Agricultural Innovations
Practical applications for sustainable agriculture
Fact Sheet
Sustainable Agriculture Research & Education

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

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

**Inside this fact sheet:**



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## Geographic Range:

Relevant to beekeepers throughout the U.S. and Canada

## Introduction

An estimated one-third of the human diet is derived directly or indirectly from insect pollinated plants. Honey bees are the world's most important insect pollinator of fruit and vegetable crops, home gardens and wildflowers. The number of bee colonies and beekeepers is steadily declining due to the inadvertent introduction of the parasitic mite *Varroa destructor* into the U.S. in 1987. Left untreated, varroa mites kill most bee colonies within one to two years.

To control the mite, beekeepers have been using pesticides (pyrethroids and organophosphates) in their bee colonies. However, that approach has generated problems, including the mites developing resistance, the enormous operating expense of purchasing and spraying pesticides in honey bee colonies and risks of contaminating honey and beeswax with residue.

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 beekeepers everywhere. A reduction in pesticide use by beekeepers will enhance environmental quality and economic viability of individual beekeeping operations; strengthen an agricultural system (beekeeping) 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 well-being of honey bees -- our honey producers and vital pollinators.

## Breeding for Resistance

We have been breeding honey bees for resistance to diseases and *Varroa destructor* since 1994. The most devastating disease of honey bees is American foulbrood (AFB), a highly infectious bacterial disease of brood (larvae). We have demonstrated that honey bees bred for hygienic behavior, a genetic trait, demonstrate good resistance to AFB and also to a fungal disease, chalkbrood [1]. Bees bred for hygienic behavior are able to detect and physically remove disease-infected brood from the colony before it becomes infectious. Hygienic bees are able to 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.

**Photo A.** Hygienic bees detect, uncap and remove a sealed, 5th instar larva that is infected with either American foulbrood or chalkbrood disease. Hygienic bees are able to detect that the larva is diseased *before* it reaches the infectious stage; in this way, hygienic bees eliminate the pathogen and avoid further disease transmission through the colony.

Our research has shown that bees bred for hygienic behavior also display resistance to *V. destructor* mites because they are able to detect and remove broods infested with the mites [2]. This mite parasite alternates between feeding on blood of 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 by eliminating the offspring of the mite developing within a wax-sealed cell (Photo A).

We have bred hygienic behavior into an Italian line of honey bees. However, the behavior is present in all races and lines of honey bees in the US (and the world!), and can be easily selected for, using the methods described below. Our "MN Hygienic Line" of bees is available commercially in the US and has become widely accepted by beekeepers. However, our hope is that beekeepers select for hygienic behavior from among their favorite line of honey bee, whether it be Carniolan, Italian, Caucasian or other species. In this way, there will be a number of resistant lines available within the U.S. to maintain genetic diversity -- the perfect way to promote the vitality of our pollinators.

Much of our research effort is in evaluating our 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 evaluate the honey production, gentleness and wintering ability of our line to ensure that it is acceptable to both commercial and hobby beekeepers [3, 4]. In the last several years, we have made great strides in increasing the degree of resistance of our line to the mites, so that the frequency of treatments to control the mites can be greatly reduced and alternative treatments (such as organic acids and botanical oils) can be used to reduce mite loads.

## Testing Honey Bee Colonies for Hygienic Behavior

It is relatively easy to determine if a colony of bees displays hygienic behavior by testing them using one of the methods described below [5, 6].

### Two best 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 x 2.5 inches) is cut from a frame and frozen for 24 hours at -10°F. The frozen comb section is inserted into a frame of sealed brood in the colony being tested (Photo B). 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

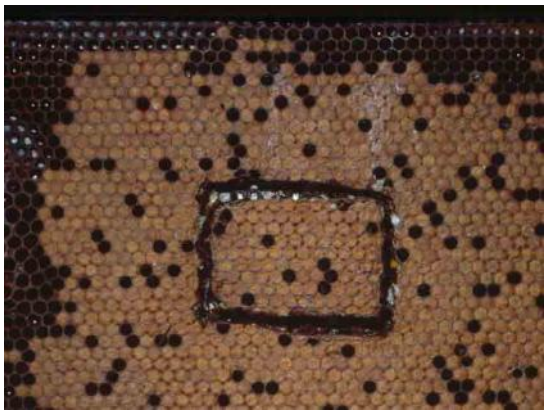

**Photo B.** A comb section of sealed brood containing approximately 100 cells on each side was cut out of the comb, frozen for at least 24 hours, and replaced in the hole left in the comb.

-killed brood insert is placed in the center of the brood nest. Two days (48 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 48 hours. A non-hygienic colony will take over six days to completely remove the frozen brood. The speed with which a colony removed dead brood is correlated with its ability to remove diseased and parasitized 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 N2 is relatively inexpensive and easy to obtain; check with your local gas and welding suppliers, veterinary practice, or livestock artificial inseminating firm. There are no laws in any state restricting the use of industrial grade liquid N2 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 -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 N2 from the supplier.

You will need to construct (or find) a hollow cylinder into which you will pour the liquid N2 to freeze a circular section of sealed brood. We have been using a 3-inch diameter PVC pipe. The cylinder must be at least 4 inches long because the nitrogen will boil on contact with the brood.

