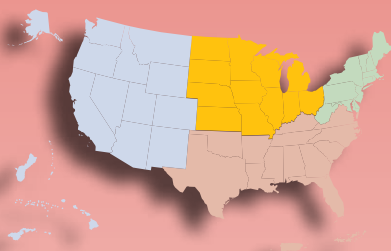


FACT SHEET

Peer-reviewed research findings and practical strategies for advancing sustainable agricultural systems



SARE FUNDING FOR THIS PROJECT

Project Number

LNC99-152.1

Project Year

1999

SARE Region

North Central

Grant Type

Research and Education

Project Coordinator

Marla Spivak

Extension Entomologist

University of Minnesota

spiva001@umn.edu

For more information, go to www.sare.org/project-reports and search by project number.

Written by Marla Spivak and Gary Reuter, University of Minnesota
Department of Entomology



www.sare.org



European honey bee with a *Varroa* mite on its back. Photo by USDA-ARS

A Sustainable Approach to Controlling Honey Bee Diseases and *Varroa* Mites

Geographic Adaptability: Relevant to beekeepers throughout the US and Canada.

Introduction

Honey bees are the world's most economically valuable insect pollinator of fruits, vegetables and other crops. Along with the more than 4,000 species of wild bees in North America, honey bees also provide significant pollination of wildflowers and cultivated gardens. The health of honey bee colonies started declining after the inadvertent introduction of the parasitic mite *Varroa destructor* into the United States in 1987. Honey bee colony health continues to be compromised by exposure to agricultural and urban pesticides, and by poor nutrition stemming from insufficient floral resources. High levels of *Varroa*, and the viruses this mite transmits from bee to bee, can lead to the death of bee colonies within one to two years if left unmanaged. To control this mite, beekeepers have resorted to using synthetic and organic miticides within their colonies, which has added both an enormous operating expense for beekeepers and the risk of contaminating honey and beeswax with residue. It has also led to the mites evolving resistance to some treatments.

Our first goal was to breed honey bees, *Apis mellifera*, resistant to diseases and parasitic mites to help reduce the amount of antibiotics and pesticides used in bee

colonies. Our second goal was to ensure that our selection methods could be easily implemented by beekeepers to enable them to select for their own resistant stocks of bees. A reduction in miticide use, and implementation of stock selection by beekeepers, will enhance environmental quality and economic viability of individual beekeeping operations; strengthen an agricultural system (beekeeping) that is based on small- and moderate-scale owner-operated farms; protect human health and safety by preventing the risk of contaminating honey and hive products; and promote the wellbeing of honey bees, our honey producers and vital pollinators.

Our Research

We began breeding honey bees for resistance to diseases and *V. destructor* in 1994. Honey bees bred for hygienic behavior, a genetic trait, demonstrate behavioral resistance to American foulbrood (AFB), a highly infectious bacterial disease of brood (larvae). Hygienic honey bee colonies also demonstrate resistance to chalkbrood, a fungal disease (Spivak and Reuter, 2001a). Bees bred for hygienic behavior can detect and physically remove disease-infected brood from the colony before it becomes infectious. Hygienic bees can in fact detect and remove diseased brood before the human eye can detect any sign of disease symptoms! When bees remove the disease in the non-infectious stage, it prevents the disease from spreading throughout the colony.

Our research has shown that bees bred for hygienic behavior also display one form of resistance to *Varroa destructor* mites because they are able to detect and remove brood infested with the mites (Spivak, 1996). This mite parasite alternates between feeding on adult bees and feeding and reproducing on the pupal stage of bees. Bees that remove mite-infested pupae from the nest interrupt the reproductive cycle of the mite and eliminate the offspring of the mite developing within a wax-sealed cell (Figure 1).

