UNDERSTANDING COLONY LEVEL PREVALENCE AND INTENSITY OF HONEY BEE GUT PARASITE, *NOSEMA CERANAE*

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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.

Pests and diseases constantly threaten honey bee health. Recently, honey bee colonies have been reported to be infected with a gut pathogen called *Nosema ceranae*. This microsporidian has historically been prevalent in the Asian honey bee, but has only recently been observed in honey bees across Europe and the United States. *Nosema ceranae* has been shown to increase winter colony losses and decrease bee immune responses to other diseases (Higes, Martín, & Meana, 2006).

The biology and epidemiology of this new pest is still relatively unknown. Unlike *Nosema apis*, *Nosema ceranae* has little or no outward symptoms recognizable to beekeepers. *Nosema ceranae* appears to have a different phenology than *Nosema apis* and looks to have displaced *Nosema apis* in the past few years (Bourgeois et al. 2012). Thus, continuing research is necessary to better understand this pathogen.

Methods

Hives were sampled from the brood area in July 2013. These samples were then tested using light microscopy techniques to examine the presence and intensity of *Nosema*. Eight *Nosema*-free hives were selected to serve as foster colonies for the experimental bees.

Brood frames were pulled only from hives in which sister queens were placed previously. The brood frames were kept within the incubator at 32^{o}C with the relative humidity at 55% to simulate conditions in the hive. Brood frames were checked every 8-12 hours for emerging bees. Newly emerged bees were then painted green, blue, white, or red according to their particular age cohort. Bees were painted with Testors $_{TM}$ enamel paint on the posterior of the thorax. Once the required number of bees had emerged (2,000 bees per cohort) and had been painted within 24 hours, they were placed in one of the *Nosema*-free foster hives.

After three weeks since the first cohort was placed in the foster colony, 1,000 painted bees from each cohort were vacuumed with the Bioquip$_{TM}$ Bug Vac and placed inside a sealed nucleus hive. The Triple Cohort Colony was established with 3 week, 2 week, 1 week and newly emerged bees painted green, blue, white and red, respectively. Each nucleus hive contained three empty frames

and one honey frame when painted bees were transferred to those nucleus hives. The following day, the entrance blockers were removed and the bees were allowed to forage. On Day 1 after the establishment of triple cohort colonies, existing non-painted bees were removed. A queen in a sealed queen cage was also placed inside the nucleus hive on Day 1. On Day 2 the sealant on the queen cage was removed so bees could release the queen on their own. On Day 3 the *Nosema ceranae* spores were fed to bees through mass inoculation methods.

The *Nosema ceranae* spore concentrate was formulated following the methods of (Fries et al., 2013). Spores were purified through centrifugation and calculated to inoculate 4,000 bees with 10,000 spores per bee. The spore inoculant was formulated in 100 ml sugar water w/v. The inoculant was provided to experimental colonies using mason jars placed on hive covers with holes.

After the spore inoculant was consumed completely, bees were fed 300 ml sugar water. Two weeks after the hives were inoculated; the hive entrances were blocked and the hives were placed inside a -20 C^o freezer. After two days the sacrificed bees were removed from hive, separated into cohorts, and counted. Only bees that were fully intact at time of removal were saved.

The prevalence and intensity of *Nosema* infection were determined by light microscopy techniques followed by (Cantwell 1970). Bee abdomens were removed and macerated by mortar and pestle. 1 ml of water was added and a 10µl drop was placed on a hemocytometer so spore intensity could be estimated.

Results

Our preliminary data analysis of 300 bees from each age cohort has shown a wide range of infection intensities. *Nosema* levels have been estimated as low as 50,000 spores/bee to as high as 67 million spores/bee. The mean spores/bee of the 3-week, 2-week, and 1-week cohorts are all greater than 12 million. However, the 1-day old cohort had a much lower average of 2 million spores/bee (Figure 1.).

About one third of the bees pertaining to the 3-week, 2-week, and 1-week cohorts appeared to be infected with *Nosema* (Figure 2.). The 1-day old cohort had a slight decrease in *Nosema* prevalence with one-fourth of the bees infected.

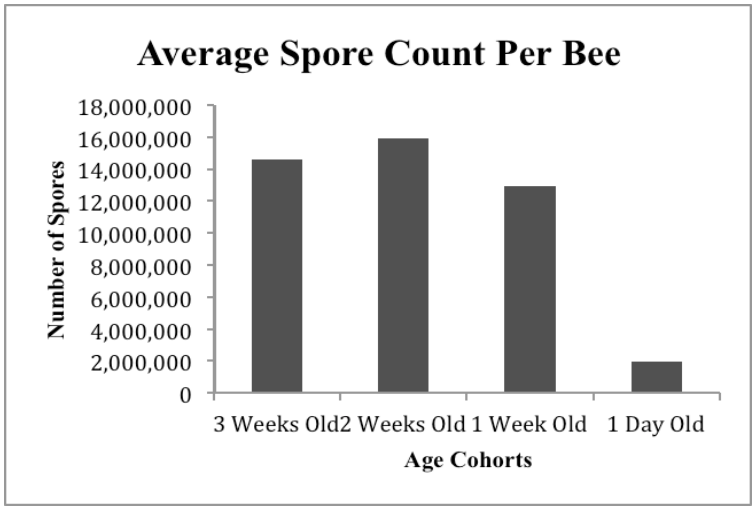

Figure 1. The average number of *Nosema* spores counted within each age cohort.

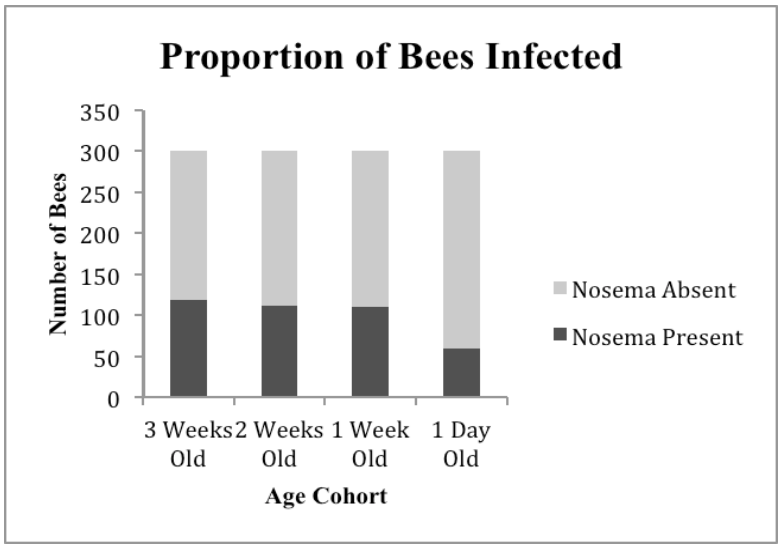

Figure 2. The prevalence of *Nosema* spores in *Nosema* inoculated honey bees.

Discussion

Data analysis is still in progress and hence we will not make any conclusions here. Results from this study will provide insights on prevalence and intensity of *Nosema ceranae*. This information regarding prevalence and intensity will help better formulate *Nosema* sampling protocol that will help beekeepers assess realistic need for colony treatment.

Literature Cited

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