EVALUATING EFFICACY OF FUMAGILIN-B® AGAINST NOSEMOSIS AND TRACKING SEASONAL TRENDS OF *NOSEMA* SPP. IN NOVA SCOTIA HONEY BEE COLONIES

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Abstract

The efficacy of the antimicrobial Fumagilin-B® against nosemosis was evaluated in both spring and autumn feeding treatments following label directions in seventy-two honey bee (*Apis mellifera*) colonies across three apiaries in Nova Scotia, Canada. The seasonal trend of *Nosema* spp. spore loads was also tracked in these same colonies throughout a thirteen-month period (February 2018 - March 2019). We found the spring Fumagilin-B® treatment to be effective at significantly suppressing *Nosema* spp. spore levels below the recommended treatment threshold. There was no effect of Fumagilin-B® treatment in the autumn based on low spore levels at this time. We detected a drastic increase in *Nosema* spp. spore loads as May progressed but a decline in spores in summer (June-September). By October, there was another increase in spore levels, but this increase did not exceed the economic treatment threshold. Across seventeen collection periods in both control and Fumagilin-B® colonies, 74% (25) of samples tested positive for *Nosema ceranae*, while 26% (9) contained no *Nosema* spp. spores. No *Nosema apis* spores were detected during this trial. Our results indicate that Fumagilin-B® is an effective management practice in the spring, but colonies should still be monitored in the autumn. Our data also support that the *Nosema* species profile is shifting to be exclusively *N. ceranae* and the treatment threshold for Fumagilin-B® may need to be updated to reflect this, as the threshold was originally developed for *N. apis*.

Keywords: Fumagilin-B®, honey bees, *Nosema* spp.

INTRODUCTION

Nosemosis is a disease caused by the microsporidia *Nosema apis* and *Nosema ceranae* that affects adult honey bees (*Apis mellifera*). Adult honey bees become infected with nosemosis through the consumption of *Nosema* spp. spores in contaminated food or water, through trophallaxis or by cleaning (Fries, 2010; Martín-Hernández et al., 2012). These spores then germinate into a vegetative stage in the ventriculus, infecting the epithelial cells within the honey bee midgut (Fries, 2010; Martín-Hernández et al., 2012). This causes various symptoms including a reduction in lifespan, colony strength, brood food production, honey production, poor

spring build-up and colony death (Higes et al., 2008; Guzman-Novoa et al., 2010; Higes, Martín-Hernández, & Meana, 2010; Goblirsch et al., 2013). High *N. apis* levels cause dysentery, and high *N. ceranae* levels have been known to cause small spring clusters, but these symptoms alone cannot be used to reliably diagnose the presence of *Nosema* spp. (Higes et al., 2008; Fries, 2010; Stevanovic et al., 2013). Since the early 2000s, the composition of *Nosema* spp. has shifted in honey bee colonies from predominantly *N. apis* to a mixture of *N. apis* and *N. ceranae* in many areas (Klee et al., 2007; Paxton et al., 2007; Emsen et al., 2016). In Canada, multiple studies and surveys have found that the majority of *Nosema* spp. positive

honey bee colonies sampled are dominated by *N. ceranae* infections rather than by *N. apis* or mixed *Nosema* spp. infections (Copley et al., 2012; Emsen et al., 2016; Canadian National Bee Health Survey, 2017). To manage *Nosema* spp. infections, beekeepers in Canada use Fumagilin-B® (originally manufactured by Medivet Pharmaceuticals, High River, Alberta and now manufactured by Can-Vet Animal Health Supplies Ltd., Guelph, Ontario; DIN 02231180), the only commercially available antimicrobial registered to treat nosemosis. In Nova Scotia (NS), 20% of surveyed beekeepers in the Canadian Association of Professional Apiculturists annual survey reported using Fumagilin-B® in a spring treatment and 30% of surveyed beekeepers reported using Fumagilin-B® in an autumn treatment; these were the highest reports of Fumagilin-B® use to manage *Nosema* spp. across Canada on a percentage beekeeper basis, except for Alberta and Saskatchewan (Ferland, 2019).

