FINAL PROJECT REPORT
WTFRC Project: CP-18-105

Project Title: Using cold storage to increase the stability of honey bee supply

PI:	Brandon Hopkins	Co-PI:	Walter Sheppard
Organization:	Washington State University	Organization:	Washington State University
Telephone:	509-335-0881	Telephone:	509-335-4142
Email:	bhopkins@wsu.edu	Email:	shepp@wsu.edu
Address:	Department of Entomology	Address:	Department of Entomology
Address 2:	PO Box 646382	Address 2:	PO Box 646382
City/State/Zip:	Pullman WA 99164-6382	City/State/Zip:	Pullman WA 99164

Cooperators: 2B Apiaries, Olson's Honey, Idaho Bee Storage

Other funding sources

Agency Name: Project Apis M
Amt awarded $60,000
Notes: Awarded becaue we have the capacity provided with the WTFRC to perfom indoor storage research on honey bee colonies. This project investigates queen storage practices using indoor storage

Agency Name: Google X
Amt awarded $35,000 + (~$150,000 in-kind for sensors)
Notes: Collaboration and sponsored research with Google's R&D company (X) began about the same time colonies were being prepared for this winter project. They wanted to help by proving sensors and in turn are getting some data about hive activity.

Agency Name: California Almond Board
Amt. awarded: $97,000
Notes: The award from the Almond Board was made possible by leveraging the money provided by the WTFRC to secure the remainder of the funding required to complete the construction of the research equipment needed for the research described in this proposal

Agency Name: USDA-NIFA
Amt. requested: $499,800
Notes: The proposal included research that is only possible because of the funding awarded by the WTFRC and the Almond Board of California. Provides funding for graduate students and bee research supplies to complement WTFRC funindg of the CA containers.

Organization Name: WSU **Contract Administrator:** Katy Roberts
Telephone: 509-335-2885 **Email:** arcgrants@wsu.edu

Item	2018	**2019**	2020
Salaries		15,290	18348
Benefits		2464	2956
Wages		6000	6000

Benefits		1560	1560
Goods and Services	100,000		
Shipping			
Supplies		3000	3000
Travel			
Plot Fees			
Miscellaneous			
Total	100,000	**28,314**	31,864

OBJECTIVES

Goals

The initial goal of this proposal is to construct three controlled atmosphere rooms capable of holding a significant number of honey bee colonies for winter and summer experiments. The second goal is to utilize the controlled atmosphere facilities to address the following objectives:

Objectives:

1. Optimize controlled atmosphere storage conditions (CO_2, humidity, temperature) to maximize Varroa mite mortality while producing healthier bees following the winter storage period.
2. Determine ideal timing and storage conditions for mid-season (summer) honey bee colony cold-storage to create a break in the brood cycle that allows beekeepers to more efficiently and effectively control Varroa.
3. Utilize the combined findings from the objectives above to develop a whole season IPM strategy for commercial tree fruit pollinators. Implement that strategy in collaboration with a commercial beekeeping operation to demonstrate the feasibility and economic benefit to the commercial beekeeping industry.
4. Leverage the new facilities to attain additional funding to expand the research/utility of indoor controlled as it applies to the stabilization of the beekeeping industry for the benefit of the tree fruit industry

SIGNIFICANT FINDINGS

Provide a bulleted list of significant findings during the prior year(s) of the project.

- Research on Objective 1 suffered a setback due to equipment failure in one chamber half way through the trial period last winter. The repeated experiment to determine if CO2 can be used to control Varroa will be completed in one week and there were no equipment failures this winter.

- Completed large body of research on Objective 2. We have a Masters student preparing her thesis on the work (graduating this Spring). In addition to the controlled experiments in our container; we completed an observational study with a commercial beekeeper in Idaho where we were able to force colonies into a broodless state and significantly improve varroa control in those colonies compared to colonies with capped brood (Fig 1).

- Produced a 1st edition of an indoor wintering "best management practices" booklet that is now available as an online resource with continual updates on this management practice. The link and document has been accessed more than 2000 times. The PDF version is currently being transferred to a more dynamic web resource with new material added quarterly

- With the combined funds from WTFRC and the Almond Board for the facilities, we were able to secure USDA-NIFA funding to expand the research associated with this project. We

also secured funding from Project Apis M to investigate queen banking, and Google X because of the research funding provided by WTFRC that provided the equipment to pursue this line of research.

