NSW GOVERNMENT Department of Primary Industries

primefact

Testing for hygienic behaviour

November 2014, Primefact 1378 first edition
Elizabeth Frost, Development Officer Bees, Livestock Systems, Paterson

Hygienic behaviour

Hygienic behaviour is a characteristic of honey bees that confers resistance to American foulbrood (AFB) and chalkbrood as well as limited resistance to varroa mites. Bees that carry the hygienic behaviour trait detect and remove dead and diseased brood before it reaches the infectious stage, thereby preventing the spread of disease within the colony. The fastest way to increase your stock's resistance to AFB and chalkbrood is to test for hygienic behaviour. Select colonies that test highly hygienic and use them as queen and drone mothers. Annual testing and continual selection is necessary to improve and maintain this trait.

Figure 1. Comb exhibiting chalkbrood mummies

The easiest way to start selecting for hygienic behaviour is to adopt a strict "no chalkbrood" policy when selecting breeder queens and drone mothers from amongst your top-performing colonies.

Every beekeeper has the ability to test for hygienic behaviour in their stock. As with most things a beekeeper does, success is won with the proper tools and know-how within the context of environmental conditions. In the case of hygienic testing, if possible, one shouldn't test during a heavy nectar flow, periods of very high or low temperatures or prolonged drought. If you anticipate robbing either don't test at that time or enclose tested, thawing frames in a screened tent and keep colonies closed while their frame is tested and allowed to thaw.

Testing

The two methods of testing are known as the **freeze-killed brood test** or the **liquid nitrogen-killed brood test**. For either test a small portion of capped brood must be frozen then returned to the colony. Twenty-four hours later the freeze-killed portion is checked to see how much dead brood the colony removed. The percentage of brood removed shows how hygienic that colony is. For example, if you freeze a section of brood with a solid pattern (no empty cells) and, after 24 hours in the colony, the workers removed 100% of the cappings and dead pupae, that colony would be considered 100% hygienic. If you want to select for hygienic behaviour this colony would be part of your breeder pool.

The **liquid nitrogen-killed brood test** is the preferred method as the **freeze-killed brood test** requires an additional day to freeze a 5cm x 6cm section of comb which must be cut out of a brood frame.

Liquid nitrogen-killed brood test

CAUTION: Liquid nitrogen can cause cold burns similar to frostbite upon contact with skin and asphyxiation in unventilated areas. Personal protective equipment (PPE) in the form of protective eyewear, waterproof safety boots, and leather/insulated waterproof gloves should be worn by the liquid nitrogen handler. The testing process requires the use of a liquid nitrogen tank and proper transport and handling of liquid nitrogen.

See the Safety Data Sheet (SDS) for further precautionary guidelines in the use of liquid nitrogen:

www.boc-healthcare.co.nz/internet.lh.lh.nzl/en/images/MSDS_Medical%20Liquid%20Nitrogen_Download434_85554.pdf

Figure 2. Test results of a hygienic colony. 97% liquid nitrogen-killed brood removed in 24 hours. Photo Courtesy: K. Lee, University of Minnesota

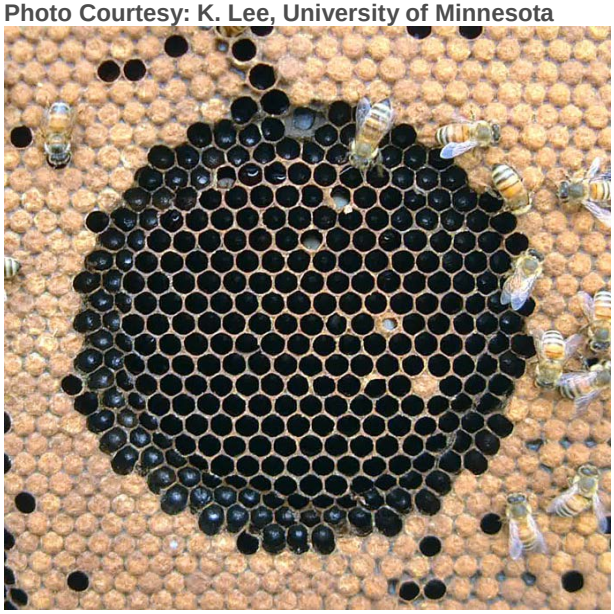

Figure 3. Test results of a non-hygienic colony. 61% liquid nitrogen-killed brood removed in 24 hours

Materials

- Thumb tacks/drawing pins or permanent marker
- PVC tubes: Pipe PVC Pressure Class 12 SWJ 80mm. Average wall thickness: 5mm. Cut into 100mm lengths, sand the end that will cut into the brood. Ten tubes will test sixty colonies.

Check your local irrigation supply or hardware store

Figure 4. Note sanded edge of PVC tube to cut into brood

- Liquid nitrogen tank (20L capacity, will test approximately 60 colonies). Tanks rentable at some suppliers.
- Liquid nitrogen (300ml per colony)

Check with regional cattle artificial insemination suppliers or gas supply store

- Metal cup (300ml capacity) with handle. Mark 300ml level inside cup.
- Gloves (leather/insulated and waterproof)
- Splash-proof eye-wear
- Safety boots (waterproof)
- Record book/Datasheets

Methods

1. Select and label colonies to be tested. Colony labels should withstand the elements and be located on a permanent component of the colony, for example front face of brood box or hive cover.
2. Select frame to be tested. Search for frame with the best brood pattern (least amount of empty cells). Avoid frames with lots of uncapped brood or emerging brood.

