Project Apis m.

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RESEARCH AGREEMENT 382

THIS RESEARCH AGREEMENT 382 (the "Agreement") is made as of August 15, 2023, by and between Project Apis m, a California nonprofit corporation (the "PAm"), and Auburn University (the "Contractor").

Recitals

A. PAm is a party to a certain Research Management Agreement (the "Funding Agreement") with CropScience LP ("Bayer"), pursuant to which PAm receives funding from Bayer to support bee-related research. PAm desires to utilize a portion of such funding to engage Contractor to conduct certain research on the terms and conditions in this Agreement.

B. Contractor desires to conduct the research as provided herein.

Agreement

NOW, THEREFORE, in consideration of the foregoing, and in reliance upon the mutual promises contained in this Agreement, the parties hereby agree as follows:

1. Research. Contractor agrees to take the actions and conduct the research as set forth on Exhibit A hereto, including providing to PAm all deliverables described therein and the Reports (defined below) (the "Project"). Dr. Geoffrey Williams shall be Contractor's project director for the Project (the "Project Director"). Contractor shall maintain communication with the designated liaison for PAm regarding the status of the Project. Contractor shall provide PAm a copy of any final publication resulting from the Project.

2. Reports. During the Term (defined below) Contractor shall provide to PAm: (i) regular project updates via email and telephone; (ii) detailed semi-annual reports due on October 1 and April 1; and (iii) and a final report following completion of the Project (each a "Report" and collectively, the "Reports"). Each Report will include all elements outlined in PAm's online submission portal. Bayer and PAm shall be identified as the funding and research sponsor in all Reports. All Reports will be of high quality by professional and academic standards (the "Performance Standards"). PAm has the right to request, and Contractor shall provide, an update to and/or resubmission of any Report which does not meet the Performance Standards. In addition to the Reports, Contractor shall provide PAm with periodic updates regarding the Project, suitable for PAm to share with the general public, regarding new developments and overall status of the Project. PAm may provide copies of all Reports and other information regarding the Project to Bayer.

3. Funding. PAm agrees to provide a total of $58,430.00 (the "Funds") to Contractor for performance of the Project, subject to and contingent upon PAm's receipt of such

Funds from Bayer pursuant to the terms of the Funding Agreement. The Funds are a fixed amount and are not subject to increase regardless of the amount expended by Contractor in performing the Project. The Funds will be distributed to Contractor, following receipt by PAm of an appropriate invoice from Contractor, in 2] installments, the first, $43,823.00 after signing this agreement], with the final installment, $14,607.00 being delivered to Contractor only following receipt by PAm of all deliverables set forth on Exhibit A and the final Report, each in form and substance satisfactory to PAm. If Contractor fails to timely produce any deliverables set forth in the Research Proposal or any Report or to resubmit any Report that does not meet the Performance Standards, then PAm may withhold any unpaid installments of Funds. No Funds may be used for overhead or indirect costs related to the performance of the Project. No Funds may be used in any manner for the purpose of influencing legislation and/or influencing governmental policy or action.

4. Term and Termination. The term of the Agreement shall commence on the date hereof and end on July 1, 2025 (the "Term"). This Agreement may be terminated prior to the expiration of the Term: (i) by PAm, if Contractor fails to produce Reports according to the Performance Standards or (ii) by PAm, if Contractor breaches or defaults in any of its representations, warranties, covenants or agreements set forth in this Agreement if such breach or default remains uncured following a ten (10) day cure period which shall commence upon PAm's written notice to Contractor of such breach or default; or (iii) by either party on thirty (30) days advance written notice to the other party. PAm shall have no obligation to pay any remaining installment payments of Funds that arise after the date of the termination of this Agreement. Upon termination of this Agreement, Contractor shall provide PAm with all complete and partially complete deliverables and Reports that exist as of the date of such termination.

5. Independent Contractor Status. PAm and Contractor agree that Contractor and PAm are acting as independent parties and that Contractor is an independent contractor. Neither party shall be considered or represent itself as a joint venturer, partner, agent or employee of the other.

6. Record Keeping. Contractor shall maintain accurate and complete books, records and accounts of all financial transactions pertaining to the Project and the Funds. Contractor shall permit PAm or its authorized representative to have access, during normal business hours, to all records necessary to support the amounts distributed for research hereunder. Contractor will use reasonable efforts to ensure that PAm receives all necessary documentation and responses to questions raised during an audit in a timely manner. PAm will provide notice to Contractor, of at least two (2) weeks prior to the commencement of any audit to allow the necessary time for Contractor to retrieve supporting documentation. Unless otherwise agreed to by the Parties in writing, Contractor shall retain its records for a period of at least five (5) years following the termination of this Agreement.