A minimum of 10 oz of liquid N2 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-oz or larger polystyrene foam coffee cup for measuring and pouring. Other materials will shatter on contact with the liquid N2.

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 (i.e. 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 1.5 to 2 oz. of the liquid N2 into the cylinder and wait for it to freeze the edges or evaporate. Then pour the remainder of the liquid N2 into the cylinder. Wait to remove the cylinder until it thaws, which may take three to five minutes (Photo C). 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 (Photo D).

**Photo C.** A 3" diameter PVC tube is twisted into the comb down to the midrib. The liquid N2 is slowly poured into the tube.

**Photo D.** 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.

Remove the frame containing the frozen brood 48 hours later, and record the number of sealed cells remaining within the circle. When testing a colony that has been re-queened, six to eight weeks must elapse after requeening for the bees in the colony to be daughters of the new queen (Photos E-1 and E-2).

**Important note**

Both of these tests should be repeated on the same colony, and it will be noticed that the results between tests may vary. 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 >95% of the brood on two consecutive tests**. The speed with which a colony removed dead brood is correlated with its ability to remove diseased and parasitized brood.

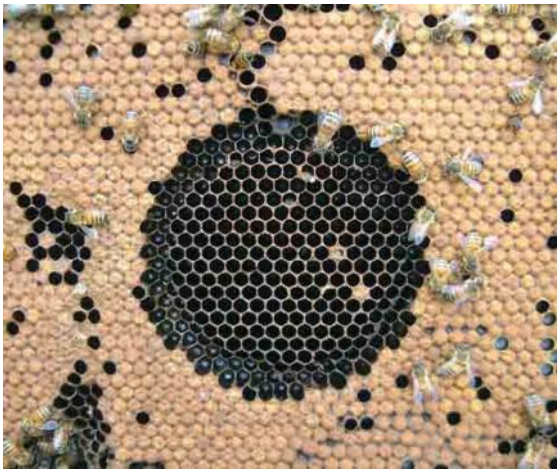

**Photo E-1.** An example of a **hygienic** colony that has uncapped and removed over 95% of the brood frozen with liquid N2 within 48 hours.

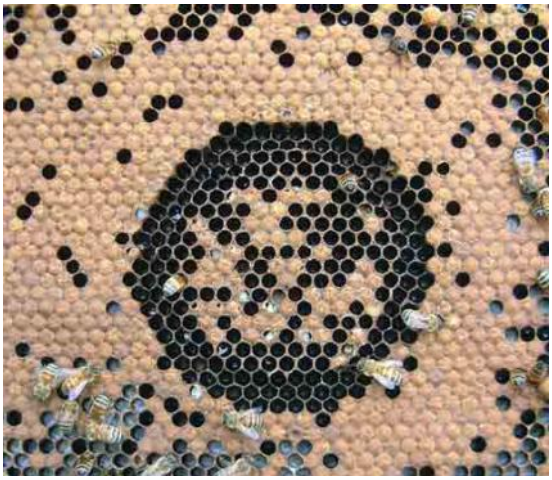

**Photo E-2.** An example of a **non-hygienic** colony that has uncapped and removed less than half of the frozen brood within 48 hours.

## Breeding for Hygienic Behavior

Any race or line of bees can be bred for hygienic behavior. We recommend that beekeepers select for hygienic behavior from among their best breeder colonies; i.e., from those that have proven to produce honey, winter well, are gentle, and display all the characteristics desired by the breeder. A breeder 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 48 hours. The colonies that remove the most freeze-killed brood within 48 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 colonies, 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.

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.

## 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. At this point, bees selected for hygienic behavior 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 [7, 8, 9].

### How is hygienic behavior inherited?

Hygienic behavior is a genetic trait. The 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 [10]. However, modern genetic analysis is revealing that hygienic behavior is controlled by a number of genes in a complex way [11].

### 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. 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 his/her drone-producing colonies. Some queen producers, particularly from Minnesota, raise and mate hygienic queens in areas where the majority of drones are also hygienic.

## SARE Research Synopsis

Our goal was 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 beekeepers everywhere. We have bred a line of bees for hygienic behavior called the "MN Hygienic line." Hygienic behavior, the ability of bees to detect and remove diseased and mite-parasitized brood from the nest, 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*.

Since 2001, we have been incorporating another trait into the MN Hygienic line called "Suppression of Mite Reproduction" or SMR. We also have been investigating the mechanism for the SMR trait to determine how bees can reduce mite reproductive success. Our results demonstrated that bees bred for SMR 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 SMR 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 Hygienic/SMR cross significantly reduces mite loads in colonies relative to the pure Hygienic line and unselected lines of bees.

We are developing a web-based course called "Healthy Bees" on sustainable methods of controlling diseases and mite pests of honey bees. The main emphasis will be on promoting the use of resistant bee stocks as the foundation for integrated pest management strategies. This will be the only such course available online to beekeepers, and is a crucial link between our research and its successful implementation.

This fact sheet is based on a SARE-funded project.
For more information, please visit www.sare.org > Project Reports > Search the database for project # LNC99-152.1

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