Survival and health improvement of Nosema infected Apis florea (Hymenoptera: Apidae) bees after treatment with propolis extract

Article *in* Journal of Asia-Pacific Entomology · February 2018

DOI: 10.1016/j.aspen.2018.02.006

CITATIONS
25

READS
757

5 authors, including:

Guntima Suwannapong
Burapha University
47 PUBLICATIONS **402** CITATIONS

SEE PROFILE

Samrit Maksong
Kanchanaburi Rajabhat University
6 PUBLICATIONS **83** CITATIONS

SEE PROFILE

Mark Eric Benbow
Michigan State University
260 PUBLICATIONS **5,305** CITATIONS

SEE PROFILE

Christopher Mayack
United States Department of Agriculture
40 PUBLICATIONS **1,009** CITATIONS

SEE PROFILE

Journal of Asia-Pacific Entomology 21 (2018) 437–444

Contents lists available at ScienceDirect

Journal of Asia-Pacific Entomology

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

Survival and health improvement of *Nosema* infected *Apis florea* (Hymenoptera: Apidae) bees after treatment with propolis extract

G. Suwannapong[a], S. Maksong[a], M. Phainchajoen[b], M.E. Benbow[c], C. Mayack[d,e,*]

[a] Biological Science Program, Faculty of Science, Burapha University, Chon Buri 20131, Thailand
[b] King Mongkut's University of Technology (Ratcha Buri Learning Center), 70150, Thailand
[c] Department of Osteopathic Medical Specialties, Michigan State University, East Lansing, MI 48824, USA
[d] Department of Biology, Swarthmore College, PA 19081, USA
[e] Molecular Biology, Genetics, and Bioengineering, Faculty of Engineering and Natural Sciences, Sabancı University, İstanbul, Turkey

ARTICLE INFO

Keywords:
Alternative treatments
Nosema ceranae
Propolis
Trehalose
Bee health

ABSTRACT

Nosema ceranae is now considered to be an emerging infectious disease of the European honey bee *Apis mellifera*. Only one antibiotic, Fumagillin, is commercially available to combat *Nosema* infections. This antibiotic treatment is banned from use in Europe and elsewhere there is a high probability for antibiotic resistance to develop. We are therefore interested in investigating the effects of a natural propolis extract on its ability to reduce *N. ceranae* infection loads in the dwarf honey bee, *Apis florea*, a native honey bee with a range that overlaps with *Apis cerana* and *Apis mellifera* that is at risk of infection. Experimentally infected caged bees were fed a treatment consisting of 0%, 50%, or 70% propolis extract. All 50% and 70% propolis treated bees had significantly lower infection loads, and the 50% treated bees had higher survival in comparison to untreated bees. In addition, propolis treated bees had significantly higher haemolymph trehalose levels and hypopharyngeal gland protein content similar to levels of uninfected bees. Propolis ethanolic extract treatment could therefore be considered as a possible viable alternative to Fumagillin to improve bee health. This natural treatment deserves further exploration to develop it as a possible alternative to combat *N. ceranae* infections distributed around the world.

Introduction

Nosemosis, is a disease caused when honey bees are infected with *Nosema ceranae* or *Nosema apis*, and it is now currently distributed around the world (Higes et al., 2013; Paxton et al., 2007; Williams et al., 2008a; Suwannapong et al., 2011a; Fries, 2010). Nosemosis is also implicated as one of the possible factors responsible for the recent decline in honey bee health (Higes et al., 2013; Higes et al., 2010a; Higes et al., 2008). *N. ceranae* is much more prevlanant and is suspected to be replacing N. apis throughout the world. In the European honey bee *Apis mellifera*, N. apis appears to have a competitive disadvantage when co-infected with the relatively new N. ceranae (Natsopoulou et al., 2015; Williams et al., 2014). The widespread invasive nature of N. ceranae is concerning because it is suspected to be a larger threat to sustaining honey bee health than previously thought. *Apis florea* can potentially get a *N. ceranae* infection from shared flower use of contaminated flowers or other food sources because it has foraging areas that overlap with *Apis cerana* and *Apis mellifera*. For this reason, there is potential for *N. ceranae* to jump from its original host, *A. cerana*, to other bee species like it has done with *A. mellifera*. If this is the case there is potential for the lowering of bee health of *A. florea* due to increased virulence in this new host like what has been found with *A. mellifera* (Higes et al., 2013; Suwannapong et al., 2011a; Higes et al., 2010b; Botías et al., 2013).

Maintaining honey bee health is critical to sustaining current food production practices. Honey bees provide important ecosystem and agricultural services as pollinators, and thus maintaining honey bee health is paramount to aid high agricultural output in order to meet the growing demand of food consumption (Breeze et al., 2014; Brittain et al., 2013; Breeze et al., 2011; Potts et al., 2010; Klein et al., 2007). *A. florea* in particular is valuable for local economic development in Thailand because this species of honey bee is the primary pollinator of many crops and wild plants (Suwannapong et al., 2011b). Although *Nosema* infected bees do not exhibit obvious external disease symptoms, they can have digestive disorders resulting in malnutrition, reduced hypopharyngeal glands, and shortened life spans (Goblirsch et al., 2013; Woyciechowski and Lomnicki, 1995; Woyciechowski and Kozlowski, 1998; Wang and Moeller, 1971; Wang and Moeller, 1969). In general, malnutrition and energetic stress have emerged to be one of the main pathological effects from a *N. ceranae* infection in *Apis*

* Corresponding author at: Sabancı University, Faculty of Engineering and Natural Sciences, Tuzla İstanbul 34956, Turkey.
E-mail address: cmayack@sabanciuniv.edu (C. Mayack).

https://doi.org/10.1016/j.aspen.2018.02.006
Received 12 October 2017; Received in revised form 5 February 2018; Accepted 9 February 2018
Available online 19 February 2018

mellifera (Dussaubat et al., 2012; Vidau et al., 2014; Mayack and Naug, 2009; Naug and Gibbs, 2009; Alaux et al., 2010). Furthermore, hives with Nosemosis demonstrate lower honey yields and depopulation of worker bees (Fries et al., 1984; White, 1919). One of the possible mechanisms suggested for the depopulation of hives is due to forager energetic stress (Mayack and Naug, 2010; Mayack and Naug, 2013; Wolf et al., 2014), as infected bees have lower haemolymph trehalose, which is the sugar used to power flight when foraging out away from the hive (Thompson, 2003; Blatt and Roces, 2001). Accompanying this reduction in trehalose is an increase in bee mortality (Mayack and Naug, 2009; Mayack and Naug, 2013; Martín-Hernández et al., 2011). The pathological effects of *Nosema* do not cause immediate death, but still can reduce pollination effectiveness and increase the likelihood of a colony collapsing (Higes et al., 2008; Wolf et al., 2014; Naug, 2014). Thus, there is a need for effective treatment control measures in order to combat *Nosema* infections on a regular basis.

