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The effect of manuka honey on two-spotted lady beetle larvae, *Adalia bipunctata* L. (Coleoptera: Coccinellidae), infected with a microsporidian pathogen

By
Kenja Ousha James

A Thesis Submitted to
Saint Mary's University, Halifax, Nova Scotia
in Partial Fulfillment of the Requirements for
the Degree of Bachelor of Science (Honours Biology).

May, 2019, Halifax, Nova Scotia

Approved: Susan Bjornson

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Professor

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Abstract

The full potential of biological control agents, such as the two-spotted lady beetle, *Adalia bipunctata* L., against insect pests greatly depends on the ability of the control agent to successfully mature to adulthood. Intracellular pathogens such as microsporidia, affect larval growth and development. Differences in food quality and quantity can also affect larval growth and development, as well as reproduction. The aim of this research is to determine whether the effects of the microsporidian pathogen, *Nosema adaliae*, is mitigated when its host, *A. bipunctata*, is reared on mixed diet of green peach aphid (*Myzus persicae* Sulzer) and antimicrobial manuka honey. Development and mortality of infected lady beetles were compared between four different treatment groups: the control group was provided aphids and water, whereas the three treatment groups were given aphids and three different dilutions of honey (5%, 10% and 15%), depending on the treatment. Larval development for the control was 15.9 ± 0.7 SE days, whereas larval development for the treatment groups was 13.8 ± 0.6, 13.8 ± 1.0, and 13.3 ± 0.9 days for larvae that were provided with 5%, 10% and 15% manuka honey, respectively. Our results indicated that the addition of manuka honey into the diet of infected *A. bipunctata* resulted in a decrease in larval development time while mortality and sex ratio were unaffected. This suggests that the addition of non-prey food such as manuka honey to an essential diet of *M. persicae* can shorten the larval development period. Further quantitative analysis would determine if there is a direct impact of manuka honey on the pathogen.

[March 29, 2019]

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Introduction

Biological Control

Biological control is the use of natural enemies such as parasites, predators and pathogens for the regulation of the population density of an organism (van den Bosch, 1971). Biological control is gaining attention as a viable means for pest control as agriculture moves towards more sustainable practices. One of the key drivers of this move is the ban of the pesticide DDT (dichloro-diphenyl-trichloroethane) during the late 1970s due to its detrimental effect on the environment and human health. Conventional pesticides are effective for insect pest control in field crops, livestock production, landscapes and for domestic use (van den Bosch, 1971). Biological control also provides effective pest control, but unlike chemical pesticides, this method incorporates the use of arthropod natural enemies, which reduces impacts to the environment, humans and non-targeted organisms, eliminates pesticide residues in food, preserves other natural enemies, and increases biodiversity (Lacey et al., 2001).

Augmentative biological control involves repeated releases of large numbers of mass-produced natural enemies to control a target species (van den Bosch, 1971). In 1951, this practice was employed in Virginia, North America to help protect threatened lumber resources against the initial spread of the invasive hemlock woolly adelgid, *Adelges tsugae,* Annad (Van Driesche et al., 2010). These adelgids were responsible for high mortality of Eastern hemlock (*Tsuga canadensis* (L.) Carriére) and Carolina hemlock (*Tsuga caroliniana* Engelm.), within temperate forests of Massachusetts. In forests where hemlock is the only species that produces dense canopy shade, these trees are vital for the survival and success of ground nesters on forest floors, including

invertebrates, small vertebrates, plants and the microbial community. The decline of the hemlock posed a rising threat to the ecology of the forest and other organisms that were dependent on these trees. A decline in adelgid density was achieved following the introduction of non-native coccinellid beetles, including *Sasajiscymnus tsugae* Sasaji & McClure, *Scymnus ningshanensis* Yu et Yao, and *Scymnus sinuanodulus* Yu et Yao and the derodontid *Laricobius nigrinus* Fender.

