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

Project No.: **14-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, and 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. Honey bee germplasm (semen) from Old World original source populations in Poland and Kazakhstan was collected in June of 2015 and hand-carried under USDA-APHIS permit into the United States. The semen was used to instrumentally inseminate domestic virgin queens produced and supplied by collaborating California queen breeders and by our own laboratory. The inseminated queens will be maintained over the coming winter (2015 - 2016) in WA, ID and CA and made available to commercial queen

producers in Spring 2016 to enhance the diversity of US honey bee populations. Previous germplasm (collected and used for insemination in 2014) was maintained in colonies in a similar manner and distributed in 2015. 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 2015, Walter Sheppard (WSS) and Brandon Hopkins (BKH) traveled to Kazahkstan and made a number of collections of semen from the subspecies *Apis mellifera pomonella* sourced from distinct locations across a 900 km range in the Tien Shan Mountains (Objective 4). Concurrently, Susan Cobey (SWC) travelled to Poland and the laboratory of Dr. Jerzy Wilde to collect semen from a large-scale breeding program that includes managed strains of *A. m. caucasica* and *A. m. carnica* (Objective 4). In addition to fresh semen taken for immediate use upon return to the US, aliquots of a number of semen samples were also cryopreserved in liquid nitrogen for subsequent use in breeding (Objective 3).

Collected germplasm (semen) was returned to the US in late June 2015 under a USDA-APHIS hand carry permit awarded to WSS. Virgin queens produced by industry collaborators (Objective 1) and by our own laboratory were inseminated by SWC with "fresh" imported pure *carnica* and *pomonella* honey bee semen. Aliquots of all semen were supplied to Dr. Judy Chen of the USDA-ARS Bee Research Laboratory in Beltsville MD for virus determination. Collection and introduction of genetic material derived from Old World endemic honey bee populations into the US has been ongoing under this project since 2008. To date, we have imported semen from three subspecies of high importance to the US beekeeping industry, including *A. m. ligustica* (Italian), *A. m. carnica* (originally derived from the Alps) and *A. m. caucasica* (Caucasian), in addition to the new 2015 introduction of the Tien Shan Mountain gray bee (*A. m. pomonella*).

Results and Discussion:

In July 2015, we received notification from the USDA-ARS honey bee laboratory that our semen collections were free of novel viruses and are currently awaiting release of the germplasm from USDA-APHIS to provide material to CA queen producers (Objective 1). 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 Washington State University (WSU) Honey Bee Germplasm Repository and funded by Project Apis M.

Based on the virus screening, a Fall 2015 release of the inseminated queens from quarantine by USDA-APHIS is pending. The queens will be overwintered in WA, CA and ID and in Spring 2016 we will produce *A. m. pomonella* and *A. m. carnica* hybrid virgin daughter queens to be inseminated with additional imported/cryopreserved semen. Imported Carniolan and Caucasian honey bee germplasm was used in domestic commercial queen production by at least 4-6 and 3 US queen producers, respectively in 2015. 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 considerable usage of propolis (collected plant resins), a material that has recently been shown to improve colony level immune function.

As noted above 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 (Obj. 1 & 4 above). 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, we anticipate that production queens (open-mated daughters of our selected inseminated stock) will be available to the general population of beekeepers. In 2015, we also conducted a beekeepers 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 2015 and will be repeated in 2016. In addition, in 2014 - 2015 WSS, SWC, BKH 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 2014 - 2015 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 2015 we produced breeder queens needed to imitate 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. 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). Leveraging of the Almond Board funds are thus directly responsible for our ability to secure a specialty crop block grant ($249k) from the USDA and WSDA to conduct a broad foraging behavior study in almonds and tree fruit crops that will begin in the 2016 field season.

The Almond Board funding received by WSS and SWC 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. In 2015, we used funds from the Washington State Tree Fruit Research Commission to initiate a collection of honey bee germplasm from a subspecies of honey bee (*Apis mellifera pomonella*) that in the Tien Shan Mountains of Central Asia. The Almond Board funds were used to support the 2014 - 2015 breeding work and collection of stocks in Poland. 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 (RBBA project above) and to increased consideration of improved honey bee genetics (WSTFRC-funded project above).

Research Effort Recent Publications:

No specific publications derived from the 2014 - 2015 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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