

**AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
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TITLE: Understanding colony level prevalence and intensity of honey bee gut parasite, *Nosema ceranae* and investigating effects of colony nutrition on persistence of *Nosema* in honey bee colonies

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SUMMARY: The microsporidian gut parasite of honey bees, *Nosema ceranae* has been reported to be the predominant species in honey bee colonies in the United States. Currently there is significant gap in knowledge regarding biology and epidemiology of this relatively new species of *Nosema* in European honey bees. Further, it is believed that nutrition plays an important role in mitigating negative impacts of this pathogen. Hence in this study we also investigated the role of pollen nutrition on *Nosema* infection and bee survival. Following were the objectives of this study: **(1)** determine within colony prevalence and intensity of *Nosema ceranae* and **(2)** investigate if optimal nutrition can inhibit prevalence of *Nosema ceranae* and reduce the intensity of infection. Our results suggest that foraging-aged bees had the highest prevalence of *Nosema* infection and the prevalence and intensity of *Nosema ceranae* infection is significantly influenced by honey bee age. Further, our study results indicate that the bees that received higher pollen quantities had higher intensities of *Nosema ceranae*, but also higher survival.

OBJECTIVES:

- 1) Determine within colony prevalence and intensity of *Nosema ceranae* (*Experiment 1*).
- 2) Investigate if optimal nutrition can inhibit prevalence of *Nosema ceranae* and reduce the intensity of infection (*Experiment 2*).

PROCEDURES:

Experiment 1 (*Nosema* prevalence and intensity): Five colonies naturally infected with *Nosema ceranae* were selected from a single Oregon State University apiary (Corvallis, OR, USA). Prior to the experiment, we identified the *Nosema* species in all five colonies as *Nosema ceranae* using the DNA analysis methods of Hamiduzzaman et al. [44]. Two or three capped combs with emerging bees were collected from each of the five colonies and were incubated in the lab under simulated hive conditions (33° C, 55% RH) for bee emergence. Incubating brood combs were checked for emerging bees 16 hours later. Newly emerged bees were color-coded by colony with a dot of Testors™ enamel paint on the thorax. Once at least 500 newly emerged bees per colony

had been painted, they were returned to their original colonies. Fifty recently emerged bees per colony were also retained to establish a baseline infection level of *Nosema ceranae*.

From each of these five experimental colonies, fifty marked (painted) bees were collected during midday 8-11 days post-emergence and again at 22-25 days post-emergence. These sampling periods represent the nursing and foraging phases, respectively, of a worker bee, as honey bees exhibit temporal polyethism. While collecting these fifty marked bees, an additional sample of fifty unmarked bees (background bees) was also collected from each colony. However, our samples of background bees consisted of an equal mix of bees from the brood area, colony cover (lid), colony entrance and outer combs in the colony, whereas Smart and Sheppard [34] collected such background bee samples only from the inner colony cover in their study. The infection intensity and prevalence of *Nosema ceranae* in these random samples of mixed aged bees were compared to the infections of the marked bees of known age.

The prevalence and intensity of *Nosema* infection in all collected bees were determined following light microscopy techniques as described by Cantwell. All marked bees collected were analyzed individually. Although background bees were collected as composite samples, they too were analyzed individually. This permitted us to observe the prevalence and intensity of infection in the bees comprising a composite sample, which is usually not known because all bees are typically analyzed together. Furthermore, individually analyzing bees from the composite samples allowed us to observe if any significant differences exist between sampling bees of known ages and traditional composite samples.

Results / Discussion

The prevalence of *Nosema ceranae* infection was significantly different between some age cohorts ($F_{5, 24} = 4.92$; $P = 0.0043$). *Nosema ceranae* infection was not detected in any of the recently emerged bees. Foraging-aged bees had a significantly higher prevalence of *Nosema ceranae* infection than both nurse-aged bees and recently emerged bees. Nurse-aged bees and recently emerged bees, however, did not have significantly different levels of *Nosema* prevalence. The prevalence of *Nosema ceranae* infection in background bees was not significantly different between sampling dates; nor did it differ from that of corresponding marked bees on any of the sampling dates. Further, there was no significant correlation ($r = 0.29$, $P > 0.05$) between the percentage of *Nosema ceranae* infected bees (prevalence) and infection level (intensity) in a composite sample.