Another instance where the practice of biological control was used against an invasive forest species was to control the cypress aphid (*Cinara cupressivora* Buckton), first reported in Malawi in 1986. The infestation of the cypress aphid, which is native to Greece, affected native cypress trees in Eastern and South Africa (Day et al., 2003). Aphid infestation of the endangered Mulanje cedar (*Widdringtonia whytei* Rendle) in Malawi and the native African juniper (*Juniperus procera* Hochst. ex Endl) in Kenya caused dieback and ultimately the death of mature trees, resulting in a loss of US $41 million dollars worth of trees in 1991. The release of a natural enemy of the aphid, the endoparasitic wasp, *Pauesia juniperorum* Stary, led to the decline of the cypress aphid in Africa during the mid-1990s (Day et al., 2003).

Lady beetles and Biological Control

Many predatory lady beetle species have been used for biological control against invasive prey, and the general biology of both ladybeetles and their prey has been well studied (Dixon, 2000). As a result, there are multiple recorded cases where lady beetles are used as control agents worldwide, with 155 attempts to control aphids, and 613 attempts to control scale insects. In general, the success of lady beetles is attributed to their high prey specificity, high searching efficiency, high relative rate of increase, and

their searching capabilities relative to the dispersion of prey (Thorpe, 1930; Smith 1939). One example of the successful use of a lady beetle for biological control, is the augmentative release of the seven-spotted lady beetle, *Coccinella septempunctata* L., to control cotton aphid., *Aphis gossypii* Glover, within cotton fields in central China. During periods when *C. septempunctata* are abundant, beetles are collected from wheat fields and transported to cotton fields to control invasive cotton aphids (Dixon, 2000). Lady beetles released at a rate of 1.5-3/m^2 can reduce aphid abundance by nearly 100% within two days.

Adalia bipunctata

Adalia bipunctata L. is a general aphid predator that is endemic to Europe, Central Asia and North America (Pervez, 2005). *A. bipunctata* feed on a wide range of aphid prey. When there is a great variety of prey available, *A. bipunctata* consume prey with higher nutritional value to support lady beetle development and reproduction. The diet of *A. bipunctata* reflects its development, which is dependent on food quality, food quantity and environmental temperature (Pervez, 2005). Hodek (1962) stated the importance of distinguishing between types of prey: 'essential prey' are those that support immature growth and development and adult reproduction, whereas 'alternative prey' provide a source of energy and nutrients for maintenance, but not the nutrition needed for growth and development. When fed a diet of the black bean aphid (*Aphis fabae* Scopoli), a prey species of low nutritional value, *A. bipunctata* larvae took longer to complete development and were reduced in weight (Blackman, 1967).

Growth of *A. bipunctata* may also differ between their sexes. According to Hemptinne et al. (1996), females consume a larger number of aphids when compared to

males, to satisfy their energy requirement needed for egg production and viability. Females reared on aphids have shorter preoviposition periods and greater fecundity than those provided with unnatural or factitious foods (frozen Mediterranean flour moth [*Ephestia kuehniella* Zeller] eggs and fresh bee pollen) because these are not common prey for *A. bipunctata* (Jalali et al., 2009).

In addition, the infection of parasitic mites and pathogens affects the development and reproductive success of some lady beetles. For example, the parasitic mite, *Coccipolipus hippodamiae* McDaniel & Morril, lives under the wing case of *A. bipunctata*, where it feeds on its host haemolymph. Parasitization of *C. hippodamiae* causes the loss of energy that is required for egg production, and this results in low fecundity and a reduction in the production of viable eggs (Hurst et al., 1995; Webberly et al., 2004). Triltsch (1999) noted that when infected with the parasitoid fly, *Phalacrotophora delageae* Disney, earlier developmental stages of *A. bipunctata* such as the pupa were vulnerable to the effects of the pathogen.

Microsporidia and its effect on hosts

Microsporidia are obligate intracellular pathogens that infect a wide range of vertebrates and invertebrates, including *A. bipunctata*. Microsporidia produce spores that are transmitted both horizontally and vertically (Smith, 2009). Horizontal transmission occurs when microsporidian spores ingested by the host are spread from the gut to other tissues (Dunn and Smith, 2001). Horizontal transfer is facilitated when susceptible hosts consume infected conspecific eggs or larvae (Dunn and Smith, 2001; Pervez, 2005). Microsporidia can be transmitted vertically from infected parent to offspring when

microsporidian spores are passed from the parent female to the zygote via the cytoplasm of the egg (Dunn et al., 2000). Once within the host, the long, coiled polar filament within the spore acts as a projectile to enter adjacent host cells and initiate infection (Keeling, 2009; Sinden and Canning 1974).

