Journal of Insect Physiology 87 (2016) 12–19

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Effects of pollen dilution on infection of *Nosema ceranae* in honey bees

Cameron J. Jack [1], Sai Sree Uppala [2], Hannah M. Lucas, Ramesh R. Sagili *

Oregon State University, Dept. of Horticulture, 4017 ALS Building, Corvallis, OR 97331, USA

ARTICLE INFO

Article history:
Received 2 September 2015
Received in revised form 6 January 2016
Accepted 19 January 2016
Available online 21 January 2016

Keywords:
Nosema ceranae
Nutrition
Honey bee
Pollen
Spore intensity
Hypopharyngeal glands

ABSTRACT

Multiple stressors are currently threatening honey bee health, including pests and pathogens. Among honey bee pathogens, *Nosema ceranae* is a microsporidian found parasitizing the western honey bee (*Apis mellifera*) relatively recently. Honey bee colonies are fed pollen or protein substitute during pollen dearth to boost colony growth and immunity against pests and pathogens. Here we hypothesize that *N. ceranae* intensity and prevalence will be low in bees receiving high pollen diets, and that honey bees on high pollen diets will have higher survival and/or increased longevity. To test this hypothesis we examined the effects of different quantities of pollen on (a) the intensity and prevalence of *N. ceranae* and (b) longevity and nutritional physiology of bees inoculated with *N. ceranae*. Significantly higher spore intensities were observed in treatments that received higher pollen quantities (1:0 and 1:1 pollen:cellulose) when compared to treatments that received relatively lower pollen quantities. There were no significant differences in *N. ceranae* prevalence among different pollen diet treatments. Interestingly, the bees in higher pollen quantity treatments also had significantly higher survival despite higher intensities of *N. ceranae*. Significantly higher hypopharyngeal gland protein was observed in the control (no *Nosema* infection, and receiving a diet of 1:0 pollen:cellulose), followed by 1:0 pollen:cellulose treatment that was inoculated with *N. ceranae*. Here we demonstrate that diet with higher pollen quantity increases *N. ceranae* intensity, but also enhances the survival or longevity of honey bees. The information from this study could potentially help beekeepers formulate appropriate protein feeding regimens for their colonies to mitigate *N. ceranae* problems.

1. Introduction

Honey bees (*Apis mellifera*) are critical pollinators of many important agricultural crops and currently face several stressors, including pests, parasites and pathogens (Klein et al., 2007; Bromenshenk et al., 2010; Potts et al., 2010; Calderone, 2012). The microsporidian *Nosema ceranae* is one such parasite that was first reported in the European honey bee in 2006 (Higes et al., 2006; Huang et al., 2007; Chen et al., 2008). *Nosema* spores ingested by honey bees germinate inside the host midgut and reproduce there intracellularly. *N. ceranae* infection has been shown to promote precocious foraging (Goblirsch et al., 2013), modify vitellogenin titres and queen mandibular pheromones in queens (Alaux et al., 2011), reduce longevity (Eiri et al., 2015), decrease immune functions (Antúnez et al., 2009) and increase colony loss (Higes et al., 2008, 2009).

Reduced longevity may be observed in infected bees as the presence of *N. ceranae* in the midgut disrupts protein metabolism and causes energetic stress. This obligate parasite obtains its energy from the bees' midgut cells and impairs midgut epithelial cells and midgut development during replication (Higes et al., 2007; Holt et al., 2013). In doing so, *Nosema* spp. infections negatively affect the midgut proteolytic enzyme activity (Liu, 1984; Malone and Gatehouse, 1998). Additionally, the presence of *N. ceranae* alters the expression of some portions of the midgut proteome responsible for energy production, protein regulation and antioxidant defense (Vidau et al., 2014). As a result of the energetic stress caused by such disruptions to nutrient digestion and metabolism, infected honey bees commonly demonstrate pronounced hunger (Mayack and Naug, 2009) and are less likely to share food with nestmates via trophallaxis (Naug and Gibbs, 2009). This, in turn, may have detrimental effects on colony health.