Experiment 2 (Nosema-Nutrition Study): Newly emerged honey bees with similar genetics (progeny of sister queens) were obtained and stocked in cages in the lab and reared in an incubator. We had a total of 36 cages for this experiment with 250 bees in each cage. Following were the diet treatments: 1:0 (pollen: cellulose); 1:1 (pollen: cellulose); 1:2 (pollen: cellulose); 1:3 (pollen: cellulose) and 0:1 (pollen: cellulose). We also had a control diet group that was provided pollen similar to 1:0 (pollen: cellulose) treatment but did not receive any *Nosema* spores. Five days after initiation of these treatments, bees in each cage were mass-inoculated

with *Nosema ceranae* spores. Once a week, we measured the consumption of diet and bee mortality was recorded every other day until the end of the experiment. Sixteen days after spore inoculation 30 bees from each cage were removed for several analysis (*Nosema* intensity, hypopharyngeal gland protein, midgut enzyme activity etc.).

Results/Discussion

Interestingly, in this study we found that the bees that received higher pollen quantities had higher intensities of *Nosema ceranae*, but also higher survival. These results may seem counterintuitive, as both *Nosema* intensity and survival increased in bees that had access to higher pollen quantities. It appears that the bees receiving higher pollen diet were able to compensate for the negative effects of *Nosema*, as higher pollen diet possibly resulted in a robust immune system and also compensated for the lost energy and nutrients in infected bees. Further, our results suggest that *Nosema ceranae* is highly dependent on the host (bees) nutrition for its development, and hence bees receiving a higher pollen diet also become an ideal host for greater reproduction of this parasite by providing an ideal nutritional environment for this parasite.

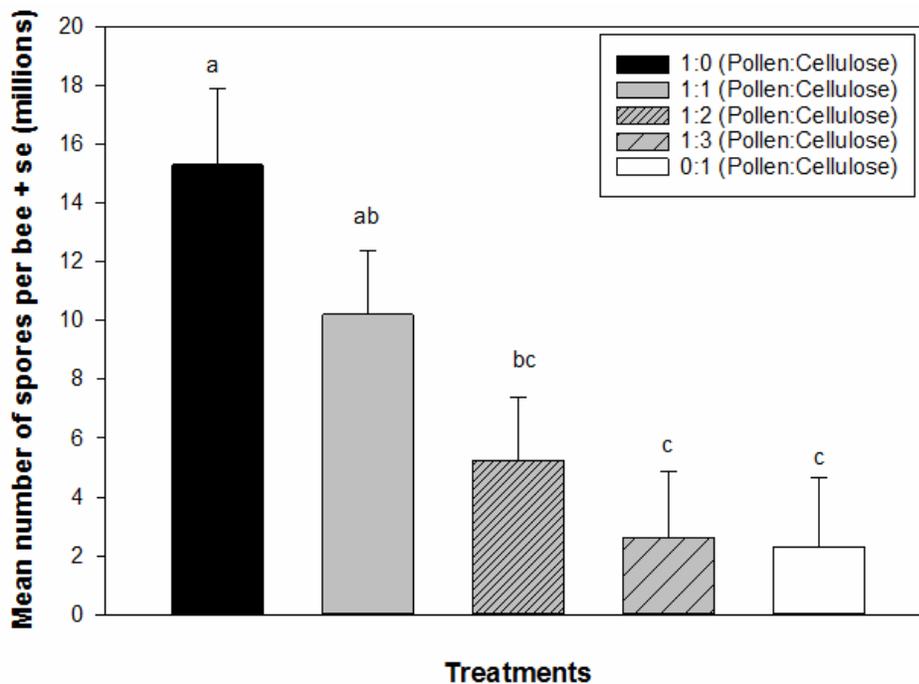


Figure 1. Mean number of *Nosema ceranae* spores per bee (+ se) fed different pollen concentrations and sampled 16 days after infection. Means with different letters indicate significant differences among treatments ($P < 0.0001$).