Microsporidia cannot grow or divide apart from their host cell, and they also lack metabolic pathways such oxidative phosphorylation, electron transport, and the tricarboxylic acid cycle. Microsporidia lack the genes for these metabolic pathways and have few genes relating to the synthesis of small molecules, such as amino acids and nucleotides (Keeling, 2009). For example, the microsporidium *Enterocytozoon bieneusi* cannot generate its own energy from sugar because it lacks the genes that are responsible for glycolysis. Microsporidia are therefore critically dependent on their host for ATP to generate energy (Dunn and Smith, 2001; Keeling, 2009). Their dependence on ATP from their hosts results in the depletion of host energy, which would otherwise be used for growth and development.

Microsporidiosis results in chronic disease that may or may not be associated with obvious signs or symptoms. However, when infected with microsporidia, some coccinellid species express reduced body size, reduced longevity and fecundity, and increased development time (Hodek et al., 2012). The microsporidian pathogens *Nosema epilachnae* and *Nosema varivestis* infected nearly 100% of a Mexican bean beetle, *Epilachna varivestis* (Mulsant), colony under laboratory conditions, decimating the colony (Brooks et al., 1980). However, microsporidiosis does not always result in direct mortality of the host. In the case of *Nosema henosepilachnae*, infection of the melon ladybird beetle, *Henosepilachna elaterii* Rossi, reduces beetle fecundity (Hodek et al.,

2012). Another case where microsporidiosis causes chronic symptoms is recorded for the convergent ladybeetle, *Hippodamia convergens* (Guérin-Méneville) where infection by *Tubulinosema hippodamiae* causes a delay in larval development (Joudrey and Bjørnson, 2007).

Honey and its antimicrobial properties

According to Juven and Pierson (1996), honey exhibits antagonistic effects against several bacteria including *Staphylococcus spp.*, a genus of bacteria that are associated with skin infections. The antimicrobial properties of honey are thought to be associated with its low pH and high osmolarity, combined with the production of hydrogen peroxide (Viuda-Martos et al., 2008; Irish et al., 2011). These antibacterial properties are divided into subcategories based on what is responsible for the adverse effect: hydrogen peroxide activity and non-peroxide activity. Hydrogen peroxide within honey is produced by glucose oxide in the presence of light and heat (Viuda-Martos et al., 2008). In some cases, active phagocytic cells within an organism such as macrophages, monocytes, and neutrophils present in epidermal cells, are known to kill invading microorganisms through the generation of toxic oxygen compounds such as hydrogen peroxide (Juven and Pierson, 1996). The cytotoxicity of hydrogen peroxide is related to its capacity as an intermediate in oxygen reduction, to generate more reactive and cytotoxic hydrogen radicals which cause damage to nucleic acid, proteins and lipids (Juven and Pierson, 1996). The non-peroxide activity of honey involves the antimicrobial activity of honey which is not related to its low pH, osmolarity or hydrogen peroxide accumulation, but is related to phytochemical components derived from the source of the

nectar in which the honey was made (Alnaimat et al., 2012; Juven and Pierson, 1996; Irish et al., 2011).

