INVESTIGATING EFFECTS OF POLLEN NUTRITION ON *NOSEMA CERANAE* INFECTION AND PERSISTENCE IN HONEY BEE COLONIES

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Introduction

There is an ever-increasing need for pollination services in our modern agriculture systems. Honey bees provide the majority of that pollination for commercial agriculture worth $ 2.5 billion in the Pacific Northwest. Healthy honey bee colonies are crucial to sustain Oregon's agricultural economy. Due to the alarming honey bee colony losses attributed to Colony Collapse Disorder, the need to understand honey bee health is dire.

Bees, along with most organisms require adequate nutrition to grow and develop properly. Since honey bees obtain their food from plants, they are directly affected by land management practices. Due to increased urbanization and current farming techniques, land use has dramatically changed and regional pollen diversity continues to decline possibly affecting bee health (Naug 2009). Previous research has shown that bees fed on a variety of different pollen types live longer (Schmidt et al. 1987) and have increased immune functions (Alaux et al. 2010).

There are many pests and diseases which constantly threaten honey bee health. *Nosema ceranae* is a microsporidian gut parasite which has recently been discovered in honey bees. The spores germinate inside the host midgut and reproduce intracellularly (Gisder 2011). *Nosema ceranae* has been shown to increase winter colony losses and decrease bee immune responses to other diseases (Higes et al. 2006). The biology and epidemiology of this new pest is still relatively unknown; thus, continuing research is necessary to better understand this pathogen.

Since immune functions can depend so heavily upon nutrition, it is important to understand the relationship between nutrition and disease load. There is no literature that we are aware of which demonstrates the relationship between nutrition and the prevalence and intensity of *Nosema ceranae*. It is our hypothesis that a nutritionally deficient diet will result in an increased rate of *Nosema ceranae* parasitization and honey bee mortality. Our objectives in this experiment were to investigate if optimal nutrition will reduce the prevalence and intensity of a *Nosema ceranae* infection.

Methods

Frames with emerging brood were pulled from sister queen hives in OSU's apiaries. Frames were randomly placed in cardboard nucleus hives and emerged in the incubator at 33^{o}C with the relative humidity at 55% to simulate conditions in the hive. Newly emerged bees (less than 24 hours old) were brushed into a large container where they were mixed thoroughly by hand to remove variances between the individual colonies. After the bees were homogenized, they were placed inside cylindrical wire cages (6306 cm^{3}) and returned to the incubator. Each cage contained 250 bees. On the top of each cage were two inverted vials of 100ml of water and 50% sucrose solution provided *ad libitum*. Bees inside the cage were also exposed to varying diet treatments.

In order to vary the amounts of nutrients amongst the cages, treatments consisted of wild flower pollen and α-cellulose powder(Sigma®) in the following ratios: 1:0, 1:1, 1:2, 1:3, and 0:1 respectively. There was also a control diet which included 1:0 and was not inoculated with *Nosema ceranae* spores. There were 6 different diet treatments with 6 replicates of each treatment for a total of 36 cages. 35 ml of 33% sucrose solution was mixed into 300g of the diet treatment mixture to hold the blend together. The mixture was then measured out to 25g and packed into petri dishes. The petri dishes were then placed at the bottom of each cage.

Five days after emergence and being exposed to the diet treatment *ad libitum,* each cage was then mass inoculated with *Nosema ceranae* spores. Spores were purified through centrifugation and calculated to inoculate 250 bees with 10,000 spores per bee. The spore inoculant was formulated in 30ml of 50% sucrose solution. The inoculums were left for 24 hours and then the feeders were topped up with uncontaminated 50% sucrose solution to total 100ml.

Every other day the consumption of water and sugar syrup from each cage was measured and replaced. The diet treatment consumption was measured and replaced weekly. Bee mortality was observed every other day and dead bees were removed at time of diet treatment replacement for convenience.16 days after the bees were inoculated with *Nosema* spores, 20 bees were culled from each cage for analysis. 20 bee abdomens were used to estimate the *Nosema* prevalence and intensity. The prevalence and intensity of the *Nosema* infection was determined by light microscopy techniques followed by (Cantwell 1970). Each bee was checked individually for *Nosema ceranae* infection.

