Honey Bee Stock Improvement Program: Importation, Preservation and Utilization of Honey Bee Germplasm

Project No.: **15-POLL7-Sheppard/Cobey**

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Objectives:

1. Continue to develop a cooperative Industry/University bee breeding program to incorporate genetic material from imported honey bee germplasm into domestic breeding stocks and to implement practical programs to evaluate and maintain these stocks.
2. Evaluate imported honey bee stocks for a number of apicultural traits directly relevant to Almond pollination, including early spring build up, flight time/temperature properties, propolis collection, mite tolerance (supported by USDA Specialty Crop Block Grant – no Almond Board funds requested).
3. Implement cryopreservation for desirable honey bee germplasm from Old World and domestic bee breeding programs for future use by queen breeders.
4. Continue the collection and importation of honey bee germplasm from endemic populations of Old World honey bees to enhance honey bee genetic improvement.
5. Continue to offer and expand specialized Technology Transfer short courses to promote the skills necessary for stock improvement.

Interpretive Summary:

This document represents the final progress report for an award of the Almond Board to W.S. Sheppard/S.W. Cobey to assist with expenses necessary to import and disseminate novel honey bee germplasm. Key findings from 2015-2016 included:

1. Determination of impact of the importation and distribution of honey bee semen through California queen producers to the beekeeping industry. Based on an extensive study of queen producer stocks, we determined that honey bee populations of queen producer operations who incorporated novel honey bee germplasm provided though Almond Board funded importation of semen, exhibited significantly higher levels of genetic diversity than non-users of the genetic material. In summary, samples taken at 10-year intervals from CA queen producers (1993-1994, 2004-2005 and 2013-2014) showed a diminishment of genetic diversity each decade, based on genetic marker analysis. However, the breeding

populations of queen producers who incorporated the novel germplasm imports made possible by Almond Board support exhibited a significant increase in genetic diversity, with 2013-2014 breeding populations reaching levels higher than the baseline 1994 populations! Higher levels of genetic diversity within a population equates to greater levels of "raw material" available for bee breeding.

2. Production of new honey bee subspecies derivatives for California pollinating beekeepers. In 2015-2016, we produced and distributed queen honey bees of derived from honey bee germplasm (semen) from Old World original source populations in Georgia and Slovenia. In addition, we grew out foundation breeder stock from the subspecies *Apis mellifera pomonella* collected from Kazakhstan in 2015. The semen was used to instrumentally inseminate domestic virgin queens produced and supplied by collaborating California queen breeders and by our own laboratory. Inseminated queens of Caucasian and Carniolan stocks were maintained over the coming winter (2015-2016) in WA, ID and CA and were made available to commercial queen producers in Spring 2016 to enhance the diversity of US honey bee populations. Through ongoing and future introductions of honey bee germplasm, this project provides a mechanism for the genetic improvement of commercial honey bee populations, a critical resource to pollinate almonds and other agricultural crops. In addition, a cooperative bee breeding program has been established with members of the California Bee Breeders Association. The purpose of the program is to develop a sustainable, self-supporting, cooperative Industry/University honey bee stock maintenance program and to incorporate the germplasm importations into domestic honey bee breeding stocks.

Maintaining adequate genetic diversity is fundamental to breeding programs directed toward the improvement of all crops and animals of agricultural significance. More than 30 years ago, resident strains of almonds were evaluated in 10 Mediterranean and Asian countries and this genetic source material was available by exchange to UC-Davis plant breeders (Kester and Asay, 1977). In fact, given that many crops have non-U.S. origins, the U.S. National Plant Germplasm System maintains over 500,000 accessions (samples) of seed, tissues and plants for plant breeders to use and still conducts an average of 15 expeditions per year to foreign countries to gather new genetic material (O'Brien, 2010). Similarly, breeding programs of economically important, livestock species, such as poultry, dairy, and swine rely on the importation of genetic material from within the original ranges of the species. Historically, the beekeeping industry has not had access to these sorts of genetic resources, a limitation that could limit the ability off bee breeders to select for resistance to *Varroa* and other pests and diseases.

Materials and Methods:

In 2016, we conducted comparative field studies in California almonds with three genetic lines of honey bees. This research was made possible by the acquisition of genetic material via Almond Board funding and the field study itself was funded by a specialty crop block grant to WSU. All selected honey bee stocks met almond pollination minimums for colony size and data were collected on foraging behavior, with special reference to temperature flight minimums. In summer 2016, rather than traveling internationally for additional germplasm collection, we concentrated on performing backcrosses of existing breeding lines of *A. m.*

carnica, *A. m. caucasica* and *A. m. pomonella* derived from Almond Board supported genetic imports. Virgin queens were produced and inseminated with cryopreserved honey bee semen from previous international collections. In addition, distribution of genetic material to US queen producers continued and contributed to a reversal of the loss of genetic variability known to exist in the Industry (see Interpretive Summary 1.)