Manuka Honey

Manuka honey derived from the manuka tree (*Leptospermum scorparium* J.R. Forst & G. Forst) in New Zealand is known for is non-peroxide activity due to the presence of glyoxal and methylglyoxal (Mavric et al., 2008). These phytochemical compounds responsible for non-peroxide antibacterial activity are derived from dihydroxyacetone, a simple saccharide that is present in high levels in manuka nectar (Irish et al., 2011). Manuka honey also has antibacterial properties related to the enzyme glucose oxidase, but its attributes are mainly related to the high amounts of methylglyoxal present (Mavric et al., 2008). Methylglyoxal is derived from the manuka tree and is responsible for providing trees with a high amount of methylglyoxide, which increases tolerance to stress. Yadav et al. (2005) found that plant species with concentrations of 30–75 μM methylglyoxal had increased tolerance to salinity, drought and cold stress conditions. This compound causes the manuka tree to be valued highly for its use in traditional medicine in New Zealand. The compound present in plants which causes increase tolerance to stress is the same compound responsible for the antimicrobial effect of manuka honey. This suggests that honey containing greater concentrations of this compound should have a greater antimicrobial property. Although the biological pathway of this compound is not yet understood, it is directly responsible for the antibacterial property of honey (Mavric et al., 2008; Yadav et al., 2005).

Research Objectives

The objective of this research is to study the effects of the microsporidium, *Nosema adaliae,* on its host, the two-spotted lady beetle, *A. bipunctata,* when it is raised on a diet that includes manuka honey. According to Evans et al., (1999), larval growth and adult reproduction is enhanced when insect predators are fed a mixed diet. I predict that if *N. adaliae*- infected *A. bipunctata* is raised on a mixed diet that includes both essential prey (green peach aphids, *Myzus persicae* Sulzer), as well as alternative diet (manuka honey), the beetle larvae will be provided with adequate energy and nutrition to counteract the effects of the pathogen. *N. adaliae*-infected *A. bipunctata* provided a mixed diet of aphids and manuka honey are expected to have shorter larval development times than those fed aphids alone, while mortality and sex ratio are likely to remain uninfluenced. By observing and comparing larval development, mortality and sex ratios of *N. adaliae*-infected *A. bipunctata* reared on a mixed diet, we can test this prediction.

Material and Methods

Uninfected and microsporidia-infected *A. bipunctata* eggs were obtained from *A. bipunctata* stock beetles that were reared within the lab for the past few years. Uninfected *A. bipunctata* larvae (48h-old) were fed different concentrations of Manuka Honey Gold MGO 400+ (Manuka Health, Newmarket Auckland, New Zealand) throughout the trial. There were four different treatments used in this experiment; *A. bipunctata* fed water (control), *A. bipunctata* fed 5% honey, *A. bipunctata* fed 10% honey and *A. bipunctata* fed 15% honey (Table 1). For each treatment group, 3 individuals were prepared each day for 5 days, yielding 15 individuals per treatment. This trial was repeated to produce a second trial yielding a total of 30 individuals per treatment (total sample size, *n*=120). Power of analysis was the main determining factor of sample size, and this was based on previous laboratory work. Throughout the trial, test larvae were provided green peach aphids (*Myzus persicae*) that were reared on nasturtium (*Tropaeolum minus* L., Dwarf Jewel Mix, Strokes Seed Ltd., ON).

Table 1. Number of *Nosema adaliae*-infected *A. bipunctata* eggs and diet provided to uninfected *Adalia bipunctata* larvae.

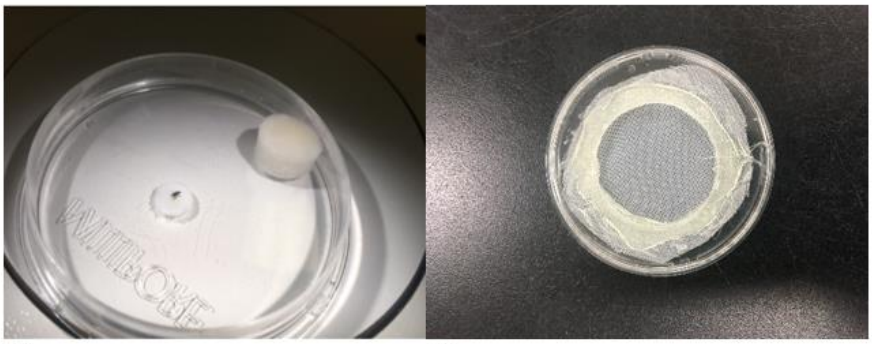
Treatments	***n***	***Nosema adaliae*-infected *Adalia bipunctata* egg**	**Diet**
Control (water)	30	1	Water, aphids
5% Honey	30	1	5% honey dilution, aphids
10% Honey	30	1	10% honey dilution, aphids
15% Honey	30	1	15% honey dilution, aphids
Total	120		