7. Publicity. Contractor shall discuss and publish its findings with respect to the Project in scientific seminars, conferences, webinars, presentations and scientific journals, including venues and journals appropriate to communicate findings to commercial beekeepers. Contractor is required to prominently acknowledge the participation of PAm and Bayer's HealthyHives in the Project at all public announcements regarding the Project and is required to

prominently display the Project Apis m. and HealthyHives names, logos and trademarks on all written materials which are publicly released regarding the Project (including on websites); provided however that the right of Contractor to display the Project Apis m. and Healthy Hives names, logos and trademarks on written materials is limited solely to public written materials regarding the Project and for no other purpose. PAm may use Contractor's name and the name of Contractor's Project Director along with PAm's reason for funding the Project.

8. Representations. Each party represents to the other that: he or it has full power, capacity and authority to enter into this Agreement; the execution, delivery, and performance of this Agreement has been duly authorized by all necessary action and constitutes a valid and binding obligation enforceable against him or it in accordance with its terms; no action, suit, or proceeding is pending or, to his or its knowledge, threatened against him or it before or by any court, administrative agency, or other governmental authority that brings into question the validity of the transactions contemplated by this Agreement. Contractor represents ~~and warrants~~ that Contractor will throughout the duration of this Agreement remain in strict compliance with all applicable laws and regulations. AV - approved.

9. Third Party Claims. From and after the date hereof, Contractor shall defend and hold PAm harmless from and against any and all claims, liabilities, damages, losses or expenses (including reasonable attorney's fees) brought against PAm by third parties that arise from or are related to the acts or omissions of Contractor in connection with this Agreement.

10. Miscellaneous. This Agreement constitutes the complete agreement between the parties with respect to the subject matter hereof and supersedes all previous agreements, whether written or oral. This Agreement may not be assigned by Contractor without the prior written consent of PAm. This Agreement may be amended by the written, mutual consent of the parties. The failure of a party to insist upon strict adherence to any provision of this Agreement on any occasion shall not be considered a waiver of such provision or deprive that party of the right thereafter to insist upon strict adherence to that provision or any other provision of this Agreement. This Agreement may be executed in counterparts, which, taken together, shall constitute the whole of the Agreement as between the parties. Electronic signatures shall have the same force and effect as manual signatures in respect of execution of this Agreement.

[SIGNATURE PAGE FOLLOWS]

SIGNATURE PAGE TO RESEARCH AGREEMENT

IN WITNESS WHEREOF, the parties hereto have executed this Agreement as of the date first set forth above.

PAm:

PROJECT APIS M

By: DocuSigned by: B706DCD1566544F...

Date: 8/2/2023

Name: Danielle Downey

Title: Executive Director

CONTRACTOR:

By: Anthony Ventimiglia, Assistant VP for Research Administration for Steven Taylor, Senior VP for Research & Economic Development

Digitally signed by Anthony Ventimiglia, Assistant VP for Research Administration for Steven Taylor, Senior VP for Research & Economic Development
Date: 2023.08.01 08:48:48 -05'00'

Date: 8/1/23

EXHIBIT A

Management and dispersal of Tropilaelaps honey bee mites experiencing a continental climate.

Problem and Significance

This work addresses PAm's mission to improve honey bee health by examining management and biology of a honey bee pest which could severely impact U.S. beekeepers. The research will address knowledge gaps concerning the management and dispersal of the mite *Tropilaelaps mercedesae* in *Apis mellifera* honey bee colonies. This information will contribute to the development of appropriate plans if Tropilaelaps is introduced to the U.S., and will identify effective Tropilaelaps management strategies should it become established. To accomplish this, we will perform two field experiments in South Korea, and communicate our findings to appropriate government agencies and the beekeeping community.

1. Principal Investigator(s)

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Collaborator

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2. Date and Duration of Proposed Study

Submission date: 1 June 2023
Duration of study: 1 January 2024 – 1 July 2025

3. Problem and Significance

The *Tropilaelaps mercedesae* mite has the potential to harm the U.S. beekeeping industry like the *Varroa destructor* mite has done since it was introduced to the U.S. in the 1980s. Reports from Asia, where the mite is currently present, suggest that Tropilaelaps parasitism causes greater colony losses than Varroa (Buawangpong et al. 2015; Ritter & Schneider-Ritter 1988). Feeding by Tropilaelaps on developing *Apis mellifera* honey bee larvae and pupae results in disease transmission, reduced honey bee weight upon adult emergence, and shortened adult honey bee life span (Khongphinitbunjong et al. 2015; 2016).

