Canadian Association of Professional Apiculturists
L'Association Canadienne des Professionels de l'Apiculture

Nosema Disease – Diagnosis and Control

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Biology

1. *Basic Biology*

- Caused by two different species of *Nosema apis* and *Nosema ceranae*. While *N. apis* has long known to infect honey bees, *N. ceranae* may have been recently introduced. Nosema belongs to a group of highly specialised parasitic fungi.
- ***Only infects adult bees***. Although all castes are susceptible, workers are more readily infected.
- The infection begins when bees ingest Nosema spores (Figure 1).
- When the spore reaches the bee gut, it explosively uncoils a long straw-like polar filament. The filament penetrates outer gut cells and, in the process, infects them. The infection gradually colonises the entire gut (within 2 weeks in *N. apis*).
- Spores pass to other workers when they are shed in worker feces. The infection spreads during periods when workers have fewest opportunities for cleansing flights (eg winter) and are forced to defecate onto the comb. Residual spores on the comb continue infect bees even after cleansing flight resumes.

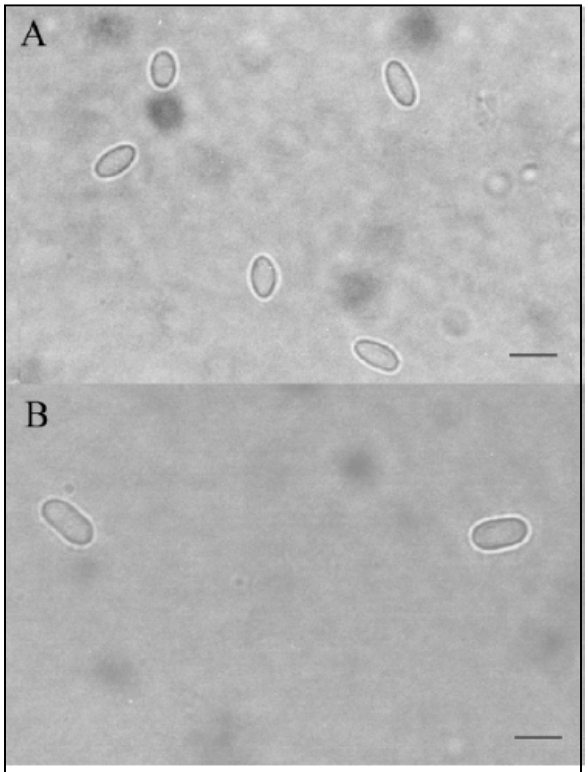

Figure. 1. Spores of *Nosema ceranae* (A) and *Nosema apis* (B) in light microscopy squash preparations. Bar = 5 μm (from Fries, 2006, *Journal of Apicultural Research* 45(3): 230–233).

2. *Seasonal Disease Cycle*

- *N. apis* infection typically increase through late winter and peak by late spring/early summer. The seasonal cycle of *N. ceranae* remains unknown.

3. *Economic Impact*

- Infected workers do not adequately develop their nursing glands and have a reduced lifespan.
- Infected queens are usually superceded, even when the infection is light.
- Overall colonies infected with *N. apis* are more likely to die overwinter, are slow to build in the spring and are poor honey producers in the summer. The impact of *N. ceranae* has not been determined, but some early research suggests its effects are more severe.

Diagnosis:

1. *Collecting Samples*

- Collect 10 to 25 bees from hive entrance. In poor weather, bees can be sampled from under the top lid or the outside of the cluster. It is important that the bees collected are older as bees less than 8 days old will not have had time to become infected.
- To estimate the overall level of *N. apis* infection for a yard it is recommended that a few bees from the hive entrance of a number of colonies in the yard be collected. Total sample size should be ~100 bees.

- Although live bees are preferable, dead bees can also be collected and examined for spores.
- Sampling can be done in the late fall or in the early spring.

2. *Mailing Samples*

- If samples are to be mailed away for diagnosis, live bees should be soaked in alcohol for at least 5 minutes and dead bees for 10 minutes. Drain off the alcohol and seal sample in a suitable container. Isopropyl (rubbing) alcohol is readily available but other types will also suffice. The alcohol will prevent the bees from decomposing prior to examination.
- It is important to also include any other observations which might provide information about the colony's problem.

  Samples for diagnosis can be sent to your provincial apicultralist.