Preparation of manuka honey solutions

To make the 5% manuka honey solution, a spatula was used to transfer 1.5 ml of manuka honey to a 50 ml beaker. The honey was mixed with 28.5 ml distilled water. To prevent contamination, care was taken to avoid double dipping the spatula into the honey container. The spatula was washed before each use. Honey solutions were stored in 20 ml disposable glass scintillation vials within a refrigerator (10^{o}C). This process was repeated for the 10% honey dilution (3 ml honey dissolved in 27 ml distilled water) and the 15% solution (4.5 ml honey dissolved in 25.5 ml distilled water).

Experimental set up

On the first day of experimental set up, each *A. bipunctata* larva was placed in a separate 50 x 9 mm sterilized, polycarbonate Petri dish (Figure 1a). The lid of each dish had a 35-mm diameter hole that was covered with a cloth mesh to allow for ventilation (Figure 1b). The pathogen, *N. adaliae,* was transmitted horizontally by providing each *A. bipunctata* larva with a single *Nosema adaliae*-infected *A. bipunctata* egg, that had been placed on a filter paper disc (6-mm diameter). Water or honey solution (depending on treatment) was provided through a non-sterile, cotton roll (Crosstex International, NY; Figure 1a). Each piece of cotton was saturated daily with either water or a honey solution that corresponded to its treatment. Petri dishes were labeled with the date, treatment, larva number, the parent identification number, and the source of the infected egg that had been provided. Larvae were reared in environmental chambers (Sanyo, MLR-350H; 16:8 L:D, 25^{o} C) for the duration of the trial. The conditions of the environmental chambers were set to match previous studies within the lab so that results yielded may be compared to these studies.



(a) (b)

Figure 1.a. A Petri dish (50 x 9 mm) base with filter paper disc (6-mm diameter) used for providing a *Nosema adaliae*-infected *Adalia bipunctata* egg to a test larva (black spot on disk). A piece of cotton in the dish was moistened with water or a solution of honey, depending on treatment. **b.** Petri dish lid with mesh for the purpose of providing ventilation.

Larval development and mortality

On the second day of the trial, (after 24 hr had lapsed), the filter paper disks were removed from the dishes. These were examined under a stereomicroscope to confirm that each larva had consumed the *N. adaliae*-infected egg that was provided earlier. Larvae that had eaten the egg were then provided a diet of green peach aphids and the cotton in each dish was replenishing with water or the appropriate solution of honey. Larvae were observed and fed every 24 hours until they emerged as adults, or for a total of 21 days. Each day, it was noted if the larvae had molted (indicated by the presence of larval exuviae), had pupated, emerged as adults, or had died.

Sex ratio

For those larvae that completed development and emerged as adults, the sex of each adult was recorded. Sex was determined by examining the terminal, abdominal sternites under a stereomicroscope, and confirmed by identifying the internal sex organs when dissected. All individuals, including emerged adults and larvae that died

prematurely, were smeared and stained with 5% Giemsa (Sigma Diagnostics, St. Louis, MO). The staining procedure was as follows: methanol (10 min), 5% Giemsa (2 h), tap water (10 min), 70% ethanol (3 min), 80% ethanol (3 min), 90% ethanol (3 min), 95% ethanol (3 min), 100% ethanol (3 min), and xylene (24 h). Specimens were then cover slipped using Permount mounting medium (Fisher Chemical, CA) and examined for microsporidian spores by light microscopy.

Data analyses

Larval development data was checked for normality using the Ryan-Joiner normality test. Because the data was not normally distributed, a Kruskal-Wallis test was used to analyze differences of mean development time between treatment groups. A Kruskal-Wallis All Pairwise Comparisons test for non-parametric data was used to determine if each treatment group differed significantly from the control. A χ^2 test was used to determine if there was a significance difference in mortality between treatment groups. Larva that did not consume the infected *A. bipunctata* egg that was provided to them, those that went missing, and those that died during the first 48 h of the trials were excluded from the data analyses. A χ^2test was also used to determine if there was a significant difference in sex ratio for newly-emerged adults.