We initially selected for hygienic behavior in an Italian-derived race of honey bees. However, the behavior is present in all races and stocks of *Apis mellifera* in the United States (and the world), and can be easily selected for using the methods described below. We bred the “MN Hygienic Line” of bees, which became widely accepted by the beekeeping industry. Much of our early research effort was devoted to evaluating the MN Hygienic Line against other lines of commercially available honey bees to ensure that it is resistant to diseases and can actively defend itself against the mite pests, resulting in lower mite levels. We also evaluated the honey production, gentleness and wintering ability of our line to ensure that it is acceptable to both commercial and hobbyist beekeepers (Spivak and Reuter, 1998a; 2001b).

In line with our second goal of ensuring that our selec-



FIGURE 1. These hygienic bees are detecting, uncapping and removing a sealed, 5th instar larva that is infected with either American foulbrood or chalkbrood disease. Notice that the larva does not appear to have symptoms of either disease to the human eye. Hygienic bees are able to detect that the larva is diseased **before** it reaches the infectious stage; that is, before it is brown and gooey with AFB or mummified with chalkbrood. In this way, hygienic bees eliminate the pathogen and avoid further disease transmission through the colony.

tion methods are accessible to beekeepers everywhere, we stopped maintaining the MN Hygienic Line at the University of Minnesota in 2009 and began working with commercial queen producers to help them select for hygienic behaviors from among their favorite stocks of honey bee, whether derived from Carniolan, Italian, Caucasian or a mixture of races. Our hope was that beekeepers would make a number of resistant lines available within the United States to maintain genetic diversity: the perfect way to promote the vitality of our pollinators. Today, many large-scale queen producers routinely select for hygienic behavior using the assay described below.

What is the Difference Between Hygienic Behavior and Varroa-Sensitive Hygiene (VSH)?

We are not alone in our efforts to select honey bees for resistance to *Varroa* mites. Researchers at the USDA-ARS Honey Bee Breeding, Genetics and Physiology Lab in Baton Rouge, La., have bred and are maintaining several stocks of bees that have demonstrated resistance to *Varroa*. One of these stocks is called VSH, which stands for Varroa Sensitive Hygiene. The VSH stock excels at detecting, uncapping and removing mite-infested brood, but interestingly, this hygienic behavior was not selected for using the assay we describe below. The VSH stock was selected using three criteria: 1) colonies with a low rate of mite population growth over the season, 2) colonies that actively removed mite-infested brood, and 3) colonies in which the mites had low reproductive success on worker pupae (Harbo and Hoopingarner, 1997; Harbo and Harris, 1999, 2005). These selection steps require more time and detailed measurements than are feasible for most commercial beekeepers. We caution here that although the tests for hygienic behavior described below are quick, they will not necessarily lead to the same degree of resistance demonstrated by VSH stocks. The good news is that new selection assays are currently being developed to help beekeepers select resistant stocks more efficiently and effectively.

Testing Honey Bee Colonies for Hygienic Behavior

It is relatively easy to determine if a colony of bees displays hygienic behavior. If you are curious whether your bees express the behavior, you can test them using one of these methods. (Also see Spivak and Downey, 1998; Spivak and Reuter, 1998b). They involve presenting bees with freeze-killed or pin-killed brood and determining the colony's rate of removal of the dead brood. The ability of a colony to quickly remove freeze-killed or pin-killed brood corresponds generally with how quickly the colony detects and removes diseased or mite-infested brood. These methods are used as an initial screen to find colonies with hygienic tendencies. This initial assay should be followed by more detailed tests of a colony's ability to detect and remove actual diseased or mite-infested brood.

