

Varroa Mite Resistance: Testing for Hygienic Behavior

This article describes the steps involved in testing honey bee colonies for hygienic behavior using a freeze-kill brood assay.



Image 1. A colony that is classified as hygienic will remove all or nearly all of the freeze-killed brood.

Introduction

Hygienic behavior is characterized by the bees' ability to remove sick, damaged, or dead brood from cells in the hive. Colonies that can quickly detect and remove diseased brood are thought to be resistant to varroa mites, chalkbrood and American foulbrood. Made popular by University of Minnesota professor Marla Spivak, bees are said to be hygienic when they receive a high score on a freeze-kill brood assay.

Hygienic behavior is tested for by freezing a patch of capped brood and returning 24 hours later to determine how much of the killed brood was removed. If the colony removes at least 95% of the killed brood, it qualifies as hygienic. This trait has been shown to be genetically inherited. Breeders can make selections based on a freeze-killed brood *assay* or test to increase the prevalence of mite removal in colonies in their operations, reducing chemical applications to control Varroa mites.

For this test, the queen must have been laying eggs in the colony for a minimum of 7 weeks and no additional bees or brood should have been added to the colony. The bees that perform this behavior are younger bees, between one and three weeks of age. In order for the test to accurately measure the queen's score, all of the brood and workers must be the

daughters of the current queen.

This test needs to be performed twice to get a queen's score. To be considered hygienic, the score should be at least 95% on both tests.

Freeze-kill assays can be performed in a number of ways. Here, we will demonstrate a method based on the procedure used by researchers at the Giovannazzo lab at Laval University and Deschambault Animal Sciences Research Centre in Quebec, Canada

Procedure

Equipment and Supplies

2 two-inch pastry rings, liquid nitrogen in a dewar, styrofoam coffee cup or dipper, cryogenic gloves, safety glasses, pen/marker, datasheet, honey, container with 0.5% bleach solution, optional: push pins, funnels, camera.



Image 2. Left. A dewar for transporting liquid nitrogen. Right. A bin containing supplies for safely conducting a freeze kill assay.

Procedure

Locating a frame of brood from the colony you wish to evaluate, the ideal frame will have a large area of capped brood. Shake or brush the bees from the frame and lay it on top of a table or on top of the colony. For higher resolution testing, the beekeeper may wish to uncap cells and identify pink- to light purple-eyed pupae, so that the same age brood is tested in each colony.

1. Find a frame of brood from the queen of interest that contains a large area of capped brood.

2. Uncap cells to locate pink-light purple-eyed pupae.
3. Shake the bees off of the frame and lay it flat on the inner cover of the hive.



Image 3. Left. A frame with a solid pattern of capped brood. Middle. Pupae of the proper age for the test. Right. A frame of brood lying on the top bars of the hive.

4. Dip the edge of the pastry rings into honey; this prevents liquid nitrogen from leaking.
5. Position the rings over the uncapped cells.
6. Press and gently twist the rings into the comb until contact with the plastic foundation.



Image 4. Left. Dipping the pastry ring in honey. Middle. Placing the ring in the brood. Right. Two rings placed in the brood.

7. Mark the frame with the location of the test with a marker or push pins.
8. Pushpins can also be used to mark the frames and funnels can be used for more accurate pouring.
9. While wearing safety goggles and cryogenic gloves, pour 35 ml of liquid nitrogen into each ring and allow it to evaporate. This cools the testing area.



Image 5. Left. A frame marked with painted arrows. Middle. Funnels for ease of pouring. Right. A dipper for measuring liquid nitrogen.

10. Pour an additional 40ml of liquid nitrogen into each ring and allow it to evaporate.
11. Allow the rings to thaw, remove them and return the frame to the brood nest of the colony.
12. Place the rings in a mild bleach solution to sanitize.



Image 6. Left. Pouring liquid nitrogen into a ring. Middle. Brood with the rings removed after freezing. Right. Sanitizing the rings after use.

13. Document the date, time, and colony number in a notebook.
14. Return exactly 24 hours later.
15. Find and remove the test frame.
16. Shake the bees off the frame and document the number of cells remaining in the test area.
17. To calculate hygienic response, subtract the number of cells remaining after 24 hours from the total number of cells tested and divide that number by the total number of cells. The 2-inch rings make calculations easy, as this is about the size of 100 cells per ring. Since two rings are being used, there is a total of 200 cells. If there are 8 cells remaining after 24 hours, the equation would look like this: $200 - 8 = 192$; $192 / 200 = 0.96$ or 96% hygienic response.



Image 7. Left. All of the brood was removed from the circles, giving a score of 100%. Middle. Half of the brood was removed from the circles, giving a score of 50%. Right. Less than half of the brood was removed from the circles, giving a very poor score.

18. It is important to note that the cells just outside the ring will also be frozen, the key with this testing is to repeat it in the same way and to choose breeding stock from your colonies that express the trait at a very high level.
19. If the colony scores above 95%, perform this test a second time. The second test needs to be above 95% for the colony to be considered hygienic.
20. Record these two test results with your colony's data. The information will be used later for selection purposes.

More information on honey bee breeding can be found at [An Introduction to Honey Bee Breeding Program Design](#).

This material is based upon work supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, through the Northeast Sustainable Agriculture Research and Education program under subaward number LNE22-447.

Authors

Kate Anton

Research Technologist

kxa138@psu.edu

814-865-9806

Robyn Underwood, Ph.D.

Extension Educator, Apiculture

rmul@psu.edu

484-268-5208

extension.psu.edu

Penn State College of Agricultural Sciences research and extension programs are funded in part by Pennsylvania counties, the Commonwealth of Pennsylvania, and the U.S. Department of Agriculture.

Where trade names appear, no discrimination is intended, and no endorsement by Penn State Extension is implied.

Please visit extension.psu.edu/alternate-format-request to request this publication in an alternative format accommodation due to a disability.

Penn State is an equal opportunity, affirmative action employer, and is committed to providing employment opportunities to all qualified applicants without regard to race, color, religion, age, sex, sexual orientation, gender identity, national origin, disability, or protected veteran status.

© The Pennsylvania State University 2025

Code: ART-8739