
Project Title

Project No.: POLL21 Hopkins

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A. Summary (*In laymen's terms – emphasize key findings and recommendations*)

We have just completed our first overwintering project (Obj. 2). Lab samples are being processed and data analysis will occur over the next few weeks. Once data analysis is complete we will prepare a manuscript for peer review publication. The key findings from this winter research is that the refrigerated cargo containers purchased with funding from this grant are well suited to meet the needs of this project (accomplished Obj 1). The colonies overwintered in the containers survived the storage period and are currently in almond orchards in California. The containers will be used again following the almond pollination season to begin work on Objective 3. Directly related to this project we produced an extension booklet to disseminate the current knowledge of indoor storage of honey bee colonies in the US. The document is being prepared for an active online version (hosted by Project ApisM) that will be updated as new information is attained through this Almond Board funded research.

B. Objectives (*300 words max.*)

1. Our goal is to develop a method utilizing indoor storage of honey bee colonies that will significantly improve the management and reliability of commercial pollination in Almonds.
We propose to accomplish this by through four objectives 1) Establish controlled atmosphere (CA) research chambers capable of holding commercially-relevant numbers of honey bee colonies. 2) Use metabolic CO₂ to increase Varroa mites mortality during the storage period. This approach will reduce honey bee miticide exposure that has been shown to cause a suite of health problems in colonies and is a major contributor to the unsustainable annual losses. 3) Utilize CA storage to force a mid-season break in brood cycle. Most of the colony losses caused by Varroa are caused by the mites' exponential growth during the summer months while bees are making honey. However, most miticides can't be applied during this time and no miticides effectively control mites during their reproductive phase in the capped brood (pupae). This forces beekeepers to either wait too long before applying miticides or requires multiple (3-4) applications to cover a full brood cycle (24 days). This is expensive considering the cost of materials, labor, and fuel to treat thousands of colonies, and ultimately, is largely ineffective. Alternatively, we will demonstrate that commercial beekeepers can move thousands of colonies into cold storage in

mid-season to create a break in brood and achieve greater mite control with only a single application. 4) Demonstrate the benefits of CA storage/cold storage disseminate the information from this work to commercial beekeepers and develop best management practices that result in greater reliability in the supply of honey bees for almond pollination

C. Annual Results and Discussion

1. Objective 1 has been completed. We established the refrigerated/controlled atmosphere cargo containers at a WSU farm site in central Washington. The results of objective 2 are still being compiled as the honey bee colonies stored this winter have now been placed in California almond orchards (as of Feb 1st) and have reassessed those colonies for hive health and varroa populations. Once results are available we plan to post a progress report through the online version of the attached booklet.
2. Discuss significance of these in terms of progress toward goals, change in approach, next steps or other conclusions based on this year's results
We are on track despite a slight delay in the arrival of the storage containers (described in the methods section below). We have confirmed the suitability of the containers and now have the full capabilities to accomplish all goals and objectives described in the grant. The next steps are to move forward with objectives 3 and 4 and to repeat objective 2 in the next winter period. The great survival of the colonies in the storage containers is very promising; as the use of such containers has not been utilized as a tightly sealed/controlled atmosphere chamber for honey bee storage prior to this winter. The dissemination of the extension booklet and online hosting of the material will be instrumental in meeting achieving success in objective 4. The interest in indoor storage of honey bees is still growing in interest and the impacts of this research and outreach are now being seen in national survey work done by BIP. The Bee Informed Partnership's most recent survey work is showing data that beekeepers utilizing indoor storage are have fewer losses than those beekeepers who do not use indoor storage.

D. Outreach Activities

Presented findings and acknowledged Almond Board of California grant funding –

1. Hopkins, B. K. (Presenter), American Honey Producers Annual Meeting, "The Future of Indoor wintering," American Honey Producers, Sacramento, CA, United States of America. (January 11, 2020).
2. Hopkins, B. K., American Beekeeping Federation National meeting, "Indoor wintering," American Beekeeping Federation, Chicago, IL, United States of America. (January 9, 2020).
3. Hopkins, B. K., Almond Board Conference, "Controlled Atmosphere improve stability of honey bee pollination," Almond Board of California, Sacramento, CA, United States of America. (December 10, 2019).
4. Hopkins, B. K., Idaho Honey Industry annual meeting, "Indoor storage and the future," Idaho Honey Industry, Boise, ID, United States of America. (December 4, 2019).