To protect U.S. honey bees, it is critical to prepare for a potential future introduction of Tropilaelaps to the U.S. We currently lack knowledge on the mite's routes of dispersal between colonies, and we lack documented tools that can eradicate the mite without destroying colonies; these are two priorities identified by USDA-APHIS as critical to developing an appropriate

response plan. Knowledge of these areas of biology and management will be essential in an eradication program, but will also be useful for continued management if the mite becomes established in the U.S. Therefore, the proposed work fulfills PAm's overall mission of improving honey bee health by investigating practical, non-destructive control solutions for Tropilaelaps mites and by examining routes of Tropilaelaps dispersal.

Similar to that of Varroa, the life cycle of Tropilaelaps has two phases – reproductive and dispersal (Traynor et al. 2020; Woyke 1994). During the **reproductive phase**, they reproduce within capped brood cells and feed on developing larvae and pupae; during the **dispersal phase**, they reside on adult bees. The Tropilaelaps mite spends only one-half to one-third as much time in the dispersal phase as Varroa (Häubermann et al. 2016; Woyke 1994). This not only increases the reproductive rate of Tropilaelaps compared to Varroa, but also limits effective chemical control since most of the mites are protected by the wax capping of a brood cell (Buawangpong et al. 2015; Woyke 1987). Unlike Varroa, there is no evidence that Tropilaelaps feeds on adult honey bees during its dispersal phase (Rinderer et al. 1994).

To completely eliminate Tropilaelaps without destroying colonies, we propose to deprive the mites of brood (eliminating their protected location and food source), and to apply chemical treatment when all mites are in the dispersal phase on adult bees. By caging the queen for 24 days, we will induce the absence of brood (**brood break**), which has not been examined for Tropilaelaps control since the early 1990s in western Asia (Woyke 1985; Woyke 1994). Treatment with formic acid alone (a U.S.-registered treatment for honey bees) provided ~90% efficacy against Tropilaelaps (Pettis et al. 2017) but was not sufficient to eliminate them completely. By combining a brood break with chemical treatment with formic acid or oxalic acid, we hope to achieve 100% efficacy against Tropilaelaps.

Also critical to developing a response to Tropilaelaps is an understanding of the ability of the mite to disperse between colonies and apiaries – to assess the risk and speed of spread from a location of first introduction. It is speculated that Tropilaelaps mites primarily disperse among colonies via exchange of brood frames during colony equalization, splitting, and consolidation (K. Khongphinitbunjong, personal communication); however, drifting adult honey bees or robbing forager honey bees may also play a role (Rath et al. 1991; Laigo & Morse 1968). During the dispersal phase, Varroa mites in colonies with low infestation rates prefer to feed on nurse bees; however, that preference disappears with mites in highly infested colonies as they attach to older workers that leave the colony, enabling dispersal to other colonies (Cervo et al. 2014). To date, no studies have evaluated natural routes of Tropilaelaps mite dispersal. This is important to evaluating the feasibility of containment, especially because feral colonies would likely be present near infested colonies.

4. Objectives

1) To determine whether the combination of a brood break and chemical treatment eliminates or reduces Tropilaelaps mite infestation in *Apis mellifera* honey bee colonies.
2) To describe natural dispersal routes of Tropilaelaps mites from *Apis mellifera* honey bee colonies.

5. Experimental Design and Methods

Objective 1: To determine whether the combination of a brood break and chemical treatment eliminates or reduces Tropilaelaps mite infestation in *Apis mellifera* honey bee colonies.

In South Korea during late summer 2024, four treatments groups each with 10 queenright *Apis mellifera* honey bee colonies will be rented from a local beekeeper. Initially, we will measure the population of brood and adult honey bees (Delaplane et al. 2013), and uncap 200 brood cells to determine the Tropilaelaps infestation rate (Dietemann et al. 2013; Pettis et al. 2017). We will also assess the infestation rate of adult honey bees using the alcohol wash method (Anderson & Roberts 2013). Colonies will be assigned to treatments, ensuring that colony strength and Tropilaelaps infestation are similar among groups. Treatment groups will include: 1) negative control, no management action, 2) brood break only, 3) brood break + oxalic acid dribble, and 4) brood break + FormicPro®. These treatment groups were chosen because a brood break only management plan was shown to be effective in previous studies (Woyke 1985; 1994). Oxalic acid should also be effective when no brood are present, and formic acid was recently shown to be effective against Tropilaelaps mites in the presence of brood (Pettis et al. 2017). On experiment day 0, colonies receiving the brood break will have their queen caged in a QCC queen cage (Thorne Beekeeping) attached to the top of a centrally placed brood frame (Büchler et al. 2020). Colonies will again be assessed on day 24 for brood and adult honey bee population size, as well as adult honey bee Tropilaelaps infestation rate (Anderson & Roberts 2013; Delaplane et al. 2013). At this time, queens will be released because all mites will be in their dispersal phase on adult honey bees due to the brood break. In FormicPro® colonies, one strip will be applied on day 24, followed by a second strip on day 34. During the brood break, oxalic acid treatment colonies will receive treatment using the dribble/solution method according to the U.S. label rate. We suspect these treatments will be adequate because no mites will be protected in brood cells. Once the queen has been laying and brood are present, 200 cells per colony will again be uncapped on day 60 to measure infestation rate in brood cells, as well as adult honey bee infestation rate, and brood and adult bee population (Dietemann et al. 2013; Pettis et al. 2017; Anderson & Roberts 2013 Delaplane et al. 2013).