The antibiotic Fumagillin was the first cost effective treatment identified for combating *N. apis* infections (Goodman et al., 1990; Moffett et al., 1969). However, Fumagillin is only effective at killing the vegetative stage of the *N. apis* life cycle, and mature spores are resistant to Fumagillin treatment (Katznelson and Jamieson, 1952; Liu, 1973). Furthermore, Fumagillin has been shown to be only temporarily effective at reducing *N. ceranae* parasite burdens in honey bee colonies (Williams et al., 2008b) and is banned from use in Europe (Higes et al., 2014). This is a pressing concern because the spread of *N. ceranae* is on the rise and this pathogen is also potentially more virulent than *N. apis* (Paxton et al., 2007; Williams et al., 2014; Martín-Hernández et al., 2011). Therefore, the use of Fumagillin, despite its limited effectiveness against *N. ceranae*, will continue to increase and consequently there is a high probability for Fumagillin resistance to develop rapidly in *N. ceranae*.

In response to this, other antibiotics, including sulpha drugs have been tested for the control of *N. ceranae* with limited success (Roussel et al., 2015). There are additional drawbacks to the use of antibiotics, including sulpha drugs as well, as they pose potential health risks for humans when consuming contaminated honey. Increased exposure to these antibiotics and their residues are likely to confer increased bacterial antibiotic resistance to human diseases such as tuberculosis (Kochansky et al., 2001). Indeed, recently it has been shown that Fumagillin and its counterpart dicyclohexylamine, which are both highly toxic to mammals, does not completely degrade in contaminated honey held under typical hive conditions, even after one year (van den Heever et al., 2015). Therefore, it has been realized that a natural product and perhaps a less toxic one to humans that kills *Nosema,* is desirable (Maistrello et al., 2008).

Given that Nosema lives primarily in the gut, a number of natural treatments have been developed and tested that are administered orally by mixing the substance in sugar syrup that is bulk fed to bees. One treatment, Nozevit works by keeping the midgut pH low and thereby prevents the midgut from becoming rigid that is detrimental for absorption of nutrients (Higes et al., 2014). In the same vein, prebiotics and probiotics have been investigated to maintain this low pH required to determine if it reduces the parasite burden in the midgut, but actually an increase in *Nosema* loads have been observed (Ptaszynska et al., 2016), these increases however can be negated with Fumagillin treatment (Maggi et al., 2013). On the other hand, natural products such as Zeolite and BeeCleanse significantly reduce *Nosema* loads, but the magnitude of the effect was marginal, with millions of spores remaining in bees after many days of treatment (Gajger et al., 2013; Gajger et al., 2015). Essential oils and other plant extracts have been found to have a more dramatic and targeted effect at reducing *Nosema* loads and extending the life-span of infected bees, providing evidence for exploring treatments along these lines to be more promising for developing alternative treatment methods to combat *N. ceranae* (Damiani et al., 2014; Costa et al., 2010; Strachecka et al., 2015; Porrini et al., 2011).

Previous work supports the notion that propolis, as a natural product obtained from plant resins by bees, can be effective at inhibiting microsporidian development and improve infected honey bee survival (Suwannapong et al., 2011b; Krol et al., 1993). Therefore, in this study we not only evaluate the potential of propolis to control *Nosema* development in *Apis florea*, but we also measure the extent in which the treatment can ameliorate its associated pathological effects by measuring trehalose levels in the haemolymph and the protein content of the hypopharyngeal gland that are known to decrease in infected bees (Wang and Moeller, 1969; Mayack and Naug, 2010; Suwannapong et al., 2010). The aim of this study was to investigate the effect of propolis on experimentally infected *A. florea* workers inoculated with *Nosema* spores due to its ability to spread to other native bee species in the local area and cause increased virulence in a new host.

Materials and methods

Preparing propolis extractions

Propolis was obtained from three colonies of the stingless bee *Trigona apicalis* in an apiary located in Chanthaburi Province, Thailand. The propolis obtained was collected from plants growing in this local area collected by managed stingless bees from a central research station. Propolis was first dried in a hot air oven at 80 °C for 72 h, and then 60 g of it was shaken with a 100 ml of 70% ethanol, followed by gravity filtration using a Whatman No. 4 filter. This crude extract was stored in a dark bottle and was considered as a stock solution of 100% propolis extract. The stock propolis extract was then diluted with distilled water to make 50% and 70% concentrations (v/v) that were used in the following experiment as propolis ethanoic extraction treatments.

Nosema spore preparation

Nosema spores were isolated from three heavily infected colonies of *Apis cerana* located in the Samut Songkhram Province, in southern Thailand. Honey bee midguts were each placed in a microcentrifuge tube containing 200 µl distilled water and homogenized using a sterile pestle. These tubes were then spun at 6000*g* for 10 min three times or until pollen grains could be separated. Spores were counted using a hemocytometer. Spores were then re-suspended in 50% (w/v) sucrose solution at a concentration required to feed 8×10^4 spores per bee. The sucrose solution containing spores was kept at 4 °C until it was needed for inoculation.

Nosema inoculation and propolis extract treatment

Bee brood comb from tree branches were obtained from three colonies of *A. florea* free of *Nosema*. To provide newly emerged worker bees for caged experiments, this comb was incubated at 34 ± 2 °C with relative humidity maintained between 50 and 55%. The newly emerged bees were carefully removed and placed in a cage (50 bees per cage). Two days after eclosion they were divided into six treatment groups (50 bees per group), each treatment group was placed in one bee cage. The first three treatment groups were randomly selected to be inoculated with *Nosema* and this was accomplished by individually force-feeding 2 µl of the 50% sucrose solution (w/v) containing 8×10^4 *Nosema* spores. These treatment groups were then provided with 0%, 50% and 70% propolis extract mixed with 20 ml 50% sucrose solution (v/v), defined as 0P, 50P and 70P, respectively. The last three treatment groups were deemed as controls. The negative control (CO) was not infected with *Nosema*, was not treated with propolis, and did not receive any ethanol. The propolis control bees (CP), were not infected with *N. ceranae*, but instead were treated with 70% propolis, without ethanol. The last control group was infected with *N. ceranae*, but treated with 49% ethanol (CE), which was based on the amount used during the extraction of the 70% propolis extraction process. All treatment groups

Table 1
All of the treatment groups with their corresponding experimental manipulations. *Nosema* spores harvested from *Apis cerana* were used for the inoculation of *Apis florea*. All treatment groups were fed the same diet and each cage started with a total of 50 bees. group contained three replicate cages from each of the three source colonies, totaling to 9.

Treatment group	*N. ceranae* inoculation (spores/bee)	Propolis treatment (% v/v)	Ethanol exposure (%)	Food provided	Number of cage replicates (*N*)
(0P) Infected with 0% propolis extract treatment	80,000	0	49	60 g of pollen mixed with 20 ml of 50% sucrose solution (w/v)	9
(50P) Infected with 50% propolis extract treatment	80,000	50	49	60 g of pollen mixed with 20 ml of 50% sucrose solution (w/v)	9
(70P) Infected with 70% propolis extract treatment	80,000	70	49	60 g of pollen mixed with 20 ml of 50% sucrose solution (w/v)	9
(CO) No infection, with no treatment	0	0	0	60 g of pollen mixed with 20 ml of 50% sucrose solution (w/v)	9
(CP) No infection with propolis extract treatment	0	70	0	60 g of pollen mixed with 20 ml of 50% sucrose solution (w/v)	9
(CE) Infection with ethanol extract treatment	80,000	0	49	60 g of pollen mixed with 20 ml of 50% sucrose solution (w/v)	9

were provided with the same food (60 g of pollen mixed with 20 ml of 50% sucrose solution (w/v)) (Table 1).

