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Effects of Queen Source and Age of Colony on Nosema (*Nosema, apis*) Spore Load in Honey Bees (*Apis mellifera*)

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Abstract: Bee colonies are being decimated by new diseases and by older diseases that have become more virulent. Nosema is a disease that contributes to these losses. Many recommendations concerning queen type in the past have focused on honey production. Extension educators need information on how queen source and colony age can be used to manage disease. Results include: bee colonies headed by queens selected for their increased hygienic behavior exhibited reduced nosema spore loads. This effect lasted during the early spring and was not detected at a later date. Older, over-wintered, hives exhibited higher spore loads than newly made colonies.

Introduction

The agricultural producers that Extension educators work with are concerned about protecting bees because of their importance in the pollination of agricultural crops such as almonds, apples, and tomatoes (Thomas, Franklin, Roger, & Timothy, 2008). Honey bees are increasingly mentioned in the national media due to the new diseases that are decimating bee colonies. One of these diseases is nosema (*Nosema, apis*). The effects of queen source on nosema spore loads have not been studied. Also, recommendations on queen source focus on honey production and bee temperament and do not always consider disease resistance issues. Extension professionals need information that will be useful in managing this disease.

Nosema is a spore forming protozoan that attacks honey bees (*Apis, mellifera*) (Shimanuki & Knox, 2000; Shimanuki, Knox, Furgala, Caron, & Williams, 2003). Nosema spores are ingested along with food and water by adult honey bees. The spores germinate and multiply in the beeâ s mid gut and are eliminated in the feces. In severe infections, the feces are defecated on or near the hive and increase the contact and spread of the infection throughout the hive. Symptoms of the disease include dysentery, increased winter loss of bees, reduced honey production, and shortened life span of the worker bees. These symptoms are most noticeable in the spring. The disease usually fades away in the summer and briefly returns again in the fall of the year. Worker bees and queen bees have been found to become infected at similar rates when inoculated with nosema spores (Thomas, Pomper, Hunt, Thacker, & Jones, 2004), but infected queen bees do not transfer the disease to their eggs (Hassanein, 1951).

Factors such as queen source and age of the colony may affect nosema spore loads (Blackiston, 2002). Some of the new breeds of queens are selected based on their hygienic behaviors (Spivak & Downey, 1998). These queens produce worker bees with an increased propensity for removing diseased or mite infested brood. Hygienic queens reduce the incidence of diseases such as foulbrood, a bacterial disease of bees (Blackiston, 2002), but it is not known if hygienic behavior affects nosema.

The study reported here hypothesized that these same hygienic behaviors will have an effect on nosema because cleanliness in the hive is essential in reducing the prevalence of this disease. There may also be differences in nosema levels between older, established hives that have survived the winter and newly made colonies. The new colonies would have modified brood levels and worker populations that may leave them stressed and susceptible to disease. Older established hives would have larger populations of brood and stable worker populations that may lead to less stress and lower disease levels. Therefore honey bee colonies were tested to determine if queen source and age of the hive influenced nosema spore loads.

Materials and Methods

Two separate experiments were conducted to evaluate nosema spore loads in honey bee colonies. The first experiment evaluated the effects of queen source. The second experiment evaluated the effects of colony age. Each study used a completely randomized block design. Treatments for the first test consisted of testing bees headed by a queen with a background of hygienic behavior and bees headed by queens that had not been selected for hygienic behavior. Treatments for the second experiment consisted of colonies that had been established for more than a single season and colonies that had recently been established. Analysis of variance following the multivariate general linear hypothesis was used to analyze the results of each test and the least significant difference method was used for mean separation.

In the spring of 2008, an apiary with 40 colonies was established in Pocatello, Idaho for the purpose of studying nosema spore loads in honey bee colonies. These colonies were managed as migratory colonies, meaning they would be transported to California for pollination of the almond crop. This was done in order to match the environmental stresses that production colonies experience.

All colonies were medicated in the spring and the fall for foulbrood using tylosin, and for mites using thymol. Excess honey was removed from the hives that fall. All colonies had 60 pounds of honey stored for the winter. The colonies spent the winter in California and returned to Idaho in the spring of 2009.

In the spring of 2009, the colonies were divided. This is a normal practice used by beekeepers to create new colonies. Division was accomplished by removing three frames of brood (young bees in the larval stage) and two frames of honey from the original hives and placing these frames into new hive boxes. These new hive boxes represent the newly made colonies. The hives from which the frames were removed represent the over-wintered hives. These migratory colonies were used to study the management of nosema in the following two experiments. Each experiment used separate colonies as test subjects.