3. *Microscopic Examination*

- Microscopic examination is the only method which will provide a definitive diagnosis of nosema.
- Place bee samples in freezer to immobilize bees. Add 1 mL water/bee and grind up sample until mixture is uniform. A mortal and pestle works nicely or samples can be sealed in a Ziploc bag and crushed using a rolling pin. For a cleaner preparation, remove heads and use only the abdomens or remove the digestive tracts from the abdomen and grind up. A good website that explains how to remove the abdomens is http://www.ars.usda.gov/is/np/honeybeediseases/honeybeediseases.pdf.
- Bees can also be examined individually to obtain a percentage of infected bees per colony.
- Place a drop of the suspension on a microscope slide and cover with a cover slip. Examine for *N.apis* spores under the high dry objective (400x) of a compound microscope. **Important:** this will only tell you whether nosema is present or not.
- To determine the number of spores per bee a counting chamber or haemocytometer can be used. It can be purchased from a scientific supply house. A haemocytometer consists of a special cover slip, a chamber that holds a specific volume of fluid (0.05 x 0.05 x 0.1 mm = 0.00025ml) and is marked with a grid pattern.
- Place a small volume of spore suspension under the cover glass, an eyedropper works nicely. When the droplet touches the cover glass and chamber the correct volume will fill the chamber. Allow spore suspension to settle for 3 minutes. Count.
- To count, find the ruled area and focus the microscope to sharply define spores. Count all spores in 5 blocks of 16 small squares. Do not count spores directly on the lower or left line of the large block but do count spores on the upper and right hand lines. A total of 80 small squares will be counted.
- Calculate number of spores per bee:

  # of spores/bee = average number of spores per little square x 4,000,000.
- A good website which shows videos on using the haemocytometer is http://www.uni-greifswald.de/~immuteach/methods/counting_chamber/counting_chamber.html#

4. *Interpretation of Results*

- Treat colonies if there are **more than 1 million spores per bee or if 50% of bees are infected**

Control:

1. *Disinfecting Comb*

- Nosema readily introduced to healthy on contaminated comb. Experimental work suggests that colonies transferred onto foundation or disinfected drawn comb maintain minimal levels of nosema without the aid of medication. Transfer of colonies to clean equipment is readily done during the spring dandelion flow (Figure 2).
- Comb can be disinfected by electron-beam irradiation, maintaining comb at 50°C (120°F) for 24h or fumigation with concentrated (80%) acetic acid (Figure 3).

2. *Medication*

- Fumagillin (dicyclohexylammonium fumagillin) is the only product registered for the control of nosema. It is marketed under the trade name Fumagilin-B (Medivet Pharmaceuticals, High River,

AB). Each gram of Fumagilin-B contains 21 g fumagillin. There are two rates on the label and treatments are fed preventatively in syrup feed:

- Wintered Colonies. Each is treated with 190mg of fumagillin in the fall (ie about 9.0 g **Fumagilin-B** per colony). Consequently treatment of 50 colonies would require the entire contents of a 454g jar of Fumagilin-B.
- Package Colonies. Each is treated with 95mg of fumagillin in the spring after hiving (ie about 4.5 g **Fumagilin-B** per colony). Consequently treatment of 100 colonies would require the entire contents of a 454g jar of Fumagilin-B.
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- Stability. Fumagillin rapidly breaks down in light. Studies have shown, however, that the breakdown products remain active against Nosema even after continuously being exposed to daylight for 6 hours. Samples of spring honey taken from colonies medicated the previous fall demonstrate that fumagillin remains active through the wintering period. Nonetheless a number of W. Canadian studies suggest that colonies treated with fumagillin in the fall may have elevated levels of nosema in spring and may benefit from a second treatment.
- Queen Production. In the close-confines of a mating nuc there is an increased chance that a queen will be infected with nosema. It is important to medicate any infected mating nuc with fumagilin before installing queen cells. Similarly, attendant bees in queen cages should be drawn from colonies that have been medicated. Nosema infected queens frequently supersede and it is important to prevent their infection. Surveys of commercial queens indicate that up to 10% of the queens sold have nosema.