Percent of infected gut cells in each bee

Three bees were randomly selected from each cage and were collected at 14 d p.i., so that their ventriculi could be processed for microscopic examination. The bee midguts were removed and fixed with Bouin's fixative for 22–24 h, then washed three times in 70% ethanol. These were then dehydrated with a standard series of ethanol concentrations (70–100%), cleared using xylene, infiltrated with mixtures of xylene and melted paraffin, and then embedded in pure melted paraffin. Tissue was cut 6 μm thick using a rotary microtome (Leica, Germany), then stained with periodic acid Schiff's reagent (PAS), and counterstained with light green for examination under a light microscope (Olympus CX50). The percentage of infected cells was calculated based on measuring a hundred ventricular cells.

Hypopharyngeal gland protein contents

Ten bees were randomly selected from each cage at six, ten, and fourteen days post inoculation (p.i.). Their hypopharyngeal glands were dissected and removed from each bee. These glands were transferred to 50 μl phosphate buffer (pH 7.4), were crushed, and then centrifuged at 1000*g* for 2 min. Supernatants were used for protein content analysis, which involved using a Bradford protein assay (Bradford, 1976) Standard curves were prepared using Bovine serum albumin (BSA), and the following concentrations, 25–500 μg/μl, were used to generate a standard curve. The absorbance was measured at a wavelength of 595 nm subtracted from a blank reagent using a Shimadzu UV–visible spectrophotometer (UV-1610).

Trehalose level in honey bee haemolymph

Ten bees were randomly selected and removed from each cage on six, ten and fourteen days post infection (p.i.), these bees were anaesthetized at −20 °C for 5 min so that their haemolymph could be collected. The immobilized bees were placed on a wax petri dish plate and mounted using a pair of insect pins that crossed over between the thorax and abdomen. Using a glass microcapillary, 5 μl of haemolymph per bee was collected, and the haemolymph was transferred to a microcentrifuge tube containing 45 μl of normal saline (0.9% NaCl). Each sample was then transferred to a 2.9 ml anthrone reagent (0.2% anthrone in 93% Sulfuric acid) and then vortexed for 30 s before being quickly placed into boiling water for 15 min followed by a cold water bath for 20 min. The samples were then read at 620 nm wavelength using a Shimadzu UV–visible spectrophotometer (UV-1610). For quantification purposes standard curves were generated using known amounts of trehalose.

Statistical analyses

The survival of caged bees within 30 days, across treatment groups, was analyzed using a Kaplan-Meier survival estimate. The infection ratio, hypopharyngeal gland protein content, and trehalose data were all found to be normal (Jarque-Bera test, $P > 0.05$). We therefore used a one-way ANOVA for the analysis of the infection ratio. We also used a General Linear Mixed Model (GLMM) for each dependent variable of infection ratio, hypopharyngeal gland protein content, and trehalose where the day after infection (6, 10, and 14 p.i.) and treatment (0% Propolis extract treatment, 50% Propolis extract treatment, 70% Propolis extract treatment, CO, Control for Ethanol, and CP) served as independent variables, cage number was considered as a random effect. A Tukey's post hoc test was performed for the Kaplan Meier estimates, day after infection, and treatment comparisons.

Results

Survival analysis

The survival of worker bees infected with *Nosema* spores was significantly lower than the propolis-treated bees. Honey bee mortality in the CE and 0P groups began on day seven p.i. Survival of the uninfected groups (CO, CP) and propolis treated infected groups (50P, 70P) were higher than the infected CE and 0P treatment groups. However, the survival rate of honey bees in the 50P and 70P was significantly lower than those of uninfected groups, CO and CP ($F = 39.48$, $df = 5$; $P < 0.0001$) (Fig. 1). The highest survival rate was found in the CO group with 89 ± 8.9%, followed by CP with 86 ± 8.4%, 50P with 54 ± 11.5%, 70P with 32 ± 2.1%, CE with 27 ± 9.6%, and 0P with 10 ± 1% survival, respectively.

Protein contents of hypopharyngeal glands

The overall protein content of the hypopharyngeal gland significantly fluctuated over time after the infection (GLMM days after infection main effect: $F_{2,53} = 19.86$, $P < 0.0001$) and the effect of the treatments was depended upon the time after post infection (GLMM interaction: $F_{10,53} = 5.49$, $P < 0.0001$). However, the control bees that were fed propolis extract (CP) had overall the highest protein content and this was significantly higher in comparison to the negative control (CO) and infected bees treated with 50% propolis extract (50P). This was followed by the infected bees treated with 70% propolis extract (70P) that had significantly higher protein content overall in comparison to infected bees treated with ethanol only (CE) and infected bees treated with 0% propolis extract (0P) (GLMM treatment main effects:

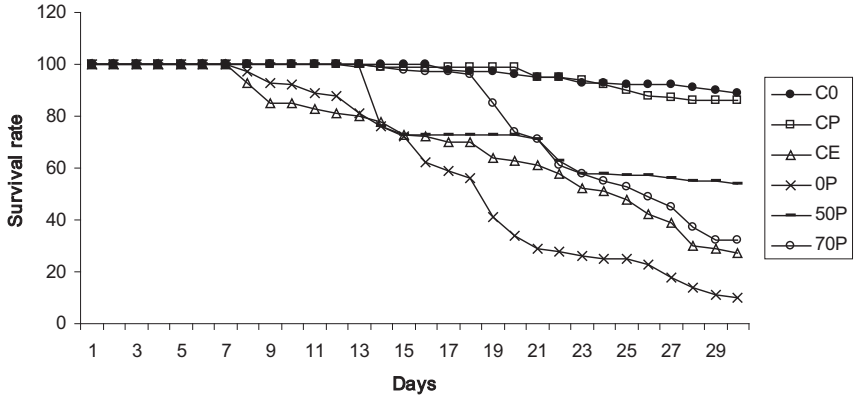

Fig. 1. Survival of honey bees (*A. florea*) after being infected with *Nosema* spores and treated with different concentration of propolis: 50% (50P), 70% (70P) and their respective controls (no propolis 0P, a negative control CO, and an ethanol control CE).