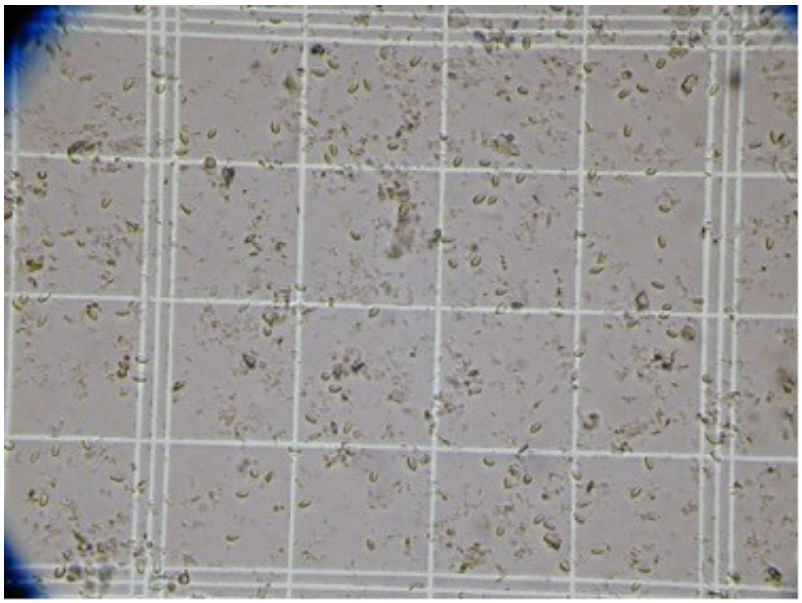
In order to study the effects of queen source on spore loads, 20 of the over-wintered migratory colonies were randomly selected. These colonies all received new queens at the time they were divided. Seven of these colonies were headed by queens with hygienic behavior, and 13 hives received queens with non-hygienic behavior. The degrees of freedom for the analysis of variance were allocated accordingly with 6 degrees of freedom being allocated for the error term.

In order to study the effects of colony age, 13 of the over-wintered hives were compared with nine of the colonies that were newly made in the spring. The degrees of freedom for the analysis of variance were allocated accordingly, with eight degrees of freedom being allocated for the error term.

All of the colonies in each experiment were analyzed for nosema spore loads on April 25, 2009. The colonies that used in the queen source experiment were analyzed again on May 15, 2009. In order to calculate nosema spore loads, 25 foraging worker bees were randomly sampled from the entrance of each colony. Young bees and older, dying bees were not included in the sample. The days when samples were taken were warm and wind speeds were below 5 mph. Bee samples were preserved in individual vials with 70% isopropyl alcohol and labeled. Bee samples were returned to the laboratory where they were analyzed. The nosema detection method used in the study relied heavily on the procedures developed by Rogers, Bishop, and MacKenzie, (2002), Oliver (2009), Cantwell (1970), and Wilson and Ellis (1966).

In the laboratory, 10 bees were strained from the alcohol, and their abdomens were removed. The bee abdomens were placed in a mortar and pestle grinder with 10 ml of distilled water and ground. Immediately following this grinding procedure, a 0.01 ml loop was used to transfer a sample of the resultant liquid to a calibrated hemacytometer. The spores were allowed 2 minutes to settle to the bottom of the hemacytometer chamber prior to counting. Nosema spores in five of the 16 squares of the hemacytometer were counted (Figure 1). Total spores per bee were calculated based on the formula described by Rogers, which is the following: Spores per bee= ((block 1+â ¦+ block 5)/80) X ($4X10^6$).

Figure 1.
Nosema Spores as Viewed on the Hemacytometer Grid



Results and Discussion

It was found that honey bee colonies headed by queens that had been selected for their increased hygienic behavior exhibited reduced nosema spore loads in the foraging worker bees (Figure 2). This effect only lasted during the early spring and was not detected at a later date when nosema symptoms historically fade (Table 1, Table 2).