In summer 2016, a collecting trip was made to several California queen producers to obtain representative genetic material for deposition into the USDA National Animal Germplasm Repository in Ft. Collins Colorado. Honey Bee semen collected from the honey bee stocks of these producers was cryopreserved and submitted to the Repository and currently represent the first and only honey bee genetic material housed within the USDA-National Animal Germplasm Program. W.S. Sheppard and B. Hopkins assisted the Director of the USDA-NAGP, Dr. Harvey Blackburn, to establish the first species committee for honey bees. This committee, composed of members representing queen breeders/producers, University bee research and USDA-ARS laboratories, are now charged with developing priorities and protocols for the collection and distribution of honey bee genetic material, respectively.

Results and Discussion:

Almond Board funding has been a critical link to acquire the new honey bee germplasm, and cryopreserved material from both domestic and imported sources is currently maintained at the WSU Honey Bee Germplasm Repository.

Imported Carniolan and Caucasian honey bee germplasm was used in domestic commercial queen production by a number of Western queen producers in 2016, with additional queen production of these strains undertaken by several by Eastern US queen producers. Caucasian honey bees exhibit behavioral characteristics of apicultural interest to beekeepers that overwinter in northern and high altitude locations in the United States, including docility, high rates of colony growth during the spring and good honey production. Another characteristic of this subspecies is extensive usage of propolis (collected plant resins), a material that has recently been shown to improve colony level immune function.

Caucasian and Carniolan genetic material is entering the US domestic honey bee population through a cooperative honey bee breeding program established with domestic queen producers in CA (Objectives 1 & 4 above). Almond Board funds made possible the importation and production of these different subspecies variants for the first time since 1922 (when stock importation to the US was restricted). A similar project based on Almond Board funded importation of Italian honey bee semen in underway with a CA queen producer who made and sold around 80,000 queens in 2015. In 2016, production queens (open-mated daughters of our selected inseminated stock) were available to the general population of beekeepers. In 2016, we also conducted a beekeeper's short course and several queen rearing course for beekeepers to help extend knowledge of the basic principles of selection and breeding in honey bees (Objective 5). These courses were fully booked in 2016 and will be repeated in 2017. In addition, in 2015-2016 W. Sheppard, S. Cobey, B. Hopkins and graduate students from the Sheppard Lab made numerous presentations to beekeeping associations in the western US (CA, OR, WA, ID) describing the research effort being supported by the Almond Board.

We report here significant progress toward the improvement of US honey bee populations based on the importation of novel honey bee genetic diversity widely accessible by the bee breeding industry. Funding provided by the Almond Board for 2015-2016 supported continued collection and additional importations of semen from Old World sources. The ability to cryopreserve semen, coupled with the established USDA-APHIS/WSU permit protocol for honey bee germplasm importation, has provided material that is maintained in the first honey bee genetic repository in the United States. Such a repository will allow practical permanent storage of genetic material for subsequent breeding use, much as has become routine in other animals of agricultural significance (dairy and meat cattle, sheep, swine, horses, etc.). The mission of the WSU Honey Bee Germplasm Repository is to maintain original source population genetic stocks imported under Almond Board funding, to conserve "top-tier" genetics of existing US commercial stocks and other specific lines of honey bees submitted by other research laboratories and queen producers.

In 2016 we produced breeder queens needed to initiate a large-scale field trial of these different genetic strains of honey bees in almonds and other crops (Objective 2). The study will compare foraging behavior and efficiency of different genetic strains of honey bees under different climatic and weather conditions during bloom.

The Almond Board funding received by W. Sheppard and S. Cobey for honey bee germplasm collection efforts continues to lay the groundwork for requests to granting agencies to support the establishment of a permanent honey bee germplasm repository. The success of the Almond Board funded honey bee germplasm research and outreach efforts by our research group was a critical prelude to both growing acceptance of the new technology and to increased consideration of improved honey bee genetics.

This was a great year – we grew out the bee stocks and moved them even more into the CA queen producers' production lines. Several Eastern bee breeders (who supply queens to commercial folks in the east who take their bees to almonds for pollination) obtained breeder stock from us in 2016.

Two main points:

1. My PhD student Megan Taylor demonstrated that queen producers who used genetic material we have supplied them over the past few years experienced a major improvement in genetic variation in their bees. Those producers who did not use the new germplasm continued to show a decline in genetic variability in their bees.

2. We started a full scale field study in California on almonds comparing some of these strains in almond pollination. The results should be available next season. This $247k grant was made possible by leveraging the outcome of the $30–35k annual funds we have received from the AB.

Research Effort Recent Publications:

No specific publications derived from the 2015- 2016 Almond Board award were forthcoming. In keeping with Almond Board policy, manuscript drafts will be submitted to the Almond Board prior to journal submission.

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