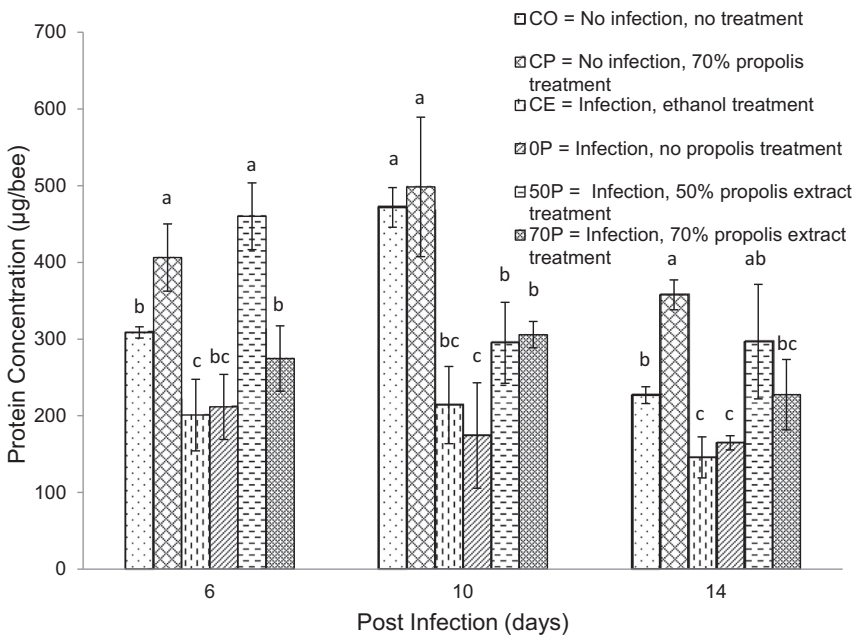

Fig. 2. Mean ± SD hypopharyngeal gland protein content of *A. florea* infected with *Nosema* at six, ten and fourteen days p.i. The treatment groups consisted of being treated with three dosages of propolis 0% (0P), 50% (50P), and 70% (70P), while the control bees did not receive any spores and these treatment groups consisted of a negative control (CO) without getting any treatment, an ethanol control (CE) that received only ethanol, and a propolis control (CP) that only received a 70% propolis extract.

$F_{5,53} = 19.86$, $P < 0.0001$, Tukey HSD, alpha = 0.05). *N. ceranae* infected bees after treatment with 50% and 70% propolis showed that propolis was associated with a significant overall increase in bee hypopharyngeal gland protein content in comparison to the CE and 0P treatment groups (Fig. 2).

Trehalose level in honey bee haemolymph

We found a significant increase and then decline in overall trehalose levels in the haemolymph as time increased after the infection (GLMM: time after infection main effect: $F_{2,53} = 69.59$, $P < 0.0001$) and the effects of the treatment depend on the time after post infection (GLMM: interaction: $F_{10,53} = 9.42$, $P < 0.0001$).

Overall across treatment groups the highest amount of haemolymph trehalose levels was found in the control uninfected bees either treated with or without propolis (CO and CP), and the infected bees treated with 70% propolis extract (70P). On the other hand, infected bees treated with 50% propolis extract (50P) had significantly lower haemolymph trehalose levels, but not as low as the infected bees treated with ethanol (CE) and the infected bees treated with 0% propolis extract (0P), as both of these groups had the lowest overall trehalose levels (GLMM treatment main effects: $F_{5,53} = 69.1$, $P < 0.0001$, Tukey HSD, alpha = 0.05) (Fig. 3).

Percentage of infected gut lining cells

The infection percentages of CO, CP, CE, 0P, 50P and 70P at 14 d p.i. were 0.0, 0.0, 66.98% ± 13.73, 72.18% ± 1.89, 16.09% ± 0.99 and 14.73% ± 0.60, respectively. The highest infection percentage occurred in the infected bees treated with 0% propolis extract (0P) and ethanol only (CE). The propolis extract treatment of 50% and 70% significantly reduced the *Nosema* load in infected bees, but these bees still had significantly higher infection loads in comparison to the uninfected bees (CO and CP) ($F_{5,17} = 62.56$, $P < 0.0001$) (Fig. 4). *Nosema* spores were distributed throughout the cell cytoplasm of all infected bees and the ventricular cells of control bees, CO and CP, showed no infection. The degeneration of the apical membrane was observed in infected bees (Fig. S3a and b). However, fewer spores were found in 50P and 70P treated bees compared to the infected bees that did not receive the propolis extract treatment (Fig. S3c and d). Transmission electron micrography of the ventricular cells of *A. florea* that were treated with the propolis extract showed deformed *N. ceranae* spores throughout the cytoplasm at day 14 p.i (Fig. S3e and f).

Discussion

Without any treatment, we confirmed that *Nosema* infection

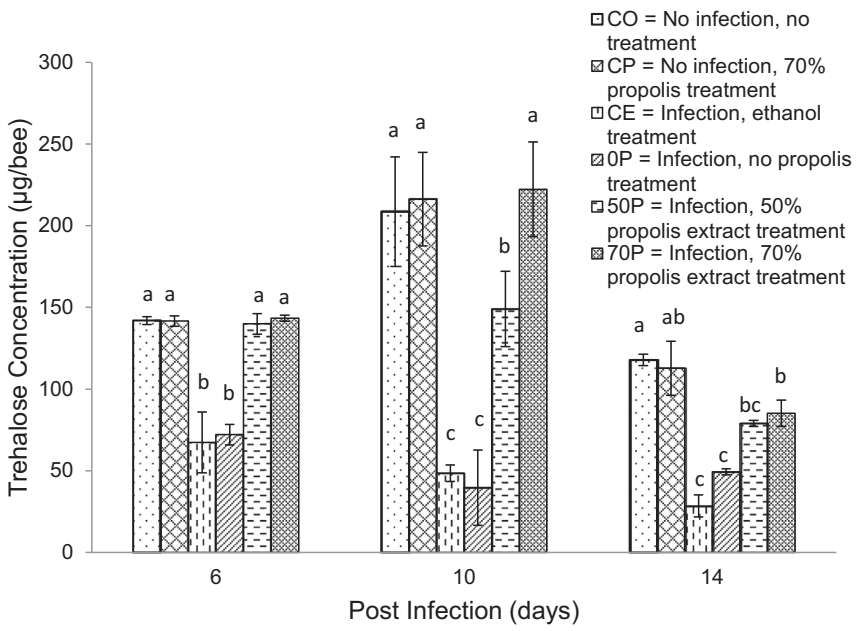

Fig. 3. Mean ± SD trehalose in the haemolymph of *A. florea* bees at six, ten and fourteen days p.i. The treatment groups consisted of infected bees being treated with 0% (0P), 50% (50P), and 70% (70P) dosages of propolis, while the control bees did not receive any spores and these treatment groups consisted of a negative control (CO) without getting any treatment, an ethanol control (CE) that received only ethanol, and a propolis control (CP) that only received a 70% propolis extract.

significantly reduces the life span of the red dwarf honey bee, *A. florea*. This finding is supported by previous studies that have demonstrated this in the closely related honey bee species *Apis mellifera* (Mayack and Naug, 2009; Mayack and Naug, 2010; Martín-Hernández et al., 2011; Maistrello et al., 2008; Suwannapong et al., 2010; Higes et al., 2007; Eiri et al., 2015) and with A. florea (Suwannapong et al., 2010). However, interestingly, bees treated with stingless bee propolis extract after infection with *N. ceranae* spores had significantly longer life spans, lowered parasite loads, but increased haemolymph trehalose and hypopharyngeal gland protein content as well. However, in this study we show that there appears to be a dose dependent effect because the higher 50P concentration of propolis extract that was used in this study causes a larger increase in survival. However, we cannot rule out the possibility that administering even higher concentrations may cause a potentially toxic effect because the 70P dose tested had significantly lower survival in comparison to the 50P treated bees and this could be due to exposure to extremely concentrated levels of terpenoids, flavonoids, phenols and other secondary plant metabolite compounds extracted from propolis (Porrini et al., 2011).

