ALBERTA TECH TRANSFER PROGRAM

NOSEMA SAMPLING

Spore Counting Procedure to Monitor the Infestation level of Nosema Microsporidians

Nosema is a microsporidian that can gravely affect a honeybee colony's health and performance. This disease can result in an increase in winter mortality, suppression of the immune system, and as a result will have a negative effect on colony productivity. Nosema affects the hypopharyngeal glans in honeybees causing a decrease in the production of larval food and brood rearing. Monitoring the level of Nosema in a colony provides an indication of the infestation level and is an indicator of colony health.

Lab Analysis Supplies

1. Microscope (compound or phase)
2. 70% ethanol
3. Pasture pipette
4. Hemocytometer with cover slip
5. Plastic bag
6. Rolling pin
7. 60 ml liquid measure
8. Filtered water

Field Sampling Supplies:

1. Sampling Jars
2. 70% Ethanol (or winter washer fluid)
3. Small bin/tub (e.g. dishwashing tub)
4. ¼ cup measuring cup

STEP 1

Choosing a Frame

Select a frame with mature bees, as they are most likely to have the highest amount of Nosema spores. These bees can be found on any frame from a honey super or an outer honey frame in the brood nest. Ensure that the queen is not present on the frame.

STEP 2

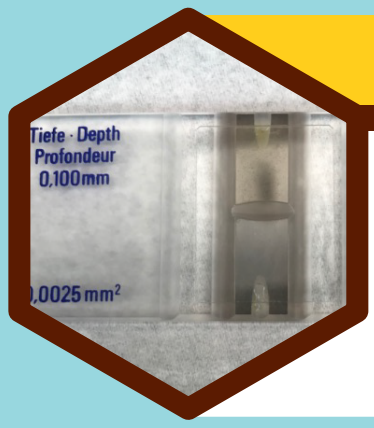
Collecting Bees

Shake the selected frame into the tub. Gather the bees into the corner and scoop out ¼ cup. Alternatively, you can gently scrape a ¼ cup of bees right from the frame. Pour the bees into a jar that is 1/3 full of ethanol (or washer fluid). Seal the jar tightly with the lid when finished.

STEP 3

Preparing the Sample

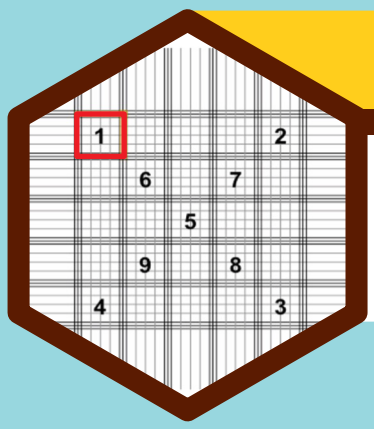
Count out 60 bees from the sample and place in a plastic bag. Macerate the bees in the bag with a rolling pin to crush into small bits (smaller the pieces the better). Add 1 ml of filtered water per bee to the bag (in this case 60 ml) and mix by squeezing the bag.



STEP 4

Setting Up the Hemocytometer

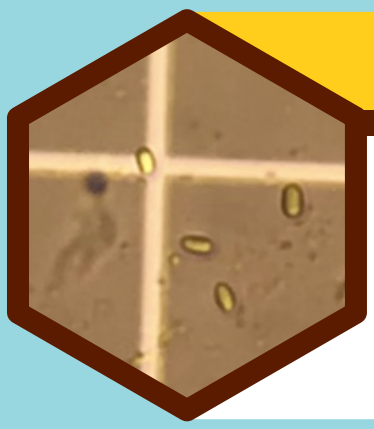
Place the hemocytometer onto a flat surface with the coverslip on top. Slowly pipette a small amount of the prepared sample into each well of the hemocytometer. If some spills out, use a kim wipe to carefully soak up excess fluid. Let the sample sit for ~2 mins so the contents can settle.



STEP 5

Focusing the Microscope

Place the sample on the stage of the microscope. Focus on square 1 using 40X objective lens. This is the ideal magnification to see Nosema spores.



STEP 6

Recognizing a Spore

Nosema spores will look like rice grains and have a distinctive ‘halo’ around them. When counting the spores, do not count it unless you can confirm it is a spore. Do not count anything that is mis-shapen or in large clumps.

CANADIAN AGRICULTURAL PARTNERSHIP
ALBERTA BEEKEEPERS COMMISSION
Alberta
Canada

STEP 7

Counting the spores

Count all the spores in the 16 smaller squares within square 1. Be sure to only count the spores that land on the top and right-hand lines (in yellow). Leave out spores on the left and bottom lines to avoid double counting. Count all the spores in boxes 1-5 and record the number after counting each box.

STEP 8

Calculate your Percent Infestation

To calculate the percent infestation, you need to know the amount of liquid in each square of the hemocytometer.
In 1 square there is 0.0000008 ml.
If you counted 5 squares, the total volume is 0.0000008 ml x 5 = 0.000004ml.
Take the total number of spores counted in all 5 boxes/ 0.000004ml = the number of spores/ml.
1ml = 1 bee, therefore the value calculated is also equal to number of spores/bee.

**The economic threshold for Nosema is 1 million spores per bee*

STANDARD PRECAUTIONS FOR NOSEMA SAMPLING

1. BE EXTREMELY CAREFUL HANDLING ETHANOL OR WINDSHIELD WASHER FLUID AROUND A LIVE COLONY
2. MAKE SURE EACH COLONY IS SAMPLED SEPERATELY
2. MAKE SURE TO RINSE THE HEMOCYTOMETER, COVER SLIP, AND PIPETTE BETWEEN EACH SAMPLE

CANADIAN AGRICULTURAL PARTNERSHIP
ALBERTA BEEKEEPERS COMMISSION
Alberta
Canada