5. Hopkins, B. K., California State Beekeepers Association Annual meeting, "Breaking the Brood cycle to improve mite control," California State Beekeepers Association, Lake Tahoe, CA, United States of America. (November 19, 2019).
6. Hopkins, B. K., Oregon State Beekeepers Annual Meeting, "Using indoor cold storage to break the brood cycle mid-season," Oregon State Beekeepers Association, Florence, OR, United States of America. (October 26, 2019).
7. Hopkins, B. K., Pacific Branch ESA, "The exciting potential for commercial indoor storage of honey bee colonies and carbon-based feed supplements for pesticide risk reduction," Entomological Society of America, Mission Bay, CA, United States of America. (April 2, 2019).
8. Hopkins, B. K. (Presenter), American Honey Producers Annual Meeting, "The Future of Indoor wintering," American Honey Producers, Tempe, AZ, United States of America. (January 11, 2019).
9. Hopkins, B. K., American Beekeeping Federation National meeting, "Indoor wintering," American Beekeeping Federation, Myrtle Beach, SC, United States of America. (January 9, 2019).

E. Materials and Methods (500 word max.):

1. Outline materials used and methods to conduct experiment(s)

To accomplish these objectives, two 20ft cargo containers (Triton International) were placed at a WSU farm site near Othello, WA (Dec 2019). Each chamber has independent control of temperature and CO₂ with real-time monitoring provided by [PurFresh](#).

Objective 2 – 120 commercial honey bee colonies were assessed in late fall for: the number of frames of bees and brood, weight, presence of disease, and a sample of bees was taken to determine varroa levels. 40 colonies were placed in each of the two chambers set at 4°C each set to a different level of CO₂ (1 and 8%), 40 colonies remained outside for the 1-month storage period. Colonies were fitted with collection cards on the bottom boards to collect and count all mites that fall during the experiment. All colonies were taken to California at the end of January and were reassessed for all factors listed above. All colonies received a miticide treatment with sticky cards on the bottom board of the hive. The final mite numbers will be compared to the number of mites before treatment and those collected during the storage period to determine mite mortality caused by respective treatments.

Objective 3 – We will collaborate with a commercial beekeeper in Washington to gather and utilize honey colonies *following* almond pollination. We will use 196 colonies. All colonies will be assessed as described above before and after storage treatment. 48 colonies will be placed in each chamber (4°C) and 48 remain outside. Colonies from chamber one will be removed at day 18, chamber 2 at day 21. Half of the hives will receive a miticide treatment, and varroa levels will be reassessed 6 weeks later.

Objective 4- The goal of this project being to increase the reliable supply of honey bees for almond pollination will require a major effort in the dissemination of the information gathered through this research. The adoption of such major shifts in management can only happen through publication and development of resources for commercial beekeepers to learn how they might best utilize new management options to decrease their annual losses. This objective will be focused on the publication and presentation

of the research. In addition to the standard peer reviewed and industry publications, we intend on creating a dedicated electronic resource devoted to the practice of indoor wintering.

2. There was a slight delay in getting the containers established and ready for honey bee colonies. The original timeline was to have the containers established in September 2019. The containers were not on site until December 2019. Honey bee colonies were loaded the same day containers arrived and the winter project continued. This delay in the arrival of the containers shortened the intended wintering storage period from 2 months to 1 month. There is potential that the shortened storage period will affect the impact of the metabolic CO₂ on varroa mortality. We have a second wintering study outlined (Winter 2020/2021) in the timeline for this current grant and with containers on site we will have an opportunity to repeat the study with a longer storage period.

F. Publications that emerged from this work

1. Peer review publications in preparation

Hopkins, B.K. Use of metabolic gases (CO₂) to increase Varroa mite mortality in overwintered honey bee colonies.

2. Extension Outreach Publication-

Hopkins, B.K. (Ed) (2019) Indoor Storage of Honey Bee Colonies in the United States (attached)