In this study we additionally demonstrate that the propolis extract treatment can cause a significant increase in the trehalose levels in the haemolymph of infected bees. These findings support the notion that stingless bee propolis extract can be used as an effective treatment to not only improve bee health by reducing *N. ceranae* parasite loads, but is also effective at counteracting one of the main pathological effects from the infection found in infected *A. mellifera*, which has been identified to be energetic stress indicated by the lowering of trehalose levels in the haemolymph (Dussaubat et al., 2012; Vidau et al., 2014; Mayack and Naug, 2009; Alaux et al., 2010; Mayack and Naug, 2010). This is important to note because energetic stress from an infection can have wide ranging effects and has been linked to risky foraging, lowered foraging frequency, and lowered foraging efficiency, which is suspected to decrease pollinator efficiency and reduce survival for the individual and the colony as a whole (Mayack and Naug, 2013; Wolf et al., 2014; Naug, 2014; Mayack and Naug, 2011). What is even more promising for using stingless bee propolis extract as a possible treatment to control *Nosema* is that the propolis extract treatment itself did not significantly reduce the life-span, protein content of the hypopharyngeal gland, or the haemolymph trehalose levels, suggesting that the propolis extract treatment itself does not appear to be harming the bee.

Other studies investigating the use of natural extracts have found that they are effective at reducing *Nosema* loads with the treatment itself not causing a decline in the survival of the honey bee *A. mellifera*, which suggests that using this approach to control *Nosema* infections is a viable option (Damiani et al., 2014; Strachecka et al., 2015). The bees treated with ethanol alone do not have significantly lower survival,

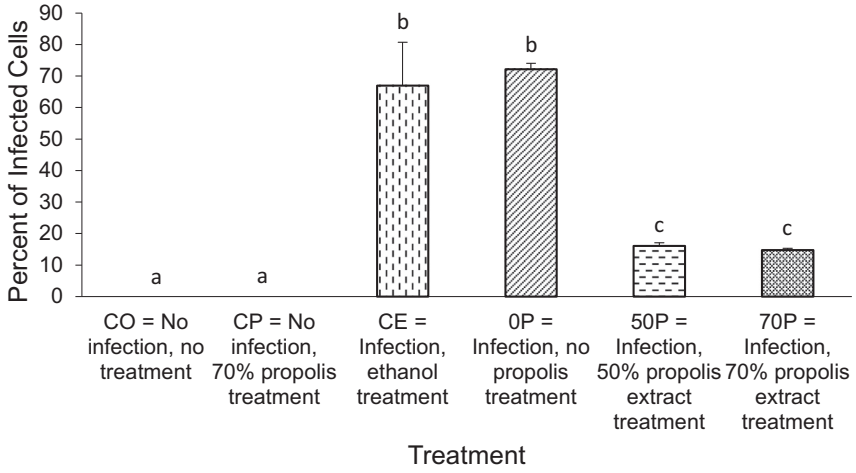

Fig. 4. Mean ± SD percentage of infected cells of the *A. florea* gut lining at 14 d p.i. The treatment groups consisted of 0% (0P), 50% (50P), and 70% (70P) propolis treatment, while (CO) the negative control did not receive any treatment, the ethanol control (CE) received only ethanol, and the propolis control (CP) only received a 70% propolis extract. Letters denote significant differences at the alpha = 0.05 level.

trehalose levels, or hypopharyngeal gland protein content either in comparison to the bees treated with 0% propolis that have a similar pathogen load, this confirms the idea that using ethanol as a solvent to extract propolis does not appear to contribute to any damaging effects beyond what *N. ceranae* would cause to the bees treated with the propolis extract. Previous findings demonstrated that a 5% ethanol treatment alone can actually synergistically increase the *Nosema* load of inoculated bees by reducing the pH of the midgut (Ptaszynska et al., 2013). Although we cannot rule out the impacts of altering the microbial gut community from administering inoculum from midguts of *A. cerana* as this could have implications for bee health (Kwong and Moran, 2016), we show that with the addition of propolis it appears to be the "active ingredient" responsible for the lowered *Nosema* loads observed.

We are confident that there is a relatively higher amount of *N. ceranae* spores in comparison to *N. apis* present in the inoculum prepared from the infected *A. ceranae* bees based on the molecular analysis. This finding corresponds to the external morphological structures observed using SEM and light microscopy. Based on visualization of spore morphology there were distinct and various spore shapes and sizes corresponding to the dimensions of the two different *Nosema* species, with a large majority of spores falling into the *N. ceranae* category (Fries et al., 1996).

The ethanol consumption could have been toxic to bees, but we do not find evidence for lowered survival in the infected bees treated with ethanol alone in comparison to the infected bees that did not receive the ethanolic propolis extract. Yet, the propolis extract did significantly increase survival of infected bees and this increased survival is likely to be result of the lowered infection, which is supported by observing the increase in trehalose levels and hypopharyngeal gland protein content. The reduced survival of *N. ceranae* infected honey bees in cages was also demonstrated previously (Williams et al., 2014; Martín-Hernández et al., 2011). This lowered survival in infected bees is known to be caused by lower trehalose levels in the haemolymph (Mayack and Naug, 2010). Therefore, the increase in trehalose levels found in propolis treated bees is likely to contribute to the increased survival observed in this study. Supporting this notion honey bee strains selected for *Nosema* tolerance have not only increased survival, but higher trehalose levels as well (Kurze et al., 2016). This suggests that the ability for the propolis treated bees to maintain higher trehalose levels in contrast to the ethanol treated bees is likely to be one reason we observe higher survival in these propolis treated bees.

Another key symptom of a *Nosema* infection is the reduction in the hypopharyngeal gland, which is correlated with lowered survival (Sagili et al., 2005), and precocious foraging, that is estimated to ultimately reduce the life-span of a *Nosema* infected honey bee by almost half (Goblirsch et al., 2013; Woyciechowski and Kozlowski, 1998; Hassanein, 1953). Therefore, the prevention of hypopharyngeal gland loss in addition to increasing survival in propolis treated bees is critical to improving bee health. *Nosema* infections have been shown to reduce the pollen foraging in bees which may compound the effects of not obtaining enough protein which leads to the reduction in the hypopharyngeal gland in *Nosema* infected honey bees (Anderson and Giacon, 1992). In the same vein, despite higher parasite loads in infected honey bees fed with a high pollen diet, a larger hypopharyngeal gland was linked to higher survival in these infected bees (Jack et al., 2016). These findings suggest that maintaining the protein content of the hypopharyngeal gland and trehalose levels are key factors to overcoming the pathological effects of a *Nosema* infection that might result from a high *Nosema* parasite load. Our findings are comprehensive in nature suggesting that propolis treatment should improve bee health on an individual and colony level. Disruption of the basic underpinnings of temporal polyethism due to the reduction of the hypopharyngeal gland may be a contributing factor to recent high colony mortality, because workers may lose flexibility in responding to environmental stressors that might lead to changes in colony demands (Goblirsch et al., 2013).