Results

Infection status

All individuals were confirmed to be infected with the *N. adaliae* pathogen except one individual within the control treatment. This individual emerged as a mature adult having a total development time of 15 days, not considering the first 48 h of the experiment (all individual larvae used at the beginning of the experiment were 48 h).

Larval development and mortality

Mean larval development of the control (15.87±0.68 days) was about two days longer than for larvae of the treatment groups (13.78±0.58, 13.5±0.93 and 13.30±0.87 days for 5%, 10% and 15% manuka honey respectively; Table 2). A Kruskal-Wallis test confirmed that development time of the experimental groups differed significantly (H=9.5; p=0.029). Further analysis using Kruskal-Wallis: All Pairwise Comparisons test indicated that each treatment group differed significantly from the control but not from each other. Larval mortality, on the other hand, was lowest for the control group (8.70%) and higher for Treatments 1, 2, and 3 (18.52%, 23.08%, 25.93%, respectively); however, there was no significant difference in mortality among treatments (χ^2=0.785, df=3, p=0.853).

Table 2. Development period (mean days ± SE) and mortality rates (%) for *Nosema adaliae*-infected *Adalia bipunctata* larvae that were provided with aphids and manuka honey.

Treatment Groups	Development time (days)		Larval Mortality (%)	
	n	Mean ± SE	n	Mortality (%)
Control (water)	23	$15.87\pm0.68_a$	2	8.70
5% Honey	27	$13.78\pm0.58_b$	5	18.52
10% Honey	26	$13.50\pm0.93_b$	6	23.08
15% Honey	27	$13.30\pm0.87_b$	7	25.93

Kruskal-Wallace (H=9.5; df=3; p=0.029). Groups with different letters are significantly different from the control.

Sex ratio

A χ^2test test analysis showed that there was no significant difference in sex ratio among treatments (χ^2=4.589, df=3, p=0.205; Table 3).

Table 3. Total number of males versus female within each treatment group of *Nosema adaliae*-infected *Adalia bipunctata* that were fed a mixed diet of green peach aphids and manuka honey.

Treatment Groups	Male	Female
Control (water)	14	7
5% Honey	12	10
10% Honey	10	8
15% Honey	11	8

Discussion

Our results indicate that the addition of manuka honey to the diet of *Nosema adaliae*-infected *Adalia bipunctata* influenced larval development. Larval development time decreased, whereas mortality and sex ratio were unaffected by honey concentration. It was impossible to determine whether the honey had a direct impact on the pathogen because microsporidian spore counts were not assessed.

Larval development

The average development time for control larvae was 15.87 days (Table 2). Considering that larvae used in the trial were 48-h old, larval development was approximately 18 days. This is similar to the mean development of *A. bipunctata* reported by Steele and Bjørnson (2012). In their study, mean development was 17.92 days for *Nosema adaliae*-infected *A. bipunctata* larvae that were fed a diet of green peach aphids (16:8 L:D; 25 C:20 C). In another study, larval development for uninfected *A. bipunctata* reared on a diet of green peach aphids and pea aphids, *Acyrthosiphon pisum* (Harris), ranged between 8-12 days (23-27^{o}C; Jalali et al., 2009). Both findings of Steele and Bjørnson (2012) and Jalali et al. (2009) indicate that the duration of larval development for uninfected *A. bipunctata* is much shorter than that of *Nosema adaliae*-infected *A. bipunctata.*

Differences in larval development on diet

Larval development of *A. bipunctata* at 23 and 27^{o}C is significantly shorter when they are reared on factitious food (*E. kuehniella* eggs and bee pollen) than on aphids (Jalali et al. 2009). In my study, the results also indicate that a difference in diet affects the development period of *A. bipunctata.* Hodek (1962) proposed that "acceptable prey"

consists of both ‘essential prey’ (prey that promotes immature growth and development, and adult reproduction) and ‘alternative prey’ (prey that serve as a source of energy and nutrients without providing the nutrients required for growth and development). Evans et al. (1999) compared the benefits of a mixed diet on *C. septempunctata* and *C. transversalis*. Those provided with aphids (essential prey) had significantly higher egg production than those provided with a mixed diet of weevil larvae (alternative prey) and sucrose water. Consumption of a limited number of aphids and no additional supplement (no sucrose) resulted in the production of a moderate number of eggs, whereas those provided with only weevil larvae, did not produce any eggs. These results suggest that when alternative prey is supplemented with sucrose, the latter serves as a primary cue to stimulate egg production. Although a mixed diet provides nutrients to support fecundity, it does not always fully substitute a diet that supports larval growth and development and adult reproduction.

