Diagnosing and Treating Nosema Disease

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Causative Agent

Nosema disease in U.S. honey bees is caused by one of two (or both) fungi named *Nosema apis* and *Nosema ceranae*. *Nosema* species are obligate, fungus-like, intra-cellular parasites that are limited to specific hosts species. *Nosema apis* and *N. ceranae* cannot be reared in laboratory culture, as is possible with most bacteria and other fungi. They can multiply in living honey bee midgut, and perhaps other, cells. There is evidence that, like *Nosema bombi* in bumble bees, the *N. ceranae* may infect other honey bee tissues, but that remains to be substantiated.

Life Cycle

When a bee ingests *Nosema* spores, the spores are filtered out of the honey sac by the proventricular valve and released into the midgut. The exact physical and chemical conditions of the honey bee midgut stimulate germination. The organism penetrates a midgut cell and grows by absorbing nutrients from that cell. The parasite increases in size until it is large enough to divide in half. Each new parasite continues this multiplication process until the nutrients in the cell begin to become exhausted. That stimulus triggers sporulation. Depending upon the species of *Nosema*, approximately 100 spores can begin to develop as early as four days post-infection or up to nine days later. Some of the early, thin walled spores appear to germinate inside the infected cells, sending their polar filaments into adjacent cells. In this manner, they can make their way through the body cavity, infecting other tissues, at least in bumble bees. The nutrient-depleted host cells rupture. Environmentally resistant, thick walled spores are released into the midgut lumen to start the process, again, or be excreted to the outside. Heavily infected worker honey bees can contain an excess of 50 million spores. Damaged intestinal tissue is subject to secondary infections and "dysentery" (brown diarrhea spots on the combs and exterior of the hive) is a common sign of infection with *Nosema apis*, but not seen with *N. ceranae*. *N. apis* infected bees also defecate inside the hive, contaminating combs with millions of infectious spores.

Effects on Colony

Nosema infections have specific negative effects on honey bees. Worker bees that ingest spores when they are less than a week old normally do not digest food well and are not capable of producing brood food secretions. Infected bees tend to skip the brood rearing phase of life and become foragers at very young ages. Their life spans can be reduced up to 78%. Young queens that ingest *Nosema apis* spores normally are superseded within a month. In climates where winter prohibits supersedures for many months, colonies often go queenless and dwindle away in early spring. Experience in Minnesota suggests that an average of one million or more *N. apis* spores per bee can lead to increased winter losses. When high percentages of workers are infected and spore counts exceed ten million spores per bee, significant numbers of colonies will die or lose queens during the winter. With *Nosema apis*, this spike in the level of infection

normally occurrs in early spring, then "goes away" as the weather improves and the bees defecate outside the hive. With *Nosema ceranae* having become the dominant species, infection and spore levels can be elevated all year. All levels of infection led to very slow spring build up with *Nosema apis*, even when forage and temperatures were ideal. Frequently, reduced honey yields followed this poor population build up. In Spain, year 'round infections with *Nosema ceranae* did not seem to interfere with spring build up and swarming, but were found to lead to very high percentages of lost colonies during summer and through to the next spring.

Diagnosis

Nosema disease is difficult to diagnose without using laboratory equipment. Pulling the last abdominal segments from a bee usually will remove the intestinal tract intact. According to some authors, a healthy midgut is tan in color, with concentric constrictions. An infected midgut will become swollen, whitish and lose its visible constrictions. There is so much variation that this method of diagnosis really cannot be trusted. Besides, other causes of dysentery, such as ingesting honeydew, fermented syrups, indigestible sugars in cola syrups, molasses and kitchen corn syrups can result in similar intestinal changes.

Scientists use a specific methodology to determine levels of infestation. Known numbers of severed abdomens are homogenized, using a mortar and pestle. The homogenate is sieved through two layers of cheesecloth into calibrated centrifuge tubes. The tubes are spun in a clinical centrifuge at 600 rpm for six minutes to drive the spores to the bottom of the tubes. The liquid (supernatant) is poured off (decanted) and the plug (pellet) at the bottom is resuspended in a specific volume of water (final calculation is spores in one ml water per bee). The plug is broken up well (resuspended) by sucking the water in and out many times through a small-tipped disposable pipette. Then a small droplet of the suspension is placed on a blood cell counting chamber (hemocytometer). The number of spores counted over certain areas of the chamber grid can be converted to millions of spores per bee. If infection levels are below 10,000 spores per bee, no spores will be seen over the entire grid and the diagnosis is determined to be "not detected" or "ND." That does not mean that there is no infection.