Our findings are also supported by the fact that stingless bee propolis has documented antimicrobial activities similar to the more well-known honey bee propolis (Farnesi et al., 2009). Moreover, previous studies have shown that the antiseptic properties of ethanol alone does not effectively reduce *N. ceranae* loads (Ptaszynska et al., 2013), suggesting that in this study it is indeed the propolis which is the "active ingredient" that is responsible for the reduced parasite load and increased survival observed in the propolis treated bees. To make this a viable option for beekeepers however, substantial improvements are still needed, one of which is perhaps using a different extraction solvent that does not adversely affect honey bee survival. An additional challenge would be to harvest the propolis from the stingless bees on a mass scale, but this harvesting of propolis is already performed with managed stingless bee colonies on a regular basis in Thailand and other countries (Silva-Carvalho et al., 2015). *A. florea* bees do not typically consume propolis and are not as heavily managed in comparison to *A. mellifera*, but they are managed for pollination services and the interest for managing them for honey production in Thailand is growing, as the cost of importing *A. mellifera* in terms of potential exotic disease transmission, is increasing (Suwannapong et al., 2011b). Propolis extract can easily be added to a high concentration of sugar syrup to an external feeder placed close to the hive, which will then often be visited by *A. florea* foraging bees to administer this treatment, if the hives are found to be infected with *N. ceranae*. In future studies, it would be interesting to see if this propolis extract treatment is just as effective for *A. mellifera* infections as this might confirm how representative this treatment might be in its antifungal properties. Our results suggest new opportunities for maintaining long-term bee health and sheds light on possible alternative ways, other than using Fumagillin, to manage *Nosema* infections. The development of new methods for the control of Nosemosis like in this study, if applicable to *A. mellifera*, will benefit beekeepers and bee scientists interested in controlling *Nosema* infections, which will facilitate new strategies that can be used to improve honey bee health.

Funding

This study was supported by the 376830 Thailand Research Fund (NRPM).

Conflict of interest

All authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants performed by any of the authors.

Acknowledgements

We would like to thank anonymous reviewers for their comments on this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aspen.2018.02.006.

References

Alaux, C., Brunet, J.-L., Dussaubat, C., Mondet, F., Tchamitchan, S., Cousin, M., et al., 2010. Interactions between *Nosema* microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). Environ. Microbiol. 12 (3), 774–782.

Anderson, D.L., Giacon, H., 1992. Reduced pollen collection by honey bee (Hymenoptera: Apidae) colonies infected with *Nosema apis* and *Sacbrood virus*. J. Econ. Entomol. 85, 47–51.

Blatt, J., Roces, F., 2001. Haemolymph sugar levels in foraging honeybees (*Apis mellifera carnica*): dependence on metabolic rate and in vivo measurement of maximal rates of trehalose synthesis. J. Exp. Biol. 204, 2709–2716.

Botías, C., Martín-Hernández, R., Barrios, L., Meana, A., Higes, M., 2013. *Nosema* spp. infection and its negative effects on honey bees (*Apis mellifera iberiensis*) at the colony level. Vet. Res. 44 (1), 25. http://dx.doi.org/10.1186/1297-9716-44-25. (PubMed PMID: PMC3640932).

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72 (1–2), 248–254. http://dx.doi.org/10.1006/abio.1976.9999. (PubMed PMID: WOS:A1976BU74400029).

Breeze, T.D., Bailey, A.P., Balcombe, K.G., Potts, S.G., 2011. Pollination services in the UK: how important are honeybees? Agric. Ecosyst. Environ. 142 (3–4), 137–143. http://dx.doi.org/10.1016/j.agee.2011.03.020. (PubMed PMID: WOS:000295245100003).

Breeze, T.D., Vaissiere, B.E., Bommarco, R., Petanidou, T., Seraphides, N., Kozak, L., et al., 2014. Agricultural policies exacerbate honeybee pollination service supply-demand mismatches across Europe. PLoS One 9 (1), e82996.

Brittain, C., Williams, N., Kremen, C., Klein, A.-M., 2013. Synergistic effects of non-Apis bees and honey bees for pollination services. Proc. R. Soc. B Biol. Sci. 280 (1754). http://dx.doi.org/10.1098/rspb.2012.2767.

Costa, C., Lodesani, M., Maistrello, L., 2010. Effect of thymol and resveratrol administered with candy or syrup on the development of *Nosema ceranae* and on the longevity of honeybees (*Apis mellifera* L.) in laboratory conditions. Apidologie 41 (2), 141–150. http://dx.doi.org/10.1051/apido/2009070. (PubMed PMID: WOS:000276461200004).

Damiani, N., Fernandez, N.J., Porrini, M.P., Gende, L.B., Alvarez, E., Buffa, F., et al., 2014. Laurel leaf extracts for honeybee pest and disease management: antimicrobial, microsporicidal, and acaricidal activity. Parasitol. Res. 113 (2), 701–709. http://dx.doi.org/10.1007/s00436-013-3698-3. (PubMed PMID: WOS:000333028100025).

Dussaubat, C., Brunet, J.-L., Higes, M., Colbourne, J.K., Lopez, J., Choi, J.-H., et al., 2012. Gut pathology and responses to the microsporidium *Nosema ceranae* in the honey bee *Apis mellifera*. PLoS One 7 (5), e37017.

Eiri, D.M., Suwannapong, G., Endler, M., Nieh, J.C., 2015. *Nosema ceranae* can infect honey bee larvae and reduces subsequent adult longevity. PLoS ONE 10 (5), 17. http://dx.doi.org/10.1371/journal.pone.0126330. (PubMed PMID: WOS:000355185600049).

Farnesi, A.P., Aquino-Ferreira, R., De Jong, D., Bastos, J.K., Soares, A.E.E., 2009. Effects of stingless bee and honey bee propolis on four species of bacteria. Genet. Mol. Res. 8 (2), 635–640 (PubMed PMID: WOS:000267938300031).

Fries, I., 2010. *Nosema ceranae* in European honey bees (*Apis mellifera*). J. Invertebr. Pathol. 103 (Supplement 1), S73–S79.

Fries, I., Ekbohm, G., Villumstad, E., 1984. *Nosema-apis*, sampling techniques and honey yield. J. Apic. Res. 23 (2), 102–105 (PubMed PMID: WOS:A1984TG45000009).

Fries, I., Feng, F., Silva, A., Slemenda, S., Pieniazek, N., 1996. *Nosema ceranae* n. sp. (Microspora, Nosematidae), morphological and molecular characterization of a microsporidian parasite of the Asian honey bee *Apis cerana* (Hymenoptera, Apidae). Eur. J. Protistol. 32 (3), 356–365.

Gajger, I.T., Tomljanovic, Z., Stanisavljevic, L., 2013. An environmentally friendly approach to the control of *Varroa destructor* mite and *Nosema ceranae* disease in Carniolan honeybee (*Apis mellifera* Carnica) colonies. Arch. Biol. Sci. 65 (4), 1585–1592. http://dx.doi.org/10.2298/abs1304585g. (PubMed PMID: WOS:000330326600039).

