A Guide to the Honey Bee's Protozoan Nosema Disease and Treatment Recommendations for Pest Control

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Abstract

Like other insects, the honey bee is subjected to many diseases and their early detection allows for prompt remedial action, and help in preventing serious outbreak and economic losses. These diseases differ in their severity, but all of them can be prevented or controlled by proper management. This handout offers information about field symptoms and diagnoses of the most common honeybee protozoan Nosema disease and treatment recommendations for pest control. There are two forms of the microsporidian Nosema associated with clinical signs of disease in honeybees, *Nosema apis* and *Nosema ceranae.* The diseases of bee are usually divided into two classes, those that attack the developing stages (the brood), and others that attack adult bees. In general, the brood diseases are more serious, and their symptoms are more definite and distinctive than those of the adult diseases. It takes experience and close observation to distinguish a diseased larva or pupa from a healthy one, or one dead from other causes. This experience can be gained only by frequent examination of the combs of a colony. For field diagnosis, Nosema incidence in honeybee colonies peaks in early fall and early spring in only adult bees by parasitizing their midgut, impairs the digestive process and causes bee's starvation. Adult bees have difficulty with controlling their fecal discharge and in heavy infestation, hive bodies are often smeared with fecal deposits. For Nosema confirmation, adult bees must be examined microscopically wherein spores are large, oblong shaped and highly uniform. For determining the level of infestation, a haemacytometer can be used to calculate the number of spores per adult bee. Though the diseases differ in their severity, but all of them can be prevented or controlled by proper management. Such management includes knowing and recognizing the symptoms of diseases, inspecting colonies regularly, and applying control measures promptly when disease is found. This is one of the reasons why the beginning beekeeper must open the colonies regularly. There are no IPM approaches specifically targeted against Nosema disease and its management, however on the maintenance of robust colonies, there should be periodic application of the antibiotic Fumagilin® B. The medication is mixed in sugar syrup according to manufacturer's recommendations and fed to bees in spring and fall. As with any medication, it is applied in the off-season when there is no chance that it will contaminate marketable honey. Drugs and antibiotics are effective in preventing disease, but cannot substitute for good management practices. They must be used at the proper time and dosage to avoid contamination of honey, and any recommended product is to be used only in accordance with the directions on the product's label.

Keywords: Nosema, Microsporidum, Protozoan, Honey bee, Colony disorder

1. Introduction

Several species of pathogens infest honey bees in various states and elsewhere, but the extent of such damage has not been measured. Some mites found in honey bee colonies in other countries, particularly in the tropics, cause serious injury to developing brood and adult. In some parts of the world, adult bees suffer from several diseases that are usually found in most colonies, but rarely cause serious damage. Those diseases that attack the developing stages (the brood), in general, are more serious and their symptoms are more definite and distinctive than those of the adult diseases (Collison et al., 2004; Sarwar, 2015). The Nosema disease is caused by the spore-forming microsporidum protozoan *Nosema apis* or *Nosema ceranae* (Sporozoa: Nosematidae), that invades the digestive tracts of honey bee workers, queens and drones. Adult bees ingest Nosema spores with food or water, spores germinate and multiply within the lining of the bee's midgut, millions are shed into the digestive tract and eliminated in the feces. When spores into the digestive tract are eliminated in the feces, fecal staining occurs on outside of hive. The Nosema disease may be present at any time during the year, but *N. ceranae* appears to be more common during summer and is sometimes referred to as 'dry nosema' as heavy load of this pathogen does not cause the characteristic fecal staining associated with *N. apis*, which appears in fall and winter. Damage to the digestive tract may produce symptoms of dysentery (diarrhea). Especially in winter, infected workers, unlike healthy workers, may defecate in or on the outside of the hive rather than out in the field. Diseased colonies usually have increased winter losses and decreased honey production. When queens become infected, egg production and life span are reduced, leading to supersedure. The loss of the queen in colonies newly started from package bees is a serious effect of the disease. Infection in worker bees inhibits digestion of food in the stomach and production of royal jelly. As a result, the productive life of the worker is shortened and its ability to produce brood food decreases, thus retarding brood production and colony development (Morse and Nowogrodzki, 1990; Flottum, 2010). The only way to positively identify Nosema disease is through the dissection of adult bees and comparison of healthy and diseased honey bee gut. The hind gut and digestive tract of diseased bees are chalky or milky white. Healthy bees, on the other hand, have amber or translucent digestive tracts. In addition, the individual circular constrictions of a healthy bee's gut are visible, whereas the gut of an infected bee may be swollen and the constrictions may not be clearly visible. More commonly, bee abdomens are masticated in a known quantity of water and an aliquot of the suspension is examined under 100 X magnification for presence of Nosema spores (Mattilla and Otis, 2006).