Figure 2.
Queen Source Effects on Nosema Spore Load ($P<0.05$)

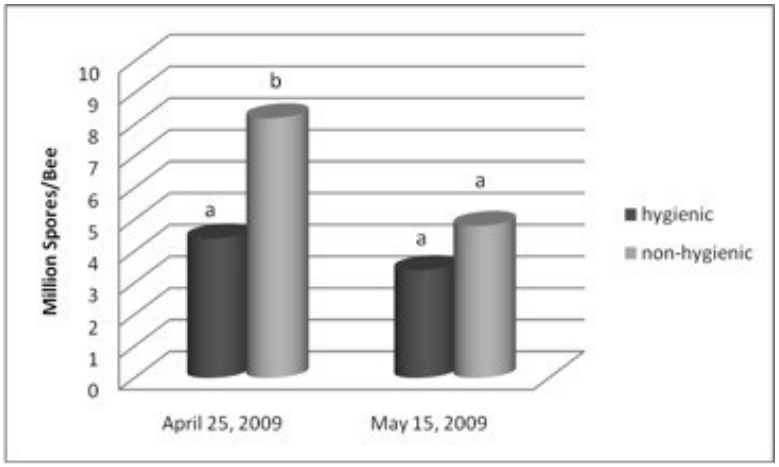


Table 1.
Analysis of Variance-Effects of Queen Source (4/25/09)

Source of variation	Sum of Squares	df	Mean Square	F-ratio	P
Replication	8.83760E+13	12	7.36466E+12	1.780	0.247
Queen source	4.82671E+13	1	4.82671E+13	11.663	0.014
Error	2.48303E+13	6	4.13838E+12		

Table 2.
Analysis of Variance- Effects of Queen Source (5/15/09)

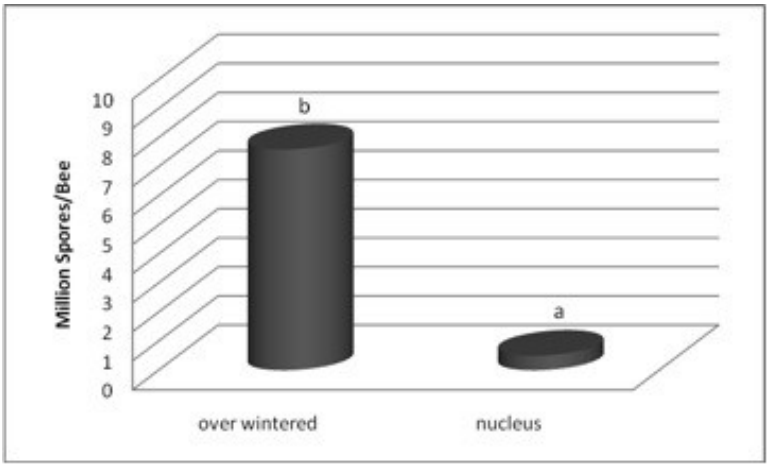
Source of variation	Sum of Squares	df	Mean Square	F-ratio	P
Replication	1.80308E+14	12	1.50256E+13	0.948	0.561
Queen source	3.40071E+13	1	3.40071E+12	0.215	0.660
Error	9.50793E+13	6	1.58465E+13		

The study also revealed statistical differences in the spore loads of worker honey bees between older established over-wintered colonies and recently established colonies (Table 3). Older, over-wintered, colonies exhibited higher spore loads in worker bees than the younger recently established colonies (Figure 3). The older colonies had longer periods of time to build nosema spore levels, which may account for the higher spore loads they exhibited. This would have been especially true in regards to levels of infection of hive equipment. The older colonies had foundation, frames, and hive boxes that had been exposed to nosema for long periods of time and therefore could have built up high spore loads. Recently established colonies did have some older frames and foundation added; however the hive boxes and most of the frames and foundation were never exposed to nosema in the past. This could have resulted in lower infection of worker populations and the observed reduced spore loads in nucleus hives.

Table 3.
Analysis of Variance- Effects of Colony Age

Source of variation	Sum of Squares	df	Mean Square	F-ratio	P
Replication	1.23751E+14	12	1.03126E+13	1.943	0.176
Colony Age	3.87934E+13	1	3.87934E+32	7.310	0.027
Error	4.24531E+13	8	5.30663E+12		

Figure 3.
Colony Age Effects on Nosema Spore Load (P<0.05)



Based on these results, Extension educators should recommend that hives be established with queens that have been selected for hygienic behavior. Educators should not focus solely on recommending queens that sire bees that have a propensity to produce an abundance of honey or that have a calm temperament. Through increased hive cleanliness, nosema can be reduced during the critical brood build up season in the spring. It is also recommended that beekeepers divide all strong hives. This can also reduce the incidence of nosema. These recommendations coupled with the use of clean boxes, frames, and foundation can help reduce disease.

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