Variation in essential foods (different aphid prey) can affect nutrition, and this may ultimately affect the duration of larval development, the number of eggs produced, and survival (Hodek et al. 2012). Blackman (1967) in his research on the effects of different aphid prey, found that a diet of black bean aphid was nutritionally inadequate for *A. bipunctata*, resulting in a delay in larval development. *A. bipunctata* reared on *A. fabae* had a 50% reduction in weight compared to being fed suitable prey. Aphid prey that are nutritionally inadequate are considered sub-optimal due to their low nutritional value and non-palatability.

The effect of manuka honey concentration on larval development

Results of my study showed that the inclusion of manuka honey into the diet *N.* adaliae-infected *A. bipunctata* larvae caused a decrease in larval development time (Table 2). Navodita et al. (2011) compared the development time of the transverse lady beetle, *Coccinella transversalis* (Fabricius), fed a combination of essential aphid prey to those fed alternative prey. Their results indicated that a diet of hibiscus mealybug (*Maconellicoccus hirsutus* Green) and honey was suitable as an alternative diet for *C. transversalis* as it resulted in a shorter larval development period and greater larval survival than a diet of *Hibiscus rosa sinensis* L. pollen and sugar.

It is difficult to determine whether the antimicrobial property of manuka honey is responsible for the reduced duration in larval development that was observed in this study. To determine whether sugar alone has an effect on larval development, further research should be done to compare the effect of *N. adaliae*-infected *A. bipunctata* that are fed an additional source of energy, one that does not contain the antimicrobial property of manuka honey. Nevertheless, recent research indicated similar results where cowpea weevil, *Callobuchus maculatus* (Fabricius) fed high honey concentrations had shorter development periods than did those not provided with honey (Adebayo and Oke, 2017).

Larval mortality

Although mortality of *Nosema adaliae*-infected *A. bipunctata* larvae was higher for those fed manuka honey compared to larvae not given honey, there was no significant difference in mortality rates (Table 2). *A. bipunctata* provided with water (control) had a mortality of 8.70% while the mortality rate of those that were provided manuka honey

ranged from 18.52 to 25.93%. An earlier study indicates that the pathogen, *N. adaliae* has no effect on the mortality of *A. bipunctata* larva (Steele and Bjørnson, 2012). The reason for the high mortality observed for those larvae that consumed manuka honey is unclear. Although mortality between treatments did not differ significantly, the highest mortality was observed for larvae fed the highest (15%) concentration of honey. The additional energy provided by the honey appears to offset the prolonged development caused by the pathogen, but there is likely a limit to the tolerance of honey in the diet, and this may be what was observed when higher concentrations of honey were included in the diet.

Sex ratio

The results of this study indicated no significant differences in sex ratios. These results were consistent with Steele and Bjørnson (2012), who report that infection of *A. bipunctata* and *H. convergens* with *Tubulinosema hippodamiae* had no effect on sex ratios. Based on this previous study, it was predicted that honey would have no effect on the *A. bipunctata* sex ratios.

Conclusion

In summary, the experimental results suggest that a mixed diet that consists of an alternative food, such as manuka honey, and essential prey (*Myzus persicae*) shortens the larval development period of *Nosema adaliae*-infected *Adalia bipunctata.* This research did not provide the information needed to conclude whether there was any impact of the manuka honey on the pathogen itself since microsporidian spore counts were not completed. Future work should focus on the effect of sugar water and other dietary supplements that do not contain antimicrobial compounds on the development of *Nosema adaliae*-infected *Adalia bipunctata.*

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