2. Nosema Disease of Honey Bees

The Nosema disease is an infection of the digestive organs of the adult bee by a single-celled organism, a protozoan called *Nosema apis*. During the normal digestive process of adult bees, healthy cells of the stomach lining are shed into the stomach. They burst open and release digestive enzymes. Infected cells are also shed in this way, but they release Nosema spores and not digestive juices. These spores can infect other healthy cells of the stomach lining and many spores pass through the intestines and are present in the feces (excreta) of the bee. Small numbers of infected bees may be found at almost any time of year in apiaries. The natural defenses of the individual and the colony against disease tend to keep it under control. However, when the bees are confined to live by poor spring weather, or subjected to stress from moving or special manipulations, such as those for queen rearing and for shaking package bees, the disease may reach damaging levels. The lives of infected bees are shortenedand affected colonies are weakened, but rarely killed. The Nosema-infected colonies do not show any symptoms that are

typical of the disease. For this reason, positive diagnosis can be made only by examination of bees for the presence of spores of Nosema. To do this, ground-up abdomens or alimentary tracts must be examined under a microscope at 400 X magnification to detect the organism (Potts et al., 2010; Neuman and Carreck, 2010).

The Nosema disease is cyclical in its severity in the bee's colony, with the greatest infection in late spring and the least in late summer or fall. The spores of the nosema organism are spread within and outside the colony with food and water. Infected bees soil the combs and spread infection within the colony. However, nosema infection does not cause dysentery, but bees suffering from dysentery may or may not have nosema disease. Empty combs contaminated with spores may be heated to 120 degrees F (49 degrees C.) for 24 hours to kill the spores. It can be controlled, at least in part, by feeding the antibiotic fumagillin (Fumidil B). Complete control is difficult because of the chronic nature of this infection in the bee's alimentary canal. The antibiotic must be available to the bees for a considerable time to rid them of the organism. Treatment is worthwhile; it reduces winter losses and creates stronger colonies in the spring. Treated colonies may also produce more honey. The antibiotic is most effective if fed in the fall when the normal level of the disease is lowest. Treatment in the spring is less effective, but may be of value for nucleus or package colonies (Van-Engelsdorp et al., 2010).

2. Dysentery (Diarrhea) Disease of Honey Bees

Bee dysentery is a health problem that affects hive individuals that do not live in hygienic conditions. This occurs when the bees have been prevented for a long time to perform cleansing flights. The diseases can also be triggered by food supplies that contain lots of impurities or by fermenting honey. Although it is not a disease, dysentery is considered here because so many beekeepers think of it as a disease symptom, especially of nosema disease. However, nosema infection does not cause dysentery, but bees suffering from dysentery may or may not have nosema disease. Bees with dysentery are unable to hold their waste products in their bodies and they release them in the hive or close to it. The condition is recognized by the dark spots and streaks on combs, on the exterior of the hive, and on the snow near the hive in late winter. Dysentery is caused by an excessive amount of water in a bee's body. The consumption during the winter of coarsely granulated honey or honey with high water content is one cause of the disease. Damp hive conditions may also contribute to the problem. Heavily infected bees may show symptoms such as disorientation, climbing grass stems, inability to fly, and they may also exhibit dysentery which can be confused with signs of Nosemosis. Low temperatures are a danger for honey bees, especially if they last for two-three weeks in a row. The bees cannot engage in cleansing flights and the chances for dysentery increase. The presence of tell-tale type of signs on combs, hive frames and hive entrance is a sign that the bees are affected by dysentery. Dead bees in the hive vicinity are also a sign of the disease. Good food and proper wintering conditions are important to prevent the problem, but there is no specific control for it once the bees are affected. The colony's recovery may be helped if it is given combs of low-moisture honey or fed heavy sugar syrup. Beekeepers having apiaries in temperate regions with harsh winters know that it is almost impossible for their bees to undergo cleansing flights in the cold season. For this reason they take out all the natural honey from combs and feed the bees with sugar syrup or a corn syrup that has a high content of fructose. These syrups are almost free of indigestible parts and thus there are fewer chances for bees to develop acute diarrhea. Bee dysentery can be treated by feeding the bees with sugar syrup enriched with medicinal plants