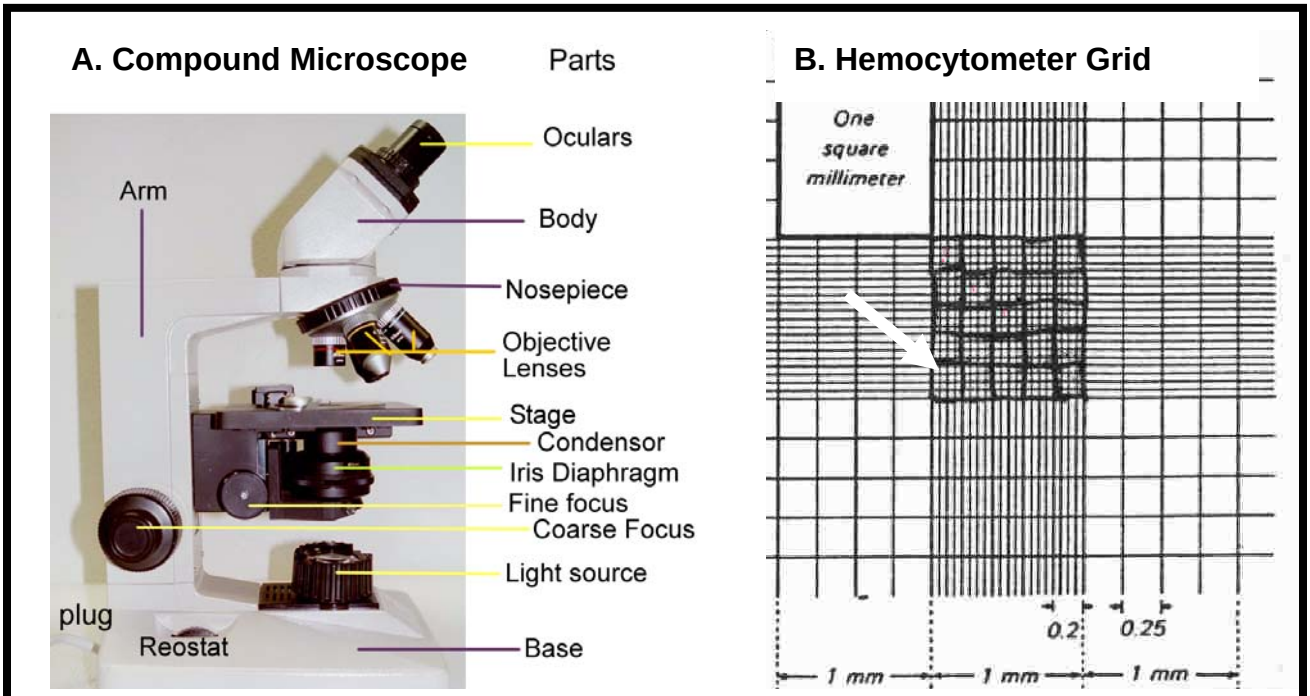

Figure 2. Tools for counting nosema spores (A) compound microscope and (B) haemocytometer. The white arrow points to a square containing 16 smaller squares. The number of spores per bee is the average number of spores per smaller square multiplied by 4,000,000.