Two Recommended Methods to Test for Hygienic Behavior

1. The Freeze Killed Brood Assay

In this assay, a comb section of sealed brood containing approximately 100 cells on each side (2 inches by 2.5 inches,

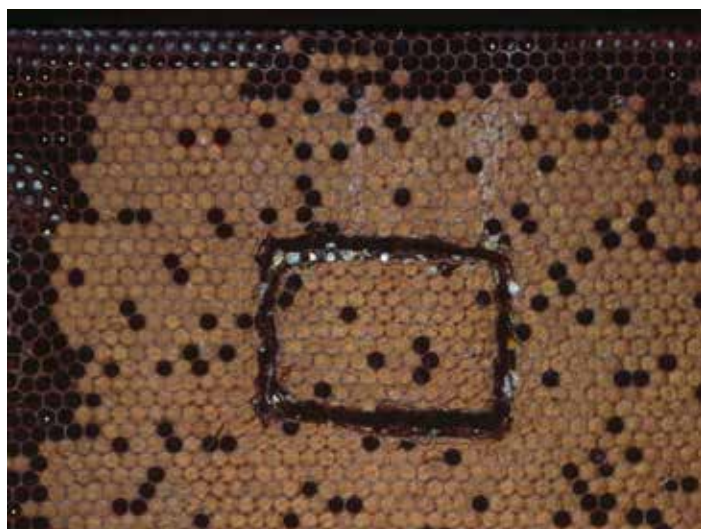


FIGURE 2. A comb section of sealed brood containing approximately 100 cells on each side was cut out of the comb, frozen for at least 24 hours, and replaced in the hole left in the comb.

or 5 centimeters by 6 centimeters) is cut from a frame and frozen for 24 hours at -10°F (-20°C). The frozen comb section is inserted into a frame of sealed brood in the colony being tested (Figure 2). Tests have shown that it does not matter if the frozen section comes from the same colony from which it was removed or from a different colony. The frame with the freeze-killed brood insert is placed in the center of the brood nest. One day (24 hours) later the frame is removed and the number of sealed cells remaining is recorded. A hygienic colony will have uncapped and removed over 95% of the frozen brood within 24 hours. A non-hygienic colony will take over six days to completely remove the frozen brood.

2. Liquid Nitrogen

Freezing the brood with liquid nitrogen is more efficient and less destructive to the combs than cutting, freezing and replacing comb inserts. Liquid nitrogen is relatively inexpensive and easy to obtain; check with your local gas and welding suppliers, veterinary practice or livestock artificial insemination firm. There are no laws in any state restricting the use of industrial grade liquid nitrogen by individuals. It must be kept in an appropriate tank (e.g., a Dewar tank, which can be purchased through gas and welding supply houses), and the tank should be securely fastened to the truck during travel to avoid spillage.

Common sense and several precautions must be used when handling liquid nitrogen. It has a boiling temperature of -195°C (-320°F), which means that it is extremely cold and will kill skin (causing severe frostbite) on contact. We recommend that users read the material safety data sheet on liquid nitrogen from the supplier.

You will need to construct (or find) a hollow cylinder into



FIGURE 3. A 3-inch diameter PVC tube is twisted into the comb down to the midrib. The liquid nitrogen is slowly poured into the tube.

which you will pour the liquid nitrogen to freeze a circular section of sealed brood. We have been using a 3-inch (75 millimeter) diameter PVC pipe. The cylinder must be at least 4 inches (100 millimeters) long because the nitrogen will boil on contact with the brood.

A minimum of 10 ounces (300 milliliters) of liquid nitrogen is needed to freeze-kill all the brood (approximately 160 cells) within a 3-inch diameter cylinder. A smaller amount will not kill all of the brood, leading to erroneous results. Use a 10-ounce or larger polystyrene foam coffee cup for measuring and pouring. Other materials may shatter on contact with the liquid nitrogen.

Select a frame with at least a 3-inch diameter circle of sealed brood containing fewer than 30 unsealed cells within the circle. Lay the frame horizontally across a support (e.g., an empty super). Twist the cylinder into the sealed brood until it reaches the midrib. Record the number of unsealed cells inside the cylinder. Pour 50–60 milliliters of the liquid nitrogen into the cylinder and wait for it to freeze the edges or evaporate. Then pour the remainder of the liquid nitrogen into the cylinder. Wait to remove the cylinder until it thaws, which may take three to 10 minutes (Figure 3). If you have additional cylinders, you can start the next test while you are waiting for previous ones to thaw. We put a drawing pin (thumbtack) in the top of the frame to mark the frame and the location of the test on the frame. Some hygienic colonies clean and repair the comb so quickly that it is hard to locate the test when you return. Place the frame in the center of the brood nest (Figure 4).