such as mint or yarrow. The colonies can be given food supplements consisting of honey and sugar cakes or sugar syrups that can contain a bit of raw milk or powdered milk (Graham, 1992; Stiglitz and Herboldsheimer, 2010).

3. Current Disease Incidence

This section provides beekeepers with further information about Nosema, which is a serious disease of brood and adult honey bees including queen bees. In some years, Nosema may cause serious losses of adult bees and colonies in autumn and spring. In recent years, another Nosema, *N. ceranae,* has been found to infect honey bees in a number of countries and information on this organism appears at the end of this annotation.

3.1. *Nosema apis* (Sporozoa: Nosematidae)

The *N. apis* is currently more prevalent in cooler climates and affects honeybees primarily in the spring and early summer It has been referred to as 'spring dwindling', with dysentery and fouling of the outside of hives with feces and it is known to age bees more rapidly in that they take on the duties of older bees and subsequently die sooner than non-infected bees. These precocious foragers have been shown to be less effective and resilient than normal foragers. The Nosema is historically considered the most serious disease of adult bees, caused by a single-celled microsporidian *Nosema apis*, which exists in two stages- a long-lived spore and a replicating vegetative stage. When spores of *N. apis* are swallowed by bees they germinate within 30 minutes inside the stomach. The organism then penetrates cells of the stomach lining and it continues to grow and multiply rapidly, using the cell contents as its food supply. Large numbers of spores are produced in the host cell in 6 to 10 days. The parasite may also penetrate and infect adjacent healthy cells. This spreads the infection further and if an adult bee ingests spores they germinate into the vegetative stage which penetrates the cells lining the bee's gut. The Nosema does not always kill a bee outright, but may trigger associated morbidities, including reduced lifespan, reduced output of brood food, and in the case of queens, increased supersedure rates. These symptoms may also be associated with tracheal mites and the collective result is colonies with low populations and sluggish spring buildup. The disease is regarded as more damaging in cold climates or under conditions that promote a protracted period of confinement in the hive (Fries, 2010).

3.2. *Nosema ceranae* (Sporozoa: Nosematidae)

A second species, *Nosema ceranae*, a natural associate of the eastern honey bee *Apis cerana*, has emerged in recent years as a problem, where it is suspected of causing large-scale colony deaths. The *N. ceranae* is known to occur in certain localities, and it probably contributes to similar occurrences of colony morbidity. It is nearly impossible to discriminate *N. apis* from *N. ceranae* without molecular techniques. Being a non-natural parasite on *A. mellifera*, there is concern that *N. ceranae* may have higher virulence toward the western honey bee, a pattern typical with many non-natural parasite relationships. However, studies have failed to firmly associate Nosema disease with wide-scale colony deaths, sometimes called colony collapse disorder. The *N. ceranae* has been first found in Asian honey bees (*Apis cerana*) and also found in European honey bees (*Apis mellifera*). As this is a relatively new parasite in honey bees, the full effect of