- Were able to leverage funding provided by the WTFRC to secure funding from the Almond Board of California to get enough funds to move forward with the facilities needed. The time to get these funds and the time for design and planning with WSU facilities has pushed the timeline of the original grant back approximately 1 year.

Year 2- indoor wintering - 2019 – completed

October -132 honey bee colonies were assessed. The number of frames of bees and brood were recorded. Samples of bees were collected in alcohol to determine the initial Varroa mite load in each colony. Those sample were used to determine tracheal mite and nosema infection (two economically important pests besides Varroa mite). 120 colonies were selected for the winter research from the initial 160 colonies screened. Colonies with too many varroa mites or too few were excluded.

December 2019- Present : Procurment and placement of CA experimental chambers (rerefigerated cargo containers.
Triton 20ft refrigerated cargo containers were placed at the WSU Irrigated Research Farm near Othello, WA

Using information about the initial Varroa mite loads for each colony; colonies were assigned to one of two controlled atmosphere rooms and a set of 40 colonies remains outdoors for the winter as an additional control.
The colonies are distributed so that each group contains, on average, the same Varroa mite load. Both containers were set at 40°F with the manipulated variable being CO_2. One container suffered from catastrophic refrigeration failure. This failure forced us to end the trial just before the end of December. We ran the functional container through the rest of the winter storage period and learned that the containers (when functioning properly) are capable of safely storing honey bee colonies. We were not able to compare high and low CO2 levels on mite levels because of the equipment failure. The refrigeration unit was repaired and functioned properly through the summer trials and is currently operating normally (see below).

Year 3 – Indoor wintering – 2020 (underway)

October – The experimental set up from winter of 2019 was duplicated in year 3, to perform the research that was lost due to equipment failure. (see above)

Controlled atmosphere Mid-season brood break for enhanced Varroa control
Year 3- Summer brood break – 2020 (completed)

April -June – 160 honey bee colonies were assessed from two different commercial beekeeping colaborators. One set of hives was utilized after almond pollination and a second set of colonies was utilized after Apple pollination. The number of frames of bees and brood was recorded. Samples of bees collected in alcohol to determine the initial Varroa mite load and tracheal and nosema infection in each colony. Each colony was weighed before and after the trial period.

With information about the Varroa mite loads for each colony; colonies will be assigned to one of two controlled atmosphere rooms and a set of colonies remained outdoors for the 3-week trial as an additional control. The colonies will be distributed so that each group contains, on average, the same Varroa mite load. They were placed in the controlled atmosphere chambers for 18 days. One room set at 40°F and in complete darkness. The second room will be held at 50°F in complete darkness. At the end of the 18-day storage period the colonies were removed and placed outdoors where a miticide treatment will applied along with a fresh "sticky card". The number of mites gathered following the miticide treatment will be compared to the total number of mites gathered on all sticky cards to determine the Varroa mite mortality caused by the treatment period. All colonies were assessed again as they were at the start of the experiment and health, colony size, mite loads were compared. The colonies were assessed again before the end of the season while they were in North Dakota. Data analysis is still ongoing as part of a Masters students thesis. They are graduating in April 2021. The work is being prepared for peer review publication.