Gajger, I.T., Ribaric, J., Matak, M., Svecnjak, L., Kozaric, Z., Nejedli, S., et al., 2015. Zeolite clinoptilolite as a dietary supplement and remedy for honeybee (*Apis mellifera* L.) colonies. Vet. Med. 60 (12), 696–705 (PubMed PMID: WOS:000366393100005).

Goblirsch, M., Huang, Z.Y., Spivak, M., 2013. Physiological and behavioral changes in honey bees (*Apis mellifera*) induced by *Nosema ceranae* infection. PLoS ONE 8 (3), e58165. http://dx.doi.org/10.1371/journal.pone.0058165. (PubMed PMID: WOS:000316936100082).

Goodman, M., Houten, At, Perry, J., Blackmann, R., 1990. Cost benefit analysis of using fumagillin to treat *Nosema*. N. Z. Beekeeper 208, 11–12.

Hassanein, M.H., 1953. The influence of infection with *Nosema apis* on the activities and longevity of the worker honeybee. Anal. Appl. Biol. 40, 418–423.

van den Heever, J.P., Thompson, T.S., Curtis, J.M., Pernal, S.F., 2015. Stability of dicyclohexylamine and fumagillin in honey. Food Chem. 179, 152–158. http://dx.doi.org/10.1016/j.foodchem.2015.01.111. (PubMed PMID: WOS:000352926400018).

Higes, M., García-Palencia, P., Martín-Hernández, R., Meana, A., 2007. Experimental infection of *Apis mellifera* honeybees with *Nosema ceranae* (Microsporidia). J. Invertebr. Pathol. 94, 211–217.

Higes, M., Martín-Hernández, R., Botías, C., Bailón, E.G., González-Porto, A.V., Barrios, L., et al., 2008. How natural infection by *Nosema ceranae* causes honeybee colony collapse. Environ. Microbiol. 10, 2659–2669.

Higes, M., Martin-Hernandez, R., Meana, A., 2010a. *Nosema ceranae* in Europe: an emergent type C nosemosis. Apidologie 41 (3), 375–392. http://dx.doi.org/10.1051/apido/2010019. (PubMed PMID: ISI:000279029200012).

Higes, M., Martín-Hernández, R., Martinez-Salvador, A., Garrido-Bailón, E., González-Porto, A.V., Meana, A., et al., 2010b. A preliminary study of the epidemiological factors related to honey bee colony loss in Spain. Environ. Microbiol. Rep. 2, 243–250.

Higes, M., Meana, A., Bartolomé, C., Botías, C., Martín-Hernández, R., 2013. *Nosema ceranae* (Microsporidia), a controversial 21st century honey bee pathogen. Environ. Microbiol. Rep. 5 (1), 17–29. http://dx.doi.org/10.1111/1758-2229.12024.

Higes, M., Gomez-Moracho, T., Rodriguez-Garcia, C., Botias, C., Martin-Hernandez, R., 2014. Preliminary effect of an experimental treatment with "Nozevit (R)", (a phytopharmacological preparation) for *Nosema ceranae* control. J. Apic. Res. 53 (4), 472–474. http://dx.doi.org/10.3896/ibra.1.53.4.03. (PubMed PMID: WOS:000344516000009).

Jack, C.J., Uppala, S.S., Lucas, H.M., Sagili, R.R., 2016. Effects of pollen dilution on infection of *Nosema ceranae* in honey bees. J. Insect Physiol. 87, 12–19. http://dx.doi.org/10.1016/j.jinsphys.2016.01.004. (PubMed PMID: WOS:000371836600002).

Katznelson, H., Jamieson, C.A., 1952. Control of *Nosema* disease of honeybees with fumagillin. Science 115 (2977), 70–71.

Klein, A., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., et al., 2007. Importance of pollinators in changing landscapes for world crops. Proc. R. Soc. Lond. B Biol. Sci. 274, 303–313.

Kochansky, J., Knox, D.A., Feldlaufer, M., Pettis, J.S., 2001. Screening alternative antibiotics against oxytetracycline-susceptible and -resistant *Paenibacillus* larvae. Apidologie 32 (3), 215–222 (PubMed PMID: WOS:000169759600002).

Krol, W., Scheller, S., Shani, J., Pietsz, G., Czuba, Z., 1993. Synergistic effect of ethanolic extract of propolis and antibiotics on the growth of *Staphylococcus aureus*. Arzneimittelforschung 43–1 (5), 607–609 (PubMed PMID: WOS:A1993LD58000021).

Kurze, C., Mayack, C., Hirche, F., Stangl, G.I., Le Conte, Y., Kryger, P., et al., 2016. *Nosema* spp. infections cause no energetic stress in tolerant honeybees. Parasitol. Res. 115 (6), 2381–2388.

Kwong, W.K., Moran, N.A., 2016. Gut microbial communities of social bees. Nature reviews Microbiology. 14 (6), 374–384. Epub 2016/05/04. https://doi.org/10.1038/nrmicro.2016.43 (PubMed PMID: 27140688; PubMed Central PMCID: PMCPMC5648345).

Liu, T.P., 1973. Effects of fumidil B on the spore of *Nosema apis* and on lipids of the host cell as revealed by freeze-etching. J. Invertebr. Pathol. 22 (3), 364–368. https://doi.org/10.1016/0022-2011(73)90165-1.

Maggi, M., Negri, P., Plischuk, S., Szawarski, N., De Piano, F., De Feudis, L., et al., 2013. Effects of the organic acids produced by a lactic acid bacterium in *Apis mellifera* colony development, *Nosema ceranae* control and fumagillin efficiency. Vet. Microbiol. 167 (3–4), 474–483. http://dx.doi.org/10.1016/j.vetmic.2013.07.030. (PubMed PMID: WOS:000330488600029).

Maistrello, L., Lodesani, M., Costa, C., Leonardi, F., Marani, G., Caldon, M., et al., 2008. Screening of natural compounds for the control of *Nosema* disease in honeybees (*Apis mellifera*). Apidologie 39 (4), 436–445. http://dx.doi.org/10.1051/apido:2008022. (PubMed PMID: WOS:000257144400007).

Martín-Hernández, R., Botías, C., Barrios, L., Martínez-Salvador, A., Meana, A., Mayack, C., et al., 2011. Comparison of the energetic stress associated with experimental *Nosema ceranae* and *Nosema apis* infection of honeybees (*Apis mellifera*). Parasitol. Res. 109 (3), 605–612.

Mayack, C., Naug, D., 2009. Energetic stress in the honeybee *Apis mellifera* from *Nosema ceranae* infection. J. Invertebr. Pathol. 100, 185–188.

Mayack, C., Naug, D., 2010. Parasitic infection leads to decline in hemolymph sugar levels in honeybee foragers. J. Insect Physiol. 56 (11), 1572–1575.

Mayack, C., Naug, D., 2011. A changing but not an absolute energy budget dictates risk-sensitive behaviour in the honeybee. Anim. Behav. 82 (3), 595–600.

Mayack, C., Naug, D., 2013. Individual energetic state can prevail over social regulation of foraging in honeybees. Behav. Ecol. Sociobiol. 67 (6), 929–936. http://dx.doi.org/10.1007/s00265-013-1517-6.