Remove the frame containing the frozen brood 24 hours later and record the number of sealed cells remaining within the circle. When testing a colony that has been requeened,



FIGURE 4. Record the number of already empty cells within the frozen circle, place a thumbtack on the top bar of the frame over the area that has been frozen, and replace the comb in the colony to be tested.

six to eight weeks must elapse after requeening for the bees in the colony to be daughters of the new queen (Figure 5a and b).

Important note: Both of these tests should be repeated on the same colony, and you may notice that the results may vary between tests. For example, a colony may remove 95% of the frozen brood on the first test, but only 50% on the second. This colony is not hygienic! It is very important that colonies be considered hygienic **only if they remove more than 95% of the brood on two consecutive tests.**

Breeding for Hygienic Behavior

Any race or stock of bees can be bred for hygienic behavior. We recommend that beekeepers select for hygienic behavior from among their best breeder colonies (i.e., those that have proven to produce honey, winter well, are gentle and display all the characteristics desired by the breeder). A queen producer can get a head start on selecting for hygienic behavior simply by rearing queens from colonies that do not have chalkbrood.

When colonies are first screened for hygienic behavior using a freeze-killed brood method, they may not remove all of the frozen brood within 24 hours. The colonies that remove the most freeze-killed brood within 24 hours should be propagated by rearing queens from them. Subsequent generations will remove the brood more quickly because hygienic queens from the first generation will produce drones for the second generation. If the hygienic queens are instrumentally inseminated with semen collected from drones from hygienic colonies, or are mated naturally in an isolated area, where all the surrounding drones are from hygienic col-

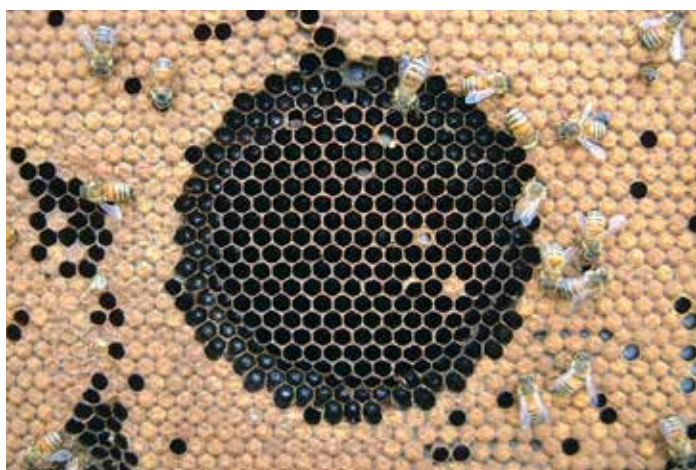


FIGURE 5a (left). An example of a hygienic colony that has uncapped and removed over 95% of the brood frozen with liquid nitrogen within 48 hours.

FIGURE 5b (right). An example of a non-hygienic colony that has uncapped and removed less than half of the frozen brood within 48 hours.

onies, it will be easier to fix the trait in your line of bees. In time, if many bee breeders select for hygienic behavior, the frequency of the trait should increase in the general population of bees, which will increase the chances that any queen will encounter drones that carry the trait.

Important note: The effects of American foulbrood, chalkbrood and *Varroa* mites can be alleviated if queen producers select for hygienic behavior from their own lines of bees. The ability of a colony to remove freeze-killed brood is correlated with disease and mite resistance; however, the actual degree of resistance can only be evaluated in controlled tests when the colonies are challenged with American foulbrood, chalkbrood or *Varroa* mites.