this species of Nosema on individual bees and colonies is still being researched. It does not appear to cause sudden, quick losses of bees as does *N. apis*. The *N. ceranae* can be detected in all four seasons, while *N. apis* occurs mostly in the milder seasons of autumn and spring. The *N. ceranae* infection affects more cells in the honey bee gut than *N. apis* infection at the same temperature. Researchers have suggested that this difference may explain why there is a higher mortality of bees when they are infected by *N. ceranae* than when they are infected with *N. apis*. The *N. ceranae* can kill bees faster than *N. apis.* Colonies infected with *N. ceranae* in summer may gradually lose adult bees resulting in reduced honey production and may even die. While dysentery may be associated with outbreaks of *N. apis*, signs of dysentery are markedly reduced in outbreaks of *N. ceranae*. The *N. ceranae* has been found in honey and pollen, and recent research has shown that *N. ceranae* spores lose viability when they are subjected to freezing and chilling (Paxton et al., 2007; Smart and Sheppard, 2012).

4. Incidence and Spread of Nosema

Infection of Nosema does not normally pass directly from infected bees to the next generation of adults. Instead, young bees become infected when they ingest spores as they clean contaminated combs. During the summer months, most honey bee colonies carry a few infected bees with little or no apparent effect on the colony. Spores may also persist on the combs and as the weather in autumn changes; these spores may initiate an outbreak of Nosema. Losses of bees at this time of the year may be very heavy. Winter losses can also be heavy when infected bees confined in their hives due to bad weather may defecate inside the hive soiling the combs and hive interior with excreta and spores. This, together with spores produced in the preceding autumn causes infection in spring. Spring outbreaks usually begin in late August or September, when temperatures begin to rise. They may last until late spring or early summer and when the warm weather comes, the disease begins to decline due to improved flight conditions. The source of infection is largely removed because the bees are able to defecate outside the hive thereby reducing the contamination of combs. Fortunately, serious Nosema outbreaks do not occur every year. Research has indicated that the subsequent conditions appear to be associated with serious autumn outbreaks and epidemics of Nosema, for instance, heavy summer rainfall, an early autumn break in the fine weather about mid-March to early April, and bees working grey box (*Eucalyptus microcarpa*), red ironbark (*E. sideroxylon*) and white box (*E. albens*). The exact reasons for these apparent relationships are not known. In these epidemics, strong colonies may be seriously weakened before winter. They may be reduced to the size of a nucleus colony in a matter of days. Infected colonies that survive the winter may require a long build-up period for the population of adult bees to reach normal numbers. Spores of *N. apis* may occur in honey or pollen and research reports indicated that honey bee workers can transmit Nosema to queens in queen mailing cages, queen banks and queen mating nuclei (Higes et al., 2009; Valera et al., 2011).

5. Effect of Nosema on Honeybee

i. Hypopharyngeal (brood food) glands of infected nurse bees lose the ability to produce royal jelly, which is fed to honey bee brood.
ii. A high proportion of eggs laid by the queen of an infected colony may fail to produce mature larvae.

iii. Young infected nurse bees cease brood rearing and turn to guard and foraging duties usually undertaken by older bees.
iv. Life expectancy of infected bees is reduced, and in spring and summer, infected bees live half as long as non-infected bees.
v. Infected queens cease egg-laying and die within a few weeks, but infected pupae are resistant to infection.
vi. An increase of dysentery in adult bees although Nosema is not the prime cause of dysentery.

It appears that Nosema spores are transmitted by a variety of routes including honey, pollen including pollen baskets from bees, wax, royal jelly and regurgitated pellets of the European bee eater (*Merops apiaster*) and mixed infections are common (Forsgren and Fries, 2010).

6. Honeybee Disease Detection

Details of apiary inspection and laboratory diagnosis carried out by researchers are held on the secure pages of bee base. This enables the production of accurate up-to-date information on the distribution of notifiable bee disease data. Bees infected with Nosema either show no symptoms, or none that are specific for this disease. Many of the so-called symptoms attributed to Nosema disease apply to other diseases or conditions of adult bees. Examination of adult bees using a light microscope is the only reliable method of diagnosing the presence of spores of Nosema (Sammataro and Avitabile, 2006; Genersch, 2010; Traver et al., 2012).