Treating Infected Colonies

Medicating for *Nosema apis* is based on the most appropriate times to prevent comb contamination and development of disease in bees that clean up fecal deposits from combs while expanding the brood nest. Later in the summer, when bees are defecating outside the hive, *N. apis* usually cannot be detected. A few bees are infected all year, but only the diseased late season bees are of consequence. When they develop high levels of infection, they defecate on the combs in October, November and December, then die.

Brood rearing never ceases in many parts of California over the winter, but as the days begin to lengthen in late December, the bees are stimulated to pick up the pace. Availability of nectars and pollens, along with warming temperatures, accelerate brood rearing. It is at this time that many bees "cleaning and polishing" cells, in anticipation of egg laying, become inoculated. How severe the disease will get in the colony population depends upon the initial spore load

(amount of contamination) and how much of the time the bees are confined to the hive by non-flight weather. So, *Nosema apis* levels can vary significantly from year to year.

In order to "cover the bases" in Minnesota, if a colony population had one million or more spores per bee in April, we fed it two gallons of fumagillin-medicated, heavy (two parts sugar : one part water) syrup the following September. If we had to "feed for weight," that was done earlier, so that the early syrup could be "ripened" and stored before the medicated syrup was applied. If the medicated syrup is mixed with other, unripened syrup, it can be diluted to ineffective concentrations. We anticipated that the medicated syrup would be consumed throughout the winter. Spore deposition on combs in early winter would be reduced and the parasite could not reproduce in medicated bees that became inoculated in the spring. The syrup would be consumed, totally, long before the bees produced any honey.

Although we have not conducted the experiments, it is likely that two gallons of medicated syrup may not be required in most of California. *Nosema apis* levels were not as high in California as they were in Minnesota. Combs should not be so badly contaminated during the winter months, since intermittent flight is possible. Therefore, first treatments with medicated syrup should coincide well with the normal practice of providing colonies with "stimulative" syrup and pollen substitute feeding in late December and January. A gallon, or so, of medicated syrup should provide protection against *Nosema apis* until the bees are flying well in March and April. Heavy nectar flows from *Manzanita*, *Eucalyptus*, mustard and radish might dilute the medication significantly, as would later feeding with non-medicated syrup.

Medicating for *Nosema ceranae* is going to be different. For one thing, fumagillin no longer is available as Fumidil-B®. A Canadian company, Medivet, sells the product as Fumigilin-B®. The new fumagillin mixes more readily into solution and it appears to be less stable in solution, if the label instructions are correct. The label suggests using the antibiotic in the fall, as always has been the ractice at that time of year for nosema control. Since *N. ceranae* tends to persist throughout the year, the label calls for increasing the dosage level, and feeding repeatedly in small amounts of sugar syrup, in the spring. Beyond that, Spanish researchers have found that *N. ceranae*-infected bees tend not to take medicated syrup from feeders, so they had to pour the syrup over the bees (called “drench”), down between the frames, to make them consume the medication.

Expected Results of Treatment

Beekeepers who have fed fumagillin to field colonies years ago had noted significant differences in colony build up. In fact, many of them stopped using fumagillin. The colonies built up too quickly and swarm control became nearly impossible. With so many colonies succumbing to CCD at this time, I doubt that swarm control will be a major issue any longer.

Decontamination of Equipment

The spores of both nosema species are susceptible to irradiation or fumigation with glacial acetic acid (details in *The Hive and the Honey Bee*). Otherwise the spores of apparently Europe-originated *N. apis* are very resistant to the elements, except sunshine, and persist for

many years. *Nosema ceranae*, on the other hand, originated in tropical Asia, and its spores are susceptible to weather, especially cold weather. The spores can be killed by refrigeration or by freezing. If possibly spore-contaminated combs are placed in a freezer and left there until it is certain that all materials in the combs have reached freezing temperatures (honey holds a lot of heat for quite a while), *N. ceranae* spores, all life stages of greater wax moth, all stages of small hive beetle, and other little critters that tend to get into stored combs will be eliminated.

I am happy to discuss *Nosema*, its consequences in colonies, and treatments. I can be reached by telephone at: (530) 752-0472 or by email at: ecmussen@ucdavis.edu. Copies of this "Bee Brief" can be downloaded at: http://entomology.ucdavis.edu/facutly/mussen/beebriefs/index.cfm.