Our experience has shown there are no apparent negative characteristics that accompany the trait. Years of research experience have shown it would greatly benefit the beekeeping industry to have productive, hygienic queens mated with hygienic drones available commercially (Spivak and Reuter, 1998a, 2001b; Ibrahim et al. 2007).

Frequently Asked Questions

Do hygienic colonies require treatments for diseases and mites?

Hygienic colonies will demonstrate good behavioral resistance to AFB and chalkbrood. This means that although hygienic colonies may become infected with these diseases, the bees will rapidly remove all evidence (clinical symptoms) of the diseases, so it appears they are completely healthy. In most cases, hygienic colonies will require NO treatments for AFB or chalkbrood; however, they will still require occasional treatments for the mites. With more widespread use of hygienic stock, however, bees will become more resistant to mites and will require fewer and fewer treatments.

How do bees detect diseased brood?

Most likely, hygienic bees detect abnormal brood by detecting abnormal odors with their antennae. Our research has shown hygienic bees have a more acute sense of smell for the odor of diseased brood than do bees that do not express hygienic behavior (Masterman et al., 2001; Gramacho and Spivak, 2003; Spivak et al., 2003; Swanson et al., 2009).

How is hygienic behavior inherited?

Hygienic behavior is a genetic trait. The famous work of Dr. Walter Rothenbuhler in the 1960s showed that it is a recessive trait, meaning that the queens and the majority of the drones she mates with must carry the hygienic genes for the workers in the colony to express the behavior (reviewed in Spivak and Gilliam, 1998). However, modern genetic analysis is revealing that hygienic behavior is controlled by a number of genes in a complex way (Lapidge et al., 2002).

Important note about genetics: If you purchase a hygienic queen, it is important to know if the majority of drones she mated with also came from hygienic colonies. If the queen did not mate with hygienic drones, the workers she produces will not express the behavior, and your colony will not be hygienic (Arathi and Spivak, 2001). To increase the chances that hygienic queens mate with hygienic drones, the drones in most of the surrounding apiaries must come from hygienic colonies. Ask your queen producer about their drone producing colonies.

Research Synopsis of SARE-Funded Work

Our goal is to breed honey bees, *Apis mellifera*, resistant to diseases and parasitic mites to reduce the amount of antibiotics and pesticides used in bee colonies, and to ensure that our breeding methods and stock are accessible to beekeep-

ers everywhere. We bred a line of bees for hygienic behavior, called the “MN Hygienic Line.” Hygienic behavior is the ability of bees to detect and remove diseased and mite-parasitized brood from the nest, and this behavior can be selectively bred into any line or race of honey bees. Our tests of the MN Hygienic Line in commercial apiaries demonstrated that they have good resistance to American foulbrood (a highly contagious and deadly bacterial disease of bee larvae) and chalkbrood (a less serious fungal disease of bee larvae). The hygienic line is partially resistant to the devastating mite, *Varroa destructor*. In 2001, we began investigating the mechanism for the Suppression of Mite Reproduction trait (now called VSH) to determine how bees can reduce mite reproductive success. Our results demonstrate that bees bred for VSH are both hygienic and have some yet-unknown property associated with their brood that reduces the number of viable offspring the mites produce. Combining the VSH trait into the hygienic line, therefore, helped increase the degree of hygienic behavior in our line and added another factor that helps suppress mite reproduction. Field trials in commercial apiaries have demonstrated that the MN Hygienic/VSH cross significantly reduces mite loads in colonies relative to the pure MN Hygienic Line and unselected lines of bees.