6.1. Nosema Field Diagnosis

i. Nosema incidence in honeybee colonies peaks in early fall and early spring.
ii. The disease only affects adult bees by parasitizing their midgut. Adult bees have difficulty with controlling their fecal discharge. In heavy infestation, hive bodies are often smeared with fecal deposits.
iii. The disease is often not detected because affected bees are either inside the colony (in winter) or in the field, where they die.
iv. Nosema impairs the digestive process and causes bee starvation.
v. Nosema is often confused with dysentery, which produces similar symptoms.

6.2. Nosema Laboratory Diagnosis

i. For Nosema confirmation, adult bees must be examined microscopically.
ii. Standard detection method is, collect 25 dead bees and place in mortar with 25 ml of water (i.e., 1 ml water for every adult bee). Grind up and collect one droplet of solution, place on slide and cover with coverslip.
iii. Examine under 100 X power of compound microscope whereby Nosema spores are large, oblong shaped and highly uniform.
iv. For determining the level of infestation, a haemacytometer can be used to calculate the number of spores per adult bee.
v. For Nosema confirmation, collect at least 25 dry bees in tissue paper (no plastic) and mail to the Apiculture office.

Infected colonies can lose adult bees sometimes at an alarming rate. Infected bees often die away from the hive and only a few sick or dead bees may be found near the hive entrance. The term 'spring dwindle' is often used to describe this condition. However, this should not be confused with the normal weakening of colonies caused by the natural dying of old, over-wintered bees in early spring. Sick or crawling bees outside the hive entrance, dead bees on the ground and excreta (dysentery) on hive components may be associated with Nosema infection, but may equally be caused by other diseases and abnormal conditions.

7. Control of Nosema in Honeybee

There are no IPM approaches specifically targeted against Nosema disease and its management is biennial application of the antibiotic Fumagilin® B over the colonies. The medication is mixed in sugar syrup according to manufacturer's recommendations and fed to bees in spring and fall. As with any medication, it is applied in the off-season when there is no chance that it will contaminate marketable honey. For detection, obtain at least 50 adult bees from the front entrance of suspected colonies and send to a bee testing laboratory for diagnosis. The new species of Nosema may be a problem throughout the season and beekeepers can monitor spore levels regularly (Martín-Hernandez et al., 2007).

7.1. Methods of Treatment

During spring, method of treatment is feeding to bees a mix of Fumagilin-B with sugar syrup in spring if there have a high level of infection (> 1 million spores/ bee). This threshold may not reflect the current virulence of Nosema. The only treatment for Nosema is fumagillin (an antimicrobial agent isolated from *Aspergillus fumigatus*), but the methods of application for treatment of *N. apis* may not be as effective for treatment of *N. ceranae* (Higes et al., 2010). Protect the Fumagilin-B medicated sugar syrup from direct sunlight when feeding to bees and the further research is in progress for addressing the virulence of Nosema through applied research. To ensure that individual colonies received the accurate dose of Fumagilin-B, mix as per label instructions and apply using direct-to-colony feeding techniques (bag feeding, pail feeding, etc.). Barrel feeding Fumagilin is not effective as the dosage is not standardized and the drug settles to the bottom of the barrel. Replace 2 to 3 old brood combs (typically darker) from the brood box to reduce the level of Nosema and accumulation of acaricides in the wax. Spring feeding with Fumagilin-B is important and current research on the seasonal patterns of Nosema demonstrates that the levels of Nosema increase in June. For the duration of late spring or summer, method of treatment is new queens and Requeen colonies when new queens are available. For the period of fall treatment material includes Fumagilin-B, and method of treatment is feeding to bees a mix of Fumagilin-B with sugar syrup as described above, and the fall feeding may protect bees during the winter season. Colonies treated with fumagillin in the autumn has significantly lower Nosema intensity in spring than did colonies that received no treatment, but by late summer there is no difference exhibited between groups (Williams et al., 2008; 2011).