Data analysis was carried out with SAS 9.3. *Nosema* intensity and prevalence data were analyzed by generalized mixed linear model analysis (PROC MIXED; SAS 9.1) for differences due to various treatments. Mean separations were performed using Fisher's protected least significant difference (LSD) test ($P < 0.05$). After statistical analysis, means were back-transformed as needed for presentation herein.

Results

Nosema prevalence (% infection in each treatment) data were transformed by square root transformation. Prevalence ranged from 23 to 27 % among various inoculated treatments which are on par with each other with no significant difference ($P = 0.3066$). Treatments that were inoculated with *Nosema* differed significantly ($P = 0.0001$) in intensity (mean spores per bee). Significantly higher intensities were observed in the 1:0 and 1:1 compared to the other diet treatments. 1:3 and 0:1 showed lowest intensity of *Nosema* and were on par with each other (Figure 1.).

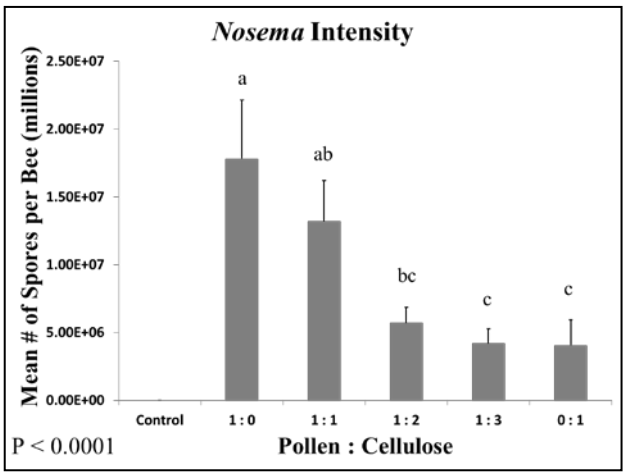

Figure 1. The mean number of spores in each treatment.

Honeybee survival data followed a normal distribution, so no data transformations were performed. Treatments differ significantly in survival ($P < 0.0001$). The total number of honey bees survived was significantly higher in the un-inoculated control (125 honey bees) which is on par with the 1:0 and 1:1 treatments compared to other treatments (Figure 2.). In general it was observed that as the pollen concentration decreased in the diet, the number of surviving honey bees also decreased. The 0:1 treatment had lowest (20) survival.

Discussion

The results of this study support the idea that nutrition plays a role in *Nosema ceranae* parasitization. The diet type provided to the bees resulted in different levels of infection and survival. This suggests that not only does the availability of pollen matter, but also the quality.

The fact that our treatments with the best nutrition had the highest intensity of infection confirms that host nutrition is vital for the parasite's success. One speculation is that the availability of pollen in the diet affects the gut pH, which is responsible for the higher reproduction of the spores. Another speculation could be that the spores, which are dependent upon the host's epithelial cells for replication, require a bee with more nutrients.

Contrary to our hypothesis, the cages with the highest number of surviving bees were the same cages that had the highest intensity of *Nosema ceranae* infection. Better nutrition seems to compensate for the parasitic damage caused by *Nosema ceranae*. This could potentially have major implications on the way beekeepers manage for *Nosema*, shifting the focus from chemical treatment of anti-biotics to increasing colony nutrition.

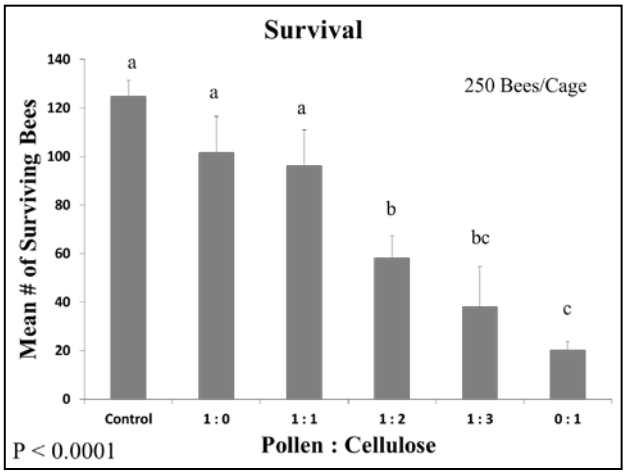

Figure 2. The mean number of surviving bees in each treatment.

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