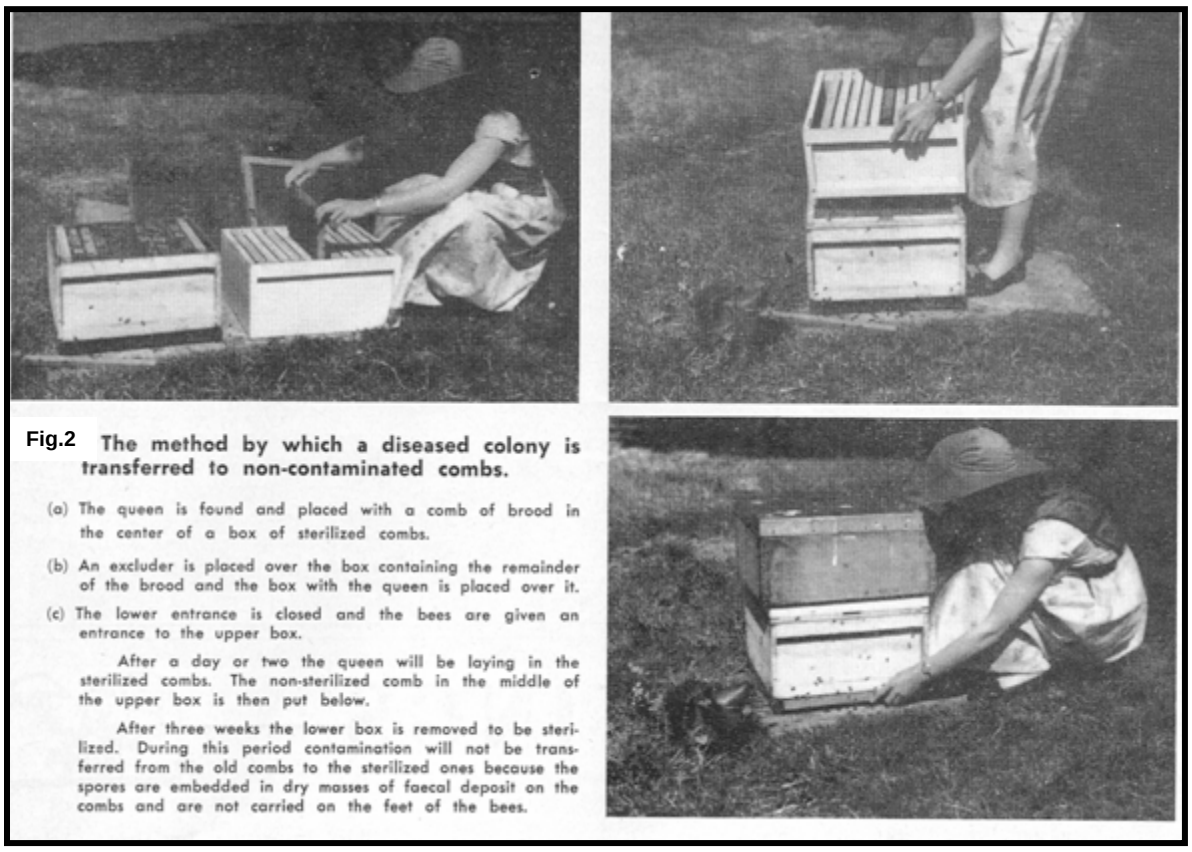

Fig.2 **The method by which a diseased colony is transferred to non-contaminated combs.**

(a) The queen is found and placed with a comb of brood in the center of a box of sterilized combs.

(b) An excluder is placed over the box containing the remainder of the brood and the box with the queen is placed over it.

(c) The lower entrance is closed and the bees are given an entrance to the upper box.

After a day or two the queen will be laying in the sterilized combs. The non-sterilized comb in the middle of the upper box is then put below.

After three weeks the lower box is removed to be sterilized. During this period contamination will not be transferred from the old combs to the sterilized ones because the spores are embedded in dry masses of faecal deposit on the combs and are not carried on the feet of the bees.

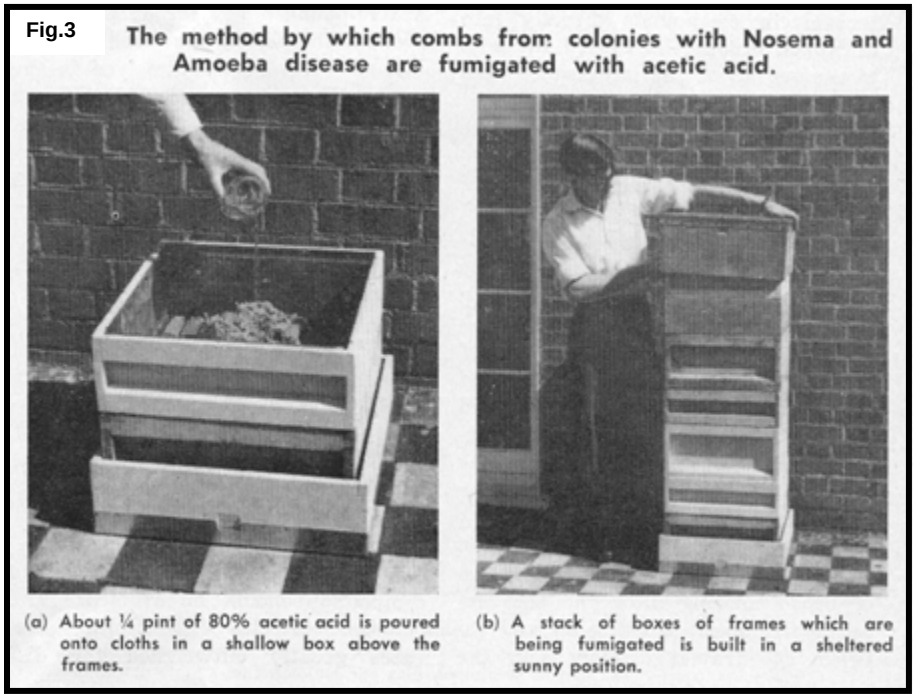

Fig.3 **The method by which combs from colonies with Nosema and Amoeba disease are fumigated with acetic acid.**

(a) About ¼ pint of 80% acetic acid is poured onto cloths in a shallow box above the frames.

(b) A stack of boxes of frames which are being fumigated is built in a sheltered sunny position.

Figures from Bailey, L. 1957. Comb fumigation for nosema diseases. American Bee Journal. January: 24-26.

A brood chamber full of empty combs is placed on an inverted lid. An empty shallow super is placed on top of this brood chamber, and absorbent material placed inside. The absorbent material is soaked with 150 ml of 80% glacial acetic acid. A further brood chamber full of combs is placed on top, and then another empty shallow super containing more rags soaked in the fumigant, and so on. The pile is eventually closed on top with a lid. The whole pile should be built out of doors during the summer, against a wall so that it is protected from strong draughts. The cracks between chambers can be ignored, but if any large holes had been present they must be blocked. The stack should be left to fumigate for 7 days followed by 7 days airing. Read the Material Safety Data Sheet for glacial acetic acid before use to determine appropriate personal protective equipment and other safety precautions.