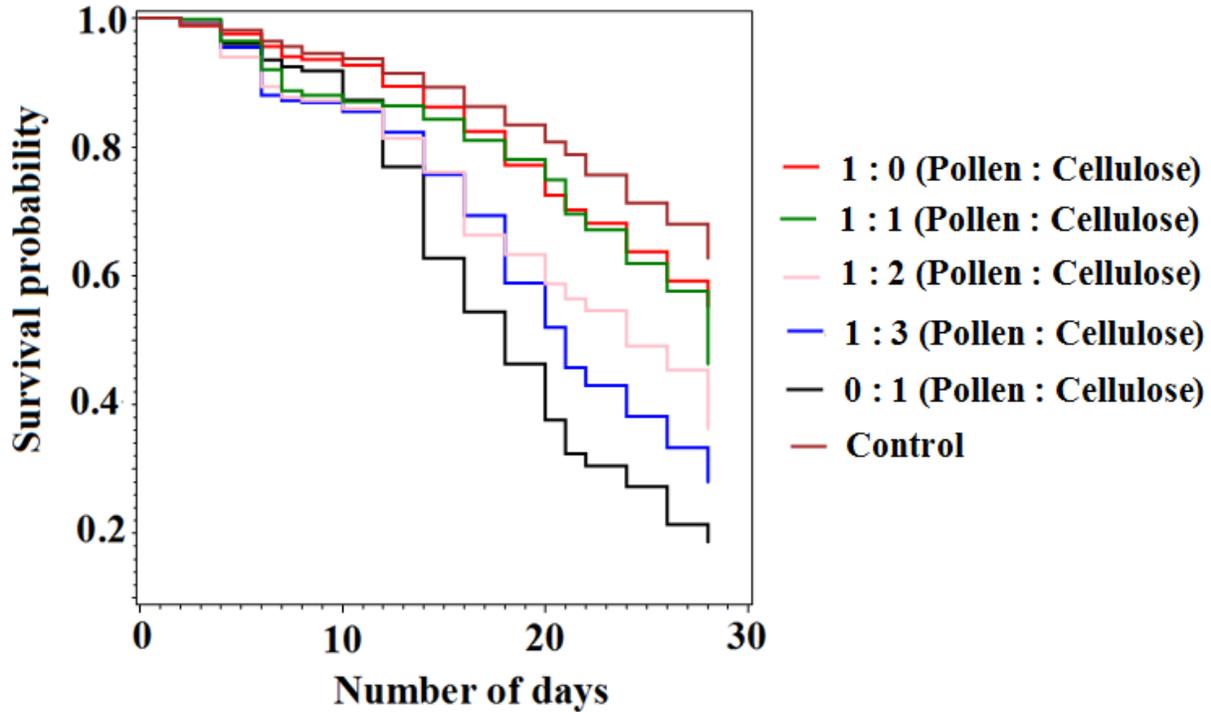


Figure 2. Survival of *Nosema ceranae*-infected bees fed different concentrations of pollen. (Control group was not infected.)

SIGNIFICANT ACCOMPLISHMENTS: Research findings from this study were disseminated to Oregon beekeepers at Oregon State Beekeepers annual meetings during 2014. Manuscript pertaining to this research was recently published in a peer refereed journal (Journal of Insect Physiology) and support of ARF has been acknowledged (<http://www.sciencedirect.com/science/article/pii/S0022191016300051>).

BENEFITS & IMPACT: Results from this study have provided new insights to beekeepers regarding the biology and epidemiology of the honey bee gut pathogen, *Nosema ceranae*. Information gleaned from this study will help beekeepers formulate appropriate *Nosema* management strategies for effective sampling and control of *Nosema* and reduce their colony losses.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM: Additional funding was received from the National Honey Board during the project term.