7.2. Management Practices

Beekeepers use management practices to minimize the incidence of Nosema because chemical treatments for control of Nosema are not registered in some states for use in honey production beehives. Use of any such treatment is illegal and could result in unacceptable residues in extracted honey. In addition, intensive research has investigated the connection between nutrition and honey bee disease and stress resistance. Both cage studies and field studies indicated that bees with poor nutrition are under more stress, more susceptible to Nosema and *Varroa destructor*, and have shorter lifespan (Frank et al., 2008; Wang et al., 2014).

i. Maintain colonies with queens having a good egg-laying potential and colonies prepared for winter should have a good population of young bees.
ii. Ensure that colonies have adequate supplies of high protein pollen in autumn and this will help to ensure good populations of young bees.
iii. Ensure that hives prepared for winter have good supplies of honey. Studies have shown that colonies with generally with half, or more, of honey have lower spore counts compared to colonies wintered with less honey.
iv. Place the hives in a sunny position in the cooler months of the year, and choose apiary sites that have good air drainage and protection from cold winds. Avoid cool shady and damp sites as research has shown that the level of Nosema infection in a colony can be reduced from about 85% to zero by placing the hive in a sun trap where it obtains maximum sun and maximum shelter from cold winds.
v. Maintain winter colonies in a minimum of hive space so that they are compact and warm, and remove supers (boxes) of combs not required by the bees.
vi. Avoid colony stress which can be caused by excessive opening of the hive, manipulation of combs, feeding and relocating colonies.
vii. Avoid stagnant water sources which may become contaminated by dead bees and bee excreta to lessen infestation.
viii. Minimize the number of squashed bees during normal hive management as any infection will be spread when their remains are cleared away by hive cleaning bees.
ix. Replace old, dark brood combs to lower the number of spores in the hive, although this will never totally eliminate the disease. Many beekeepers remove two or more old combs from the brood nest each spring, replacing them with sheets of beeswax foundation available from beekeeping supply shops.
x. Once a colony is infected with the disease, it will usually progress until the colony dies. Shaking of bees onto fresh comb has been demonstrated to be a good control method for lowering of the-infection rates.

Over the time, progress in resistance development has been made for stocks of honeybee to pests, predators and diseases with encouraging results. These stocks have been found resistant to heat, cold and to disinfectants, and are viable for many years for honey making in old combs or derelict hives (Rinderer et al., 2001; Robertson and Albert, 2006; 2007).

8. Conclusion

Honeybees are our most important honey producer or pollinator, and many kinds of pathogens, parasites, predators affect most stages of bee development, so, strong hives are important to maintain healthy colony. A healthy honeybee colony has three distinct types of individuals, a

queen, workers and drones. Each type of bee has a distinct role in the colony and collectively, they make up the members of a honey bee colony. The key to protecting honey bee colonies from diseases, parasites, and other harmful conditions is the ability to identify and deal with problems early. This article contributes information about the location of confirmed cases of Nosema so that beekeepers can remain vigilant when knowing the whereabouts of diseased apiaries (10 km square basis). These pages also provide details on the surveillance for exotic threats and recent disease trends in honey bee colonies. The honey bee colony diseases are caused by the spore forming microsporidian, *Nosema apis* and *Nosema ceranae* , and spores of these organism can only be seen using a light microscope. Increased monitoring and research are starting points to shed some light on the factors involved in recent honey bee colony losses. Beekeepers have responsibilities in preventing losses to their bees and in learning to accept some damage, especially in providing pollination services. In some areas, honey bee losses must be anticipated and the risk weighed against the possible returns from honey or pollination fees. Beekeepers should be familiar with commonly used pesticides and their toxicity to bees. They should know as much as possible about the relationships between their bees and the nectar and pollen plants in their territory. It is essential that the owners of bees can be located easily when a nearby crop or the surrounding area is being treated with toxic materials. Therefore, a beekeepers should provide ther names, address and telephone numbers to owners of land on which the bees are located. This information should also be posted in the apiary in large, readable letters. Beekeeper's organizations should compile directories of apiary locations and their owners in each county, and make them available together with marked maps at the office of the extension adviser or county agent.

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