Moffett, J.O., Lackett, J.J., Hitchcock, J.D., 1969. Compounds tested for control of *Nosema* in honey bees. J. Econ. Entomol. 62 (4), 886–889. http://dx.doi.org/10.1093/jee/62.4.886.

Natsopoulou, M.E., McMahon, D.P., Doublet, V., Bryden, J., Paxton, R.J., 2015. Interspecific competition in honeybee intracellular gut parasites is asymmetric and favours the spread of an emerging infectious disease. Proc. R. Soc. B Biol. Sci. 282 (1798), 20141896. http://dx.doi.org/10.1098/rspb.2014.1896.

Naug, D., 2014. Infected honeybee foragers incur a higher loss in efficiency than in the rate of energetic gain. Biol. Lett. 10 (11). http://dx.doi.org/10.1098/rsbl.2014.0731.

Naug, D., Gibbs, A., 2009. Behavioral changes mediated by hunger in honeybees infected with *Nosema ceranae*. Apidologie 40 (6), 595–599. http://dx.doi.org/10.1051/apido/2009039. (PubMed PMID: ISI:000273050200001).

Paxton, R.J., Klee, J., Korpela, S., Fries, I., 2007. *Nosema ceranae* has infected *Apis mellifera* in Europe since at least 1998 and may be more virulent than *Nosema apis*. Apidologie 38, 558–565.

Porrini, M.P., Fernandez, N.J., Garrido, P.M., Gende, L.B., Medici, S.K., Eguaras, M.J., 2011. In vivo evaluation of antiparasitic activity of plant extracts on *Nosema ceranae* (Microsporidia). Apidologie 42 (6), 700–707. http://dx.doi.org/10.1007/s13592-011-0076-y. (PubMed PMID: WOS:000297707400004).

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E., 2010. Global pollinator declines: trends, impacts and drivers. Trends Ecol. Evol. 25 (6), 345–353.

Ptaszynska, A.A., Borsuk, G., Mulenko, W., Olszewski, K., 2013. Impact of ethanol on *Nosema* spp. infected bees. Med. Weter. 69 (12), 736–740 (PubMed PMID: WOS:000327925300007).

Ptaszynska, A.A., Borsuk, G., Zdybicka-Barabas, A., Cytrynska, M., Malek, W., 2016. Are commercial probiotics and prebiotics effective in the treatment and prevention of honeybee nosemosis C? Parasitology Research. 115 (1), 397–406. http://dx.doi.org/10.1007/s00436-015-4761-z. (PubMed PMID: WOS:000370868600041).

Roussel, M., Villay, A., Delbac, F., Michaud, P., Laroche, C., Roriza, D., et al., 2015. Antimicrosporidian activity of sulphated polysaccharides from algae and their potential to control honeybee nosemosis. Carbohydr. Polym. 133, 213–220. http://dx.doi.org/10.1016/j.carbpol.2015.07.022. (PubMed PMID: WOS:000361920900028).

Sagili, R.R., Pankiw, T., Zhu-Salzman, K., 2005. Effects of soybean trypsin inhibitor on hypopharyngeal gland protein content, total midgut protease activity and survival of the honey bee (*Apis mellifera* L.). J. Insect Physiol. 51 (9), 953–957.

Silva-Carvalho, R., Baltazar, F., Almeida-Aguiar, C., 2015. Propolis: A Complex Natural Product with a Plethora of Biological Activities That Can Be Explored for Drug

Development. Evid.-Based Complement. Altern. Med. 29http://dx.doi.org/10.1155/2015/206439. (PubMed PMID: WOS:000356266200001).
Strachecka, A.J., Olszewski, K., Paleolog, J., 2015. Curcumin stimulates biochemical mechanisms of *Apis mellifera* resistance and extends the apian life-span. J. Apic Sci. 59 (1), 129–141. http://dx.doi.org/10.1515/jas-2015-0014. (PubMed PMID: WOS:000355623500013).
Suwannapong, G., Maksong, S., Seanbualuang, P., Benbow, M.E., 2010. Experimental infection of red dwarf honeybee, *Apis florea*, with *Nosema ceranae*. J. Asia Pac. Entomol. 13 (4), 361–364.
Suwannapong, G., Yemor, T., Boonpakdee, C., Benbow, M.E., 2011a. *Nosema ceranae*, a new parasite in Thai honeybees. J. Invertebr. Pathol. 106 (2), 236–241.
Suwannapong, G., Benbow, M.E., Nieh, J.C., 2011b. Biology of Thai honeybees: natural history and threats. In: Florio, R.M. (Ed.), Bees: Biology, Threats and Colonies. Nova Science Publishers Inc., Hauppauge, pp. 1–98.
Thompson, S.N., 2003. Trehalose - the insect 'blood' sugar. Adv. Insect Physiol. 31, 205–285.
Vidau, C., Panek, J., Texier, C., Biron, D.G., Belzunces, L.P., Le Gall, M., et al., 2014. Differential proteomic analysis of midguts from *Nosema ceranae*-infected honeybees reveals manipulation of key host functions. J. Invertebr. Pathol. 121, 89–96. http://dx.doi.org/10.1016/j.jip.2014.07.002.
Wang, D.-I., Moeller, F.E., 1969. Histological comparisons of the development of hypopharyngeal glands in healthy and *Nosema*-infected worker honey bees. J. Invertebr. Pathol. 14, 135–142.
Wang, D., Moeller, F.E., 1971. Ultrastructural changes in the hypopharyngeal glands of work honey bees infected by *Nosema apis*. J. Invertebr. Pathol. 17, 308–320.
White, G.F., 1919. *Nosema* disease. Bull. US Dept. Agric. 780, 1–59.
Williams, G.R., Shafer, A., Rogers, R., Shutler, D., Stewart, D., 2008a. First detection of *Nosema ceranae*, a microsporidian parasite of European honey bees (*Apis mellifera*), in Canada and central USA. J. Invertebr. Pathol. 97, 189–192.
Williams, G.R., Sampson, M.A., Shutler, D., Rogers, R.E.L., 2008b. Does fumagillin control the recently detected invasive parasite *Nosema ceranae* in western honey bees (*Apis mellifera*)? J. Invertebr. Pathol. 99, 342–344.
Williams, G.R., Shutler, D., Burgher-MacLellan, K.L., Rogers, R.E.L., 2014. Infra-population and -community dynamics of the parasites *Nosema apis* and *Nosema ceranae*, and consequences for honey bee (*Apis mellifera*) hosts. PLoS One 9 (7), e99465.
Wolf, S., McMahon, D.P., Lim, K.S., Pull, C.D., Clark, S.J., Paxton, R.J., et al., 2014. So near and yet so far: harmonic radar reveals reduced homing ability of *Nosema* infected honeybees. PLoS One 9 (8), e103989.
Woyciechowski, M., Kozlowski, J., 1998. Division of labor by division of risk according to worker life expectancy in the honey bee (*Apis mellifera* L.). Apidologie 29, 191–205.
Woyciechowski, M., Lomnicki, A., 1995. Honey bee workers parasitized by *Nosema apis* choose riskier foraging strategies. Pszczelnicze Zeszyty Naukowe. 39 (1), 209–210.