References

- Arathi, H.S. and M. Spivak. 2001. Influence of colony genotypic composition on the performance of hygienic behavior in the honey bee (*Apis mellifera* L.). *Animal Behavior* 62: 57–66.
- Gramacho, K.P. and M. Spivak. 2003. Differences in olfactory sensitivity and behavioral responses among honey bees bred for hygienic behavior. *Behav. Ecol. Sociobiol.* 54: 472–479.
- Harbo, J.R. and R.A. Hoopingarner. 1997. Honey bees (*Hymenoptera: Apidae*) in the United States that express resistance to *Varroa jacobsoni* (*Mesostigmata: Varroidae*). *Journal of Econ. Entomol.* 90: 893–898.
- Harbo, J.R. and J.W. Harris. 1999. Selecting honey bees for resistance to *Varroa jacobsoni*. *Apidologie* 30: 183–196.
- Harbo, J.R. and J.W. Harris. 2005. Suppressed mite reproduction explained by the behaviour of adult bees. *Journal of Apicultural Research* 44(1): 21–23.
- Ibrahim, A., G.S. Reuter and M. Spivak. 2007. Field trial of honey bee colonies bred for mechanisms of resistance against *Varroa destructor*. *Apidologie* 38: 67–76.
- Ibrahim, A. and M. Spivak. 2006. The relationship between hygienic behavior and suppression of mite reproduction as honey bee mechanisms of resistance to *Varroa destructor*. *Apidologie* 37: 31–40.
- Lapidge, K., B. Oldroyd and M. Spivak. 2002. Seven suggestive quantitative trait loci influence hygienic behavior of honey bees. *Naturwissenschaften* 89: 565–568.
- Masterman, R., R. Ross, K. Mesce and M. Spivak. 2001. Olfactory and behavioral response thresholds to odors of diseased brood differ between hygienic and non-hygienic honey bees (*Apis mellifera* L.). *J. Comp Physiol. A* 187: 441–452.
- Spivak, M. 1996. Honey bee hygienic behavior and defense against *Varroa jacobsoni*. *Apidologie* 27: 245–260.
- Spivak, M. and D. Downey. 1998. Field assays for hygienic behavior in honey bees (*Apidae: Hymenoptera*). *J. Econ. Entomol.* 91: 64–70.
- Spivak, M. and M. Gilliam. 1993. Facultative expression of hygienic behaviour of honey bees in relation to disease resistance. *J. Apicul. Res.* 32(3/4): 147–157.
- Spivak, M. and M. Gilliam. 1998. Hygienic behaviour of honey bees and its application for control of brood diseases and *varroa* mites. Parts I and II: Hygienic behaviour and resistance to American foulbrood. *Bee World* 79: 124-134; 165–182.
- Spivak, M., R. Masterman, R. Ross and K.A. Mesce. 2003. Hygienic behavior in the honey bee (*Apis mellifera* L.) and the modulatory role of octopamine. *J. Neurobiol.* 55: 341–354.
- Spivak, M. and G.S. Reuter. 1998a. Performance of hygienic honey bee colonies in a commercial apiary. *Apidologie* 29: 285–296.
- Spivak, M. and G.S. Reuter. 1998b. Honey bee hygienic behavior. *Amer. Bee J.* 138: 283–286.
- Spivak, M. and G.S. Reuter. 2001a. Resistance to American foulbrood disease by honey bee colonies, *Apis mellifera*, bred for hygienic behavior. *Apidologie* 32: 555–565.
- Spivak, M. and G.S. Reuter. 2001b. *Varroa jacobsoni* infestation in untreated honey bee (*Hymenoptera: Apidae*) colonies selected for hygienic behavior. *J. Econ. Entomol.* 94: 326–331.
- Swanson, J., B. Torto, S. Kells, K. Mesce, J. Tumlinson and M. Spivak. 2009. Volatile compounds from chalkbrood *Ascosphaera apis* infected larvae elicit honey bee (*Apis mellifera*) hygienic behavior. *J. Chem. Ecol.* 35: 11088–1116.

This publication was developed by the Sustainable Agriculture Research and Education (SARE) program with funding from the National Institute of Food and Agriculture, USDA. Any opinions, findings, conclusions or recommendations expressed here do not necessarily reflect the view of the U.S. Department of Agriculture.