A growing body of literature supports the notion that *N. ceranae* infection also affects hypopharyngeal gland structure and function. In honey bees, hypopharyngeal glands secrete major components of royal jelly and larval brood food (Patel et al., 1960), as well as

* Corresponding author.
E-mail addresses: cjack@ufl.edu (C.J. Jack), saisreeu@gmail.com (S.S. Uppala), hannah.lucas@oregonstate.edu (H.M. Lucas), Ramesh.Sagili@oregonstate.edu (R.R. Sagili).
[1] Present address: University of Florida, Dept. of Entomology and Nematology, 1881 Natural Area Drive, PO Box 110620, Gainesville, FL 32611, USA.
[2] Present address: Texas A&M AgriLife Research and Extension Center, 1509 Aggie Drive, Beaumont, TX 77713, USA.

http://dx.doi.org/10.1016/j.jinsphys.2016.01.004
0022-1910/

synthesize enzymes involved in the conversion of sucrose to simple sugars and honey production (White et al., 1963; Ohasi et al., 1999). Explorations into the effects of *N. ceranae* parasitism on honey bee hypopharyngeal glands have demonstrated that infection induces gland atrophy (Alaux et al., 2010a). The parasite, however, has not been detected in the hypopharyngeal gland tissue (Huang and Solter, 2013). However, functional development and activity of hypopharyngeal glands in honey bees is reliant on the ability to ingest and digest pollen (Haydak, 1970; Mohammedi et al., 1996). Thus, changes in the hypopharyngeal glands of *N. ceranae*-infected honey bees are potentially indirect manifestations of the effects infection has on protein digestion and metabolism.

In the wake of deteriorating honey bee colony health and unsustainable colony declines (vanEngelsdorp et al., 2012; Steinhauer et al., 2014; Lee et al., 2015; van der Zee et al., 2015), bee nutrition has garnered more attention and attained greater importance (DeGrandi-Hoffman and Chen, 2015; Fleming et al., 2015; Vaudo et al., 2015). The honey bee, as with any animal, requires adequate nutrition to thrive (Haydak, 1970). Honey bees get the vast majority of their required protein, lipids and vitamins from pollen (Hrassnigg and Crailsheim, 1998; Brodschneider and Crailsheim, 2010). However, the pollen nutrition obtained by the honey bee is plant-dependent (Crailsheim, 1990; Huang, 2012). Diminished pollen quantity and diversity within the forage available to honey bees leads to a nutritional deficit that negatively impacts colony survival (Naug, 2009) by hindering brood production, inhibiting gland development, facilitating the presence of diseases, reducing individual bee weight, decreasing immunocompetence and shortening individual life span (Schmidt, 1984; Schmidt et al., 1987, 1995; Alaux et al., 2010b; Avni et al., 2014; Wang et al., 2014). Pollen quantity, quality and diversity have also been shown to affect the survival of honey bees parasitized by *N. ceranae* (Eischen and Graham, 2008; Di Pasquale et al., 2013).

Protein and carbohydrates are critical macronutrients that influence growth, performance and survival of insects, and most insects regulate nutrient intake when provided an opportunity (Behmer, 2009). The geometric framework (GF) has been used in many organisms to determine optimal balance of nutrients (Raubenheimer and Simpson, 1999; Behmer, 2009). Recently, some studies have used the geometric framework for nutrition in social insects to determine their intake targets and have examined how ratios of protein to carbohydrate or essential amino acids to carbohydrates affect the survival and physiology. Paoli et al. (2014) reported that honey bee nutritional requirements are dependent on age and behavioral role of adult bees, and bees receiving high amino acid diets had poor longevity. Pirk et al. (2010) found that honey bee adults survived longer on lower protein to carbohydrate ratio diets, and the survival was longest on a pure carbohydrate diet. In black garden ants, Dussutour and Simpson (2012) demonstrated that diets with high protein and low carbohydrate reduced ant worker lifespan by up to 10-fold due to higher intake of protein. Interestingly, in another study, Archer et al. (2014) found that honey bees fed with high protein diet (1:3 P:C) had lower mortality when exposed to stressors such as low temperatures and nicotine. In this study the authors speculated that protein conferred a survival benefit in the stress challenged bees by enhancing their ability to mount a stress response.

