losses of colonies across the nation, without recognizable underlying causes. This phenomenon has been termed: "fall-dwindle disease', and threatens the pollination industry and production of commercial honey in the United States. In this proposal, we will test treatment regimes that prevent the onset of "fall dwindle disease", a significant yet poorly understood problem for beekeepers.

Our recent and ongoing collaborative investigations have uncovered high rates of microbial infection in colonies suffering from fall dwindle disease (See Appendix 1). At present, the exact agent(s) of infection has not been fully identified. However, we believe that the organisms responsible for infection are opportunistic microbial diseases that kill colonies whose immune systems have been compromised by other stressors. In the proposed studies, we will identify the potential stressors that facilitate the onset of fall dwindle disease and determine management practices to reduce the spread of the causative agent of the disease.

There are several factors that can contribute to colony stress, including parasite load (particularly varroa mites and the many viruses vectored by them), nutritional stress, and pathogen/chemical build-up in brood comb. Through interviews with some of the beekeepers (# interviewed/estimated # suffering losses) who suffered major losses this past fall, case histories suggest in most cases that varroa mite populations were maintained at levels below economic injury levels, and so varroa infestations cannot fully explain colony collapse. Our inability to identify viruses in the collapsed colony food stores also suggests that colony losses were not due to viruses commonly associated with colony death. Common themes in the operations that suffered losses were 1) a high rate

of colony "dead outs" over the summer, 2) the practice of placing equipment from "dead outs" on strong colonies to facilitate split production, and 3) some form of nutritional stress, either induced by a poor nectar flow or by moving bees to and from crops for commercial pollination. We therefore propose to test various strategies that minimize the likelihood of opportunistic infection from the putative causal microbial agents responsible for fall dwindle disease and the associated colony failure.

Objectives:

This multi-facetted proposal has three major objectives:

1) Test various comb treatment strategies/ chemotherapy options to prevent fall dwindle disease.
2) Beta test a multiple commercial operation disease agent surveillance and detection program

Methods:

Objective 1: Comb treatment strategies: the effectiveness of comb irradiation, acetic acid fumigation, and Fumagillan feeding on colony health.

One apiary of 16 colonies will be established. The equipment used to establish these colonies will originate from donated frames from fall 2006 dead out colonies (thus increasing the likelihood that the equipment will be contaminated with the causative agent of fall dwindle disease). Before colonies are established, the equipment will be divided into four treatment groups.

1) No treatment
2) Combs irradiated prior to package introduction
3) Combs fumigated with Acetic Acid prior to package introduction
4) Colonies fed the antibiotic Fumagillan during the summer dearth

Colonies will be established from three-pound packages following standard practices, and they will be sampled monthly for the prevalence of multiple parasites (including varroa mites, honey bee tracheal mites, nosema load, and flagellates). Moreover, we will measure several immune response indicators (e.g., melanization of sting gland [see Figure 6, Appendix 1] and pyloris [Figure 4]), pathogen load in honey stores, and viral infection of queens as determined by egg sampling. Colony size, both at the beginning and end of the experiment, will be quantified to determine the relationships between these measures of disease and colony productivity.

Irradiation should kill any pathogen or parasite in the equipment and remove any microbial agent underlying the colony collapses. Fumagillan (registered for use in honeybees for nosema control) has been reported to also control amoeba disease in other animals and may also be effective against fungal diseases given that nosema is related to fungi.

Objective 3: Beta test a large-scale honey bee disease surveillance and detection program.

In interviews of the beekeepers experiencing fall dwindle, all reported that prior to colony collapse the colonies looked strong and healthy. In terms of their operational practices, all these beekeepers were actively involved in producing splits, either to replace their

own losses or to increase the size of their operation. The act of splitting colonies is known to amplify infections of microbial diseases (e.g., nosema and amoeba disease) by

a) Transferring potential contaminated brood frames
b) Disrupting the age profile of a colony's worker bee population (as a result of lag time after queen introduction), causing a unnaturally high rate of contact between newly emerged and older (infected) bees
c) Crushing bees while manipulating frames. If these crushed bees are infected with a disease agent it, can be transferred to house cleaning bees attempting to remove them from the nest.

Beekeepers would benefit if low levels of disease could be detected prior to engaging in techniques that amplify infection and this would allow for preventive measures to be taken. In this beta-test program, up to 5 co-operating beekeepers will take ¼ cup of bees from 100 sentinel colonies in their operation every time they open the colony's brood nest. Additionally, 50 ml samples of honey will be collected at different times from a sub-group of 20 colonies out of these 100 sentinels. Honey samples will be analyzed for disease loads (see Objective 1), and bees from those colonies will have their patriline diversity quantified and monitored using standard molecular paternity analyses. Potentially decreased genetic diversity in workers could underlie increased disease susceptibility.

To protect the privacy and ensure better disclosure of management practices, the name and revealing details of individual operations will remain confidential. Efforts will focus on developing sample handling protocols, developing high-throughput sample analysis, and communicating results back to beekeepers within a meaningful time frame.

Potential outcomes:

1. Identify cost effective practices that reduce parasite load in brood frames, thus reducing colony stress and susceptibility/exposure to the opportunistic infections thought responsible for fall dwindle disease.
2. Develop a disease-monitoring lab, which could process beekeeper samples. This service is envisioned to become a pay-for-use, self-sustaining service.

Publications and Presentations:

We intend to present findings at the joint AHPA, ABF, AIA, and AAPA meeting to be held in January 2008. Results will also be published in a scientific peer reviewed journal (such as the *Journal of Economic Entomology*) followed by an *American Bee Journal* general-interest summary article.

Calender of Activities:

Objective	Month of Activity	Activity

1. Comb Treatment	March 2007	Equipment and apiary secured
	April 2007	Apiary establishment
		Initial sample collection
	May 2007	Sample collection and processing
	June 2007	Sample collection and processing
	July 2007	Sample collection and processing
	August 2007	Sample collection and processing
		Fumagillan treatment
	September 2007	Sample collection and processing
	October 2007	Sample collection and processing
	November 2007	Sample processing
	December 2007	Sample collection and processing
	Jan – March 2008	Publication preparation
2. Surveillance	March 2007	Co-operators enlisted and trained
		Sentinel colonies identified and surveyed (in Florida)
		Sample collection
	April 2007	Co-operators interviewed
		Sample processing/report generation
	May 2007	Co-operators interviewed
		Potential sample collection
		Sample processing/report generation
	June 2007	Co-operators interviewed
		Sentinel colonies surveyed (in Maine/New Jersey)
		Sample processing/report generation
	July 2007	Co-operators interviewed
		Sample processing/report generation
	August 2007	Co-operators interviewed
		Sentinel colonies surveyed (in New York/Pennsylvania)
		Sample processing/report generation
	September 2007	Co-operators interviewed
		Sentinel colonies surveyed (in Maine/New Jersey)
		Sample processing/report generation
	October 2007	Co-operators interviewed
		Sentinel colonies surveyed (in Florida)
		Sample processing/report generation
	November 2007	Co-operators interviewed
		Sentinel colonies surveyed (in Florida)
		Sample processing/report generation
	December 2007	Co-operators interviewed
		Sample processing/report generation

	Jan – March 2008	Co-operators interviewed
		Sentinel colonies surveyed
		Sample processing/report generation

Responsibilities:

Dennis vanEngelsdorp:	Project co-ordination
	Apiary set up and sample collection
	Varroa and parasite monitoring
Dr. Dianna Cox-Foster:	Virus processing, disease analysis
Dr. David Tarpy:	Protein analysis
	Patriline monitoring