Figure 5. Select frame with best brood pattern

3. Uncap a few cells to find pupae between 3-10 days old (from just pupating to white to light tan with purple eyes). Any pupae within this range can be tested. Label top-bar of selected frame with thumb tack or scrape messy top bar with hive tool and mark with permanent marker. This will aid in locating the frame later on.

Figure 6. Uncap a few cells to confirm age of pupae

4. Place frame on flat surface (an upturned cover or an empty super) and insert PVC tube. Press and twist the PVC tube into the selected brood area. Press until the midrib of the comb is reached.

Figure 7. Place frame on flat surface and insert PVC tube

5. Pour 300ml of liquid nitrogen into metal cup.

Figure 8. Pour 300ml of liquid nitrogen into metal cup

An initial small pour (approximately 50ml) into the PVC tube will allow a seal to form at the base of the tube if there are gaps. When the first pour is nearly evaporated pour the remaining liquid nitrogen into the tube.

Figure 9. Pour liquid nitrogen into PVC tube

6. Move onto the next colony while the liquid nitrogen evaporates and the frame thaws. Repeat steps 1-5. Thawing is dependent on weather. On a cool/cloudy day the frames may need to thaw for 10 minutes or more. Depending on foraging conditions it may be necessary to keep thawing frames in a screened tent to prevent robbing.

Figure 10. While liquid nitrogen evaporates, test another colony

7. Gently twist the PVC tube off once thawed and record number of unsealed cells.

Figure 11. Gently twist the PVC tube off once thawed

Count and record the number of unsealed/uncapped cells in the tube area after twisting off the PVC tube in case a cell capping is damaged during removal. This number is your uncapped count (0HR).

Figure 12. Count and record the number of unsealed/uncapped cells

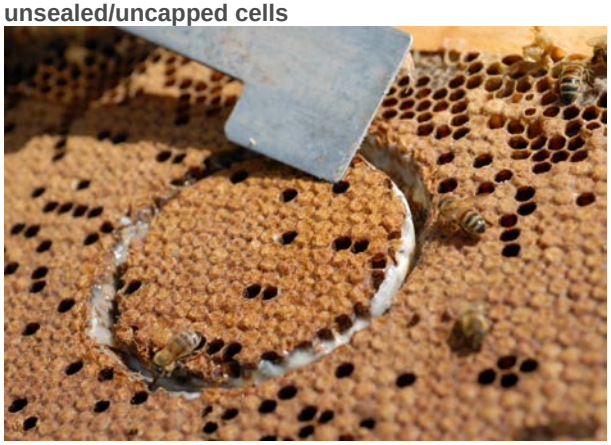

Figure 13. Count and record the number of unsealed/uncapped cells

8. Return the frame to the colony and record the time of return. Recording the time helps keep the pace on the second day of testing, ensuring that one doesn't speed up and check tests before 24 hours has passed.

Figure 14. Return the frame to the colony and record the time

9. Twenty-four hours later check the comb section for test results. Count cells still capped or still containing dead pupae (whole and parts). This number is your 24HR count. A colony is hygienic if it has cleaned out 95% or greater of the frozen pupae and cappings within 24hrs.

Figure 15. 24 hours later, check test frame

IMPORTANT: This colony did not test well and would not be considered hygienic

Figure 16. Count remaining sealed cells and pupae parts

10. Return frame to colony (remove thumbtack if used). Removing thumbtack will prevent confusion during future hygienic testing.
11. Calculate hygienic behaviour. You will need your uncapped count (0HR), 24HR count and total number of cells within the PVC tube.

First, calculate the total number of cells within your PVC tube. Within the 80mm PVC tube the average count will be close to 150 cells. For increased accuracy, in Step 6, count all whole cells (capped and uncapped) within the area of the PVC tube from three different tested frames and then get the average of the three. This average is the total number of cells within the PVC tube.

Next, calculate 0HR count by subtracting uncapped count (0HR) from the total number of cells within the PVC tube. Subtract 24HR count (remaining capped cells and pupae parts) from the 0HR count. Divide this sum by the 0HR count. Move the decimal over twice to the right to get total % removed.

Equation

([0HR count] – [24HR count]) / [0HR count] = ___% removed

Example:

Total number cells in PVC: 150

Uncapped count (0HR): 12

0HR count: 138 (That is, 150 total cells minus 12 uncapped)

24HR count: 4

Given the equation and cell counts above:

([138] – [4]) / [138] = .97

This colony removed 97% of the liquid nitrogen-killed brood and is considered hygienic.

Don’t want to count cells and use equations?

Simply look at the test 24 hours later and determine if the freeze-killed brood is closer to 100% uncapped (highly hygienic) or closer to 100% capped (highly unhygienic). Those colonies that uncap the most cells by 24 hours after the freeze-killed brood is returned to the colony will be the most hygienic and the colonies to select as breeder queens if they also have other desirable characteristics (i.e.-productivity, temperament).

Acknowledgments

This Primefact was reviewed by NSW DPI Technical Specialist Honey Bees Dr. Doug Somerville.

For updates go to www.dpi.nsw.gov.au/factsheets

Published by the NSW Department of Primary Industries.

PUB14/265