Resistance to pathogens can heavily depend upon nutrition (Behmer, 2009; Alaux et al., 2010b; Mao et al., 2013). Thus, understanding the relationship between bee nutrition and pathogen infection (intensity and prevalence) is paramount. Much of the research regarding bee nutrition and pathogen infection has focused on *Nosema apis* because our knowledge of *N. apis* infections in *A. mellifera* significantly predates that of *N. ceranae* (Bailey, 1955). For example, Rinderer and Elliot (1977) reported that feeding protein to honey bees infected with *N. apis* increased the spore development of the pathogen, but also improved the longevity of infected bees. But Mattila and Otis (2006) reported contradictory findings. In their field experiment, Mattila and Otis found there was no increase in the longevity of workers from *N. apis*-inoculated colonies that had received protein supplements. In recent years, however, researchers have documented many significant differences between *N. apis* and *N. ceranae* regarding infectivity, epidemiology, biology, phylogeny, pathology, genetics and distribution (Chen et al., 2013; Milbrath et al., 2015). Furthermore, in temperate climates *N. ceranae* has been reported to be the predominant species with occasional mixed infections of both *N. ceranae* and *N. apis* (Paxton et al., 2007; Fries, 2010; Natsopoulou et al., 2015). Some bee research has focused on the interactions between *N. ceranae* infection and pollen nutrition. Porrini et al. (2011) found that the presence of pollen in honey bee diets increased the intensity of *N. ceranae* spores. Zheng et al. (2014) reported that increased pollen feeding intensified *N. ceranae* spore loads in bees; and longevity of infected bees was lower than that of uninfected bees—with or without pollen feeding. In another study, Basualdo et al. (2014) found higher levels of hemolymph protein and survival in bees fed with bee bread when compared to protein substitutes, despite higher parasite development. More recently Fleming et al. (2015) reported higher *Nosema* levels in bees fed with commercial protein substitute diets than bees fed with wildflower pollen.

While these studies have explored how *N. ceranae* infection levels and infected bee longevity are influenced by protein/pollen nutrition, a great deal of uncertainty remains regarding the role of pollen quantity consumption on *N. cernanae* infection and survival of honey bees. To our knowledge, currently there is a dearth of comprehensive studies that have explored the effects of pollen quantity consumption on *N. ceranae* infection, prevalence, and survival of infected bees while simultaneously examining critical physiological parameters such as hypopharyngeal gland protein content and midgut enzyme activity. Here we hypothesize that *N. ceranae* infected bees with access to higher pollen quantities will have lower *N. ceranae* intensity, lower prevalence, higher hypopharyngeal gland protein, higher midgut enzyme activity and higher survival than infected bees receiving less pollen in their diet. To test these hypotheses, we examined the effects of diets containing different quantities of pollen on: (a) the prevalence and intensity of *N. ceranae* and (b) longevity and physiological parameters (hypopharyngeal gland protein and midgut proteolytic enzyme activity) of bees inoculated with *N. ceranae*.

2. Materials and methods

2.1. N. ceranae prevalence, intensity and survival analyses

In June 2014, capped combs with emerging bees were obtained from eight honey bee colonies headed by sister queens at Oregon State University apiaries (Corvallis, OR, USA). Sister queen colonies were used to control any variation in *Nosema* infection attributed to genetics of the bees. These combs with emerging bees were placed in an incubator under simulated hive conditions (33 °C, 55% RH) for bee emergence. Twenty four hours later we gently brushed newly emerged bees into a large container and mixed them thoroughly by hand. After the bees were mixed, 250 individual bees were placed inside cylindrical wire cages (3069 cm^3) and returned to the incubator. To provide caged bees with *ad libitum* access to water and 50% sucrose solution, two 25-ml glass vials (one containing each liquid) were covered with two layers of cheesecloth and then secured, inverted, to the top of each cage. On alternate days, we measured the consumption of both water and sucrose solution and replaced the vials. Varying dilutions of pollen diet were also provided to the bees in experimental cages.