Pitfalls: A potential challenge to the proposed work is finding colonies with sufficient Tropilaelaps infestation rates. However, the South Korean collaborator has excellent connections with local beekeepers so we anticipate that appropriate colonies will be identified; they will work with beekeepers several months in advance of the trial to ensure success. Furthermore, the trial will be performed in late summer when mite populations are historically high. Lastly, is potential that our queens do not resume laying after 24 days of caging. This should be alleviated by our use of the large queen cage (33x12x80 mm), which is designed for extended caging events (Büchler et al. 2020). Nonetheless, we will have mated queens available in case of issues with queen re-establishment.

Objective 2: To describe natural dispersal routes of Tropilaelaps mites from *Apis mellifera* honey bee colonies.

At least 15 *Apis mellifera* honey bee colonies will be inspected by uncapping 200 brood cells to evaluate Tropilaelaps infestation rates (Dietemann et al. 2013; Pettis et al. 2017), and then assigned to three treatment groups: 1) normal-functioning colonies with a brood infestation rate of 1-5%, 2) normal-functioning colonies with a brood infestation rate of >10%, and 3) normal-functioning colonies with a brood infestation rate of >10% to be experimentally

weakened to encourage robbing. Treatment groups were chosen based on Varroa mites' preference for nurse bees in colonies with a low mite infestation rate, which disappears with increasing infestation (Cervo et al. 2014). If Tropilaelaps dispersal is like that of Varroa, colonies with higher infestation rates will be more likely to have Tropilaelaps on exiting adults, and the weakened colonies would be most likely to have dispersal mites on exiting adults (including robber honey bees). Each colony will be fitted with a funnel trap designed to collect exiting adult honey bees (Medrzycki 2013). Traps will be engaged for 10-15 minutes once a week for two months so that adult worker and drone honey bees can be collected. We will assess the infestation rate of these trapped bees using the alcohol wash method (Anderson & Roberts 2013).

Pitfalls: The major potential pitfall is improper functioning of the funnel trap. To avoid this, we are currently testing prototypes in Auburn after discussing with the researcher who originally designed the trap. As with Objective 1, low colony Tropilaelaps infestation rates could limit this work, but again we think this will be overcome with the connections of the South Korean collaborator to identify suitable colonies; additionally, colonies from his existing research apiary can be used.

6. Intended Outcome

The intended outcome of this work is twofold: first, to support the development of appropriate Tropilaelaps response guidelines by USDA-APHIS, and second, to inform U.S. beekeepers of effective Tropilaelaps management strategies. Knowledge concerning non-destructive honey bee colony actions, as well as natural dispersal of the mite, are critical to the development of an appropriate response plan.

This work will be done in conjunction with similar research scheduled to be conducted in Thailand. Together, the proposed experiments in South Korea and the planned experiments in Thailand will prepare for an introduction of Tropilaelaps in cooler or warmer weather, respectively. By testing formic acid and oxalic acid in both these conditions, we will test management actions that suit the diverse climatic regions of the U.S. Furthermore, most work on Tropilaelaps biology and management has been performed in Thailand, but traits affecting management and dispersal could vary among regional populations of Tropilaelaps. By replicating the work in South Korea, we importantly would determine whether the Tropilaelaps dispersal behavior is consistent between these two potential source populations of Tropilaelaps mites.

Dissemination of findings: Results from these experiments will be reported to USDA-APHIS to address their current knowledge gaps, and will be communicated through talks at national entomology and beekeeper meetings (e.g. Entomological Society of America, American Beekeeping Federation, American Honey Producers Association) and in scientific journals.

