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Nosema diagnosis

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Introduction

Nosema disease is the most important adult bee disease but is mostly overlooked by beekeepers as there are no characteristic obvious symptoms. Hence, nosema disease is also referred to as 'the silent killer'. The repercussions of this infection have been considered to equal or exceed the losses caused by all of the other diseases, including the more easily diagnosed brood diseases.

This Primefact complements Primefact 699 *Nosema disease*.

Diagnosis

The microscopic examination of bees or their faecal samples is the only method that provides a definitive diagnosis of nosema regardless of the level of infection. There are a number of methods by which infection can be determined and these are all based on the detection of Nosema spores (Figure 1).

Materials required for carrying out counts:

- compound microscope with x400 objective
- microscope slides
- cover slips

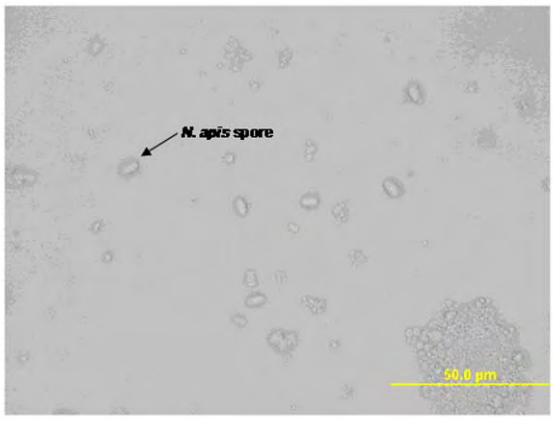

Figure 1: Wet preparation of N. apis spores prepared from whole adult bee sample

- mortar and pestle or equivalent
- bacteriological loops
- pipettes
- counting chamber.

Quick routine examinations can be carried out by examining whole bees or the abdomens from 10 bees or more.

The following procedure is a reliable method for determining the nosema spore count of infected honey bees.

Collect from 10 to 25 bees from under the lid, from outside the cluster or from the hive entrance just before or after flight. (Newly emerged bees have not had time to become infected and do not contain spores. These are unsuitable for nosema spore counts.)

The bees can be collected in 70% alcohol (methylated spirits can also be used) if they need to be stored or submitted to a laboratory.

After the bees have been immobilised by freezing they are placed in a mortar or dish with one millilitre of water per bee. Alternatively, the abdomens of the bees can be removed and used as the sample rather than whole bees.

The bees are then ground with a pestle or other suitable implement until an even suspension is formed. (The mortar and pestle should be thoroughly cleaned before being used again.)

A wet preparation is prepared by placing a drop of the resulting suspension on a microscope slide, covering the drop with a cover slip and examining the resultant preparation under the high dry objective (x400) of a compound microscope. Nosema spores have a rice grain appearance (Figure 1). This provides a non-quantitative assessment of spore numbers but is adequate for determining whether infection is present.

Alternatively, a counting chamber such as an improved Neubauer counting chamber (approximate cost $50.00 and available from scientific suppliers) can be used to determine the number of spores per bee. This chamber consists of a cover glass and a chamber that holds a

specific volume of fluid and is marked with a grid pattern for ease of counting.

- Ensure chamber is clean before use.
- Inoculate suspension using a loop or pipette under the cover glass (Figure 2).
- The material will flow under the cover glass and fill the chamber (do not overload and avoid producing bubbles).
- Let the suspension settle (about 3 minutes).
- Then count the spores in 5 large squares (80 small squares). See Figures 3 and 4.
- The number of spores per bee is determined according to the following formula.

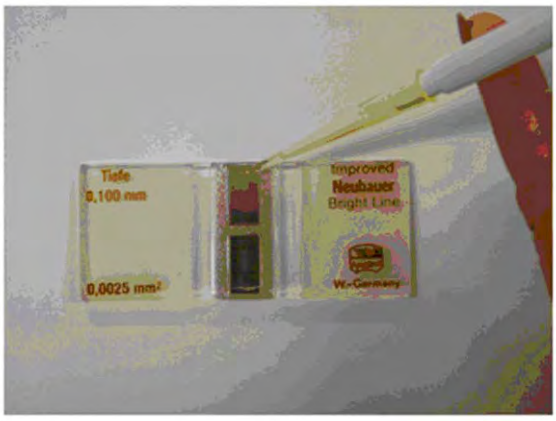

Figure 2: Inoculation of improved Neubauer counting chamber to facilitate the counting of nosema spores

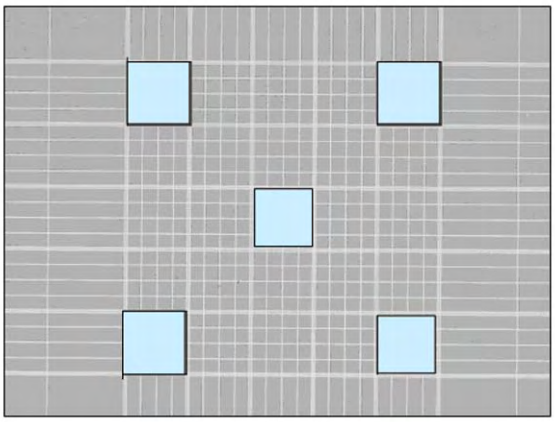

Figure 3: Five large squares (each containing 16 smaller squares) in which nosema spores are counted to determine infection levels of adult bees.

Calculation

$$\frac{\text{Total number of spores counted}}{\text{Number of squares counted}} \times 4 \times 10^6$$

= Number of spores per bee

or more simply:

Number of spores per bee = Number of spores in 5 large squares (80 small squares) x 50,000

In Figure 4 there are 47 spores in the 16 small squares. Assuming that the other 4 large squares (see Figure 3) also contained 47 spores the nosema spore count would be:

47 x 5 x 50,000 = 11,750,000 spores per bee

NB: Spores that touch lines A should be included in the count. Spores that touch lines B should not be counted.

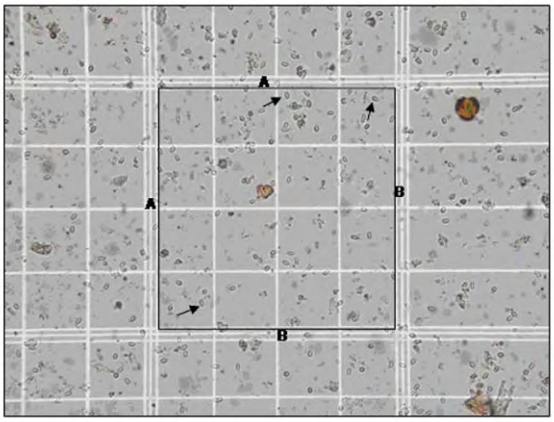

Figure 4: One large square containing 16 smaller squares and nosema spores. Small arrow heads point to 3 nosema spores.

References

Cantwell GE (1970) Standard methods for counting nosema spores. *American Bee Journal*. 110: 222-223.

Hornitzky M (2008) *Nosema Disease – Literature review and three surveys of beekeepers – Part 2.* Rural Industries Research and Development Corporation. Pub. No. 08/006

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