Year 4 - 2021

Using findings from the experiments described above we will engage with commercial beekeeper collaborator to follow and study 400 honey bee colonies using the CA overwintering facilities of our collaborator (Olson's Honey) in Yakima WA. We will assess all 400 as described in methods above during the period of almond pollination in California. All colonies will be given a numbered tag. At the end of the almond pollination season, all colonies will be treated with the most widely used registered commercial product for Varroa control (Apivar). Hives will again be assessed while colonies are in apple orchards during pollination. Any colony issues (queenless, bacterial or fungal disease) other than those caused by Varroa mite will be remedied and recorded. Nutritional issues and swarm control will be decided on by the beekeeper, consistent with his normal operating procedures. Colonies will be assessed again in the summer after canola seed pollination during which time the honey crop will be removed. After this assessment colonies will be divided into 4 treatment groups with the average Varroa load and colony strength equally distributed across all 4 treatment groups. Treatment group 1 (commercial standard control) will be treated with industry standard miticide treatment in conjunction with rest of the commercial operation and will be moved to California "holding yard" in November when the rest of the groups are moved to controlled atmosphere storage for the winter. Treatment group 2 will be treated the same as group 1 except that it will be placed in controlled atmosphere storage for the winter months before almond pollination begins. Treatment group 3 will be moved into a controlled atmosphere facility for 18 days to create a break in the brood cycle. After which it will be treated with a single application of miticide. Treatment 4 will remain outside isolated from the rest of the commercial operation and left untreated. All colonies will be assessed again in October as they are prepared for winter. Colony strength, Varroa loads and survival will be compared between treatment groups after the October sampling and again in January as they are prepared for placement in almond orchards.

RESULTS & DISCUSSION

The initial funding of this project allowed for the ability to acquire additional funding and planning to prepare for arrival of the new equipment. We utilized the funding provided by the WTRC to leverage the additional funds need for the controlled atmosphere chambers from the Almond Board of California ($100,000). The chambers will provide a wealth of research potential moving forward and the combined funding that provided these chambers allowed us to secure a USDA-NIFA funding that utilizes these chambers for honey bee research. All preliminary evidence suggests that this line of

research will provide valuable information for the commercial beekeeping industry to help keep more colonies alive and stabilize the supply of commercial pollinators.

We now have colonies inside the containers and will be moving the colonies from inside the containers directly to California where they will be assessed and the first winter's experiment will be completed. There are a series of exciting experiments that will go through the containers throughout the year and results from this winter will be used to design the experiments planned for summer and winter 2020/2021.

One of the comments/feedbacks we received about the concept of forcing period of broodlessness was that our preliminary work was done in August and many beekeepers are still producing honey at that time. We performed an additional observational study with a commercial beekeeper in southern Idaho who allowed us to follow a set of his colonies after almond pollination (April). We demonstrated the ability to stop brood rearing and significantly increase Varroa mite control. It is likely that the use of controlled atmosphere or refrigerated spaces to hold bees at times other than winter months could become the most significant management tool for increasing colony survival.

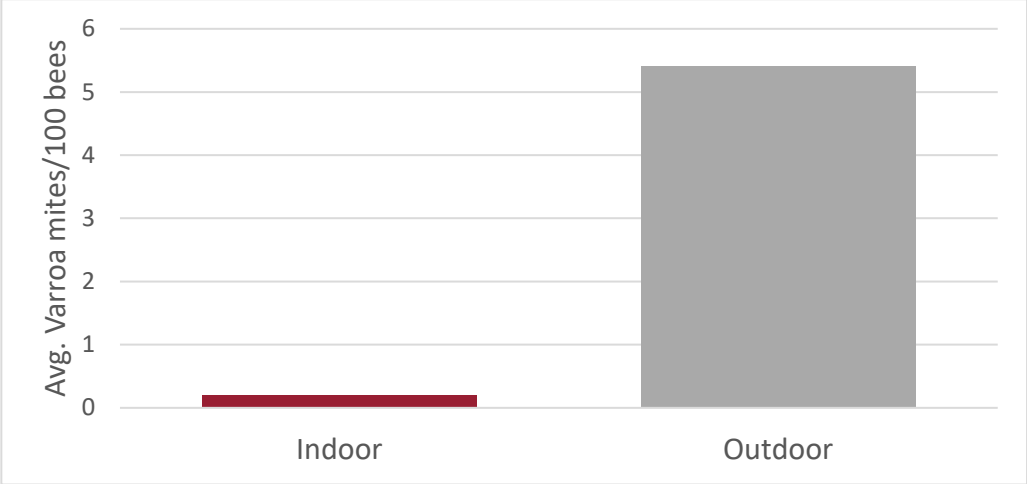

Figure 1: Average Varroa mite populations in colonies that experienced a break in brood production compared to colonies outdoors that continued brood rearing. All colonies experienced the same varroa treatment. The only difference being whether they were moved indoors for 18 days or remained outdoors.