7. Economic Feasibility for New Products

We do not anticipate that any new products will be developed from this research. However, we anticipate identifying new uses for miticides (Formic Pro® and oxalic acid) and procedures (brood breaks) that are currently registered and employed in the U.S. If effective, both miticides could immediately be used by beekeepers against Tropilaelaps, should it be detected in the U.S.,

because products can be applied to a new target pest if they are applied according to label rates (FIFRA 2012).

8. Project Timeline

The proposed project timeline is for funding over 18 months to allow for all experiments to be conducted, data to be analyzed, and results be written and submitted to a scientific journal for publication (Table 1). Research will be conducted in 2024 with funds provided through June 2025 to account for the open access publishing fee and communication of results to beekeepers.

Table 1. Anticipated timeline of the proposed work. X denotes activity.

Research Step	2024				2025	
	Jan-Mar	Apr-Jun	July-Sept	Oct-Dec	Jan-Mar	Apr-Jun
Experimental design finalization	X	X				
Colonies first inspected for Tropilaelaps		X				
Data collection			X	X		
Data analysis				X		
Dissemination of results				X	X	X

9. Information Regarding Prior or Simultaneous Submissions

A proposal has been submitted to USDA-APHIS to conduct a similar study in Thailand during Spring 2024. The purpose of performing similar work in different geographic regions of Asia is to ensure that Tropilaelaps and *Apis mellifera* populations from Thailand are not over-represented when building an understanding of this important parasite. It is possible that slight differences in genotype-environment interactions could misinform response guidelines and the U.S. beekeeping community (Büchler et al. 2014). The purpose of performing work in different climatic regions and at different periods of the year is to improve our understanding of how two active ingredients commonly used in the U.S. against Varroa – formic acid and oxalic acid – will perform against Tropilaelaps under conditions likely to be experienced by U.S. beekeepers. It is well-documented that the effectiveness of formic acid against Varroa is affected by ambient temperature (Steube et al. 2021); this is likely also the case for Tropilaelaps.

10. Matching Funds Proposal

This proposal does not include a request for matching funds.

11. References

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12. Budget Request

Budget Summary

Description	Year 1 ($)	Year 2 ($)	Total ($)
Salary	25,453	3,041	28,494
Benefits	3,359	934	4,293
Equipment	0	0	
Supplies	650	0	650
Travel (Domestic)	1,262	1,131	2,393
Travel (International)	5,300	0	5,300
Contractual	15,300	0	15,300
Other	0	2,000	2,000
Total Direct	**51,324**	**7,106**	**58,430**
Indirect	**0**	**0**	**0**
Total	**51,324**	**7,106**	**58,430**

Budget Justification

Personnel

Total funds requested are $28,494. This includes funds for Williams (PI) for 0.5 month totaling $5,994 to coordinate the project (experimental procedures, data collection and analyses, and communication of results). Funds also requested for a post-doc for 5 months, totaling $22,500; this person will be responsible for day-to-day operations, especially data collection and analysis, as well as preparation of a manuscript and delivery of presentations.

Fringe benefits
Total funds requested are $4,293. This includes funds for Williams' fringe (30.7%) totaling $1,840 and post-doc fringe (10.9%) totaling $2,453.

Equipment
No funds are requested.

Supplies
Total funds requested are $650. This includes beekeeping treatment applications totaling $125, lab supplies (e.g. gloves, forceps, sealable bags, magnifiers and lights) for $100, beekeeping supplies (e.g. beesuit, smokers, hive tools) for $200, and bee entrance collectors $225 ($15 x 15 hives).

Travel
Domestic: Total funds requested are $2,393. This includes funds for return shuttle between Auburn and ATL ($131 x 3 return trips) totaling $393. One trip is for travel to Korea, the other two trips is for travel to the two national beekeeper organization meetings to present results. Funds are further requested for flights and accommodation for the two beekeeper meetings (each of AHPA and ABF - $500 for travel and $500 for accommodation).
International: Total funds requested for travel and accommodation associated with field work in Andong, Korea totaling $5,300. This includes $2,200 for the flight, $100 for return transport between Seoul and Andong, and $3,000 for accommodation ($1,000 x 3 months).

Contractual
Funds are requested so that contractual services can be provided by Andong University, South Korea for a total of $15,300. This is to support the project with local personnel and colony rental fees to perform the work.

Salary: Total request is for $7,800. This is to fund 2 local students to support the project (each person: $1,300 x 3 months).
Fringe: No fringe is required for the salary requests
Other: Total request is $7,500 to rent honey bee colonies for use in the trial ($150 x 50 colonies).

Other
Funds are requested to cover open access publication costs totaling $2,000.