Fumagilin-B® has been used for more than five decades to manage *Nosema* spp. infections, but concerns about resistance (Huang et al., 2013) and short-lived efficacy (Williams et al., 2008) have arisen. Furthermore, as *N. ceranae* becomes more prevalent in honey bee colonies in some areas (Klee et al., 2007; Paxton et al., 2007; Chen et al., 2008; Copley et al., 2012; Emsen et al., 2016), beekeepers want to know if Fumagilin-B® is still effective against *N. ceranae* as well as *N. apis*, and whether the optimal window of treatment is in spring or autumn. The objectives of our project were to evaluate the efficacy of Fumagilin-B® applied at label rate against *Nosema* spp. in NS honey bee colonies during the spring and autumn and to monitor the seasonal trends (prevalence and abundance) of *N. apis* and *N. ceranae* over a thirteen-month period in these same colonies.

MATERIAL AND METHODS

Experimental design

Sampling was carried out from February 2018 to March 2019 in Colchester County and Kings County, Nova Scotia, Canada. Three apiaries of twenty-four colonies each were used for this

study, and all colonies were managed according to typical local practices by a commercial beekeeper. Within each apiary, there were two treatment groups (Fumagilin-B® and control) of twelve colonies each ($n = 12$). Hives used in this study were managed in a double brood chamber and were used for honey and nucleus colony production but not pollination. No hives were moved during this trial. All colonies were headed with Buckfast queens locally bred that were less than two years old. Colonies were divided as necessary to prevent swarming, and honey supers were added accordingly. If colonies became queenless, weak, or died, they were excluded from the study. Colonies were managed as two hives per pallet, and pallets were randomly assigned as either Fumagilin-B® treatment colonies or control colonies. Colonies were wintered in double brood chambers and protected with black plastic wrap.

Sampling

Colonies were sampled once monthly, except during treatment with Fumagilin-B® from February 2018 to March 2019 (Tab. 1). Samples of approximately 150 bees were collected from each colony by the inner cover being lifted and bees collected using a 250 mL measuring cup. We collected older forager and guard bees, as they are more likely to have nosemosis (Meana, Martín-Hernández, & Higes, 2010), and older bees were expected to be directly under the inner cover. Once collected, the bees were placed in a plastic bag, labelled, and placed on ice for transport back to the laboratory, where they were stored in a -20°C freezer until microscopy could occur (Fries et al., 2013).

Spring Fumagilin-B® Treatment

Colonies in the Fumagilin-B® group of each apiary ($n = 12$) received a spring dose of Fumagilin-B® mixed in sugar syrup as per label directions (4 L of Fumagilin-B® medicated 1:1 sugar syrup), while the twelve control colonies in each apiary received 4 L of unmedicated 1:1 sugar syrup. Each Fumagilin-B® colony received 100 mg of active ingredient in the spring.

Table 1.

Honey bee sampling schedule and description from February 2018 to March 2019 in Nova Scotia colonies

Date	Description
28 February 2018	First sample collection
27 March 2018	Regular monthly sample collection
23 April 2018	Pre spring treatment
24 & 25 April 2018	Spring treatment and feeding (no samples collected)
27 April 2018	2-3 days post spring treatment
01 May 2018	5-6 days post spring treatment
07 May 2018	12-13 days post spring treatment
10 May 2018	15-16 days post spring treatment
29 May 2018	34-35 days post spring treatment/Regular monthly sample collection
26 June 2018	Regular monthly sample collection
26 July 2018	Regular monthly sample collection
29 August 2018	Regular monthly sample collection
03 October 2018	Pre fall treatment
05 & 06 October 2018	Fall treatment and feeding (no samples collected)
10 October 2018	4-5 days post fall treatment
16 October 2018	10-11 days post fall treatment
19 October 2018	13-14 days post fall treatment
29 October 2018	23-24 days post fall treatment
14 March 2019	Final sample collection

Autumn Fumagilin-B® treatment

Colonies in the Fumagilin-B® group of each apiary (n = 12) received Fumagilin-B® mixed in sugar syrup as per label directions (8 L of Fumagilin-B® medicated 2:1 sugar syrup), while the twelve control colonies in each apiary received 8 L of unmedicated 2:1 sugar syrup (Williams et al., 2011). Each Fumagilin-B® colony received 200 mg of active ingredient in the autumn.

Microscopy

Nosema spp. spores were detected and quantified as per Cantwell (1970) and Human et al. (2013). Thirty bees from each sample were counted and placed in a separate plastic bag, and 30 mL of water were added to this bag (equivalent of 1 mL per bee) (Williams et al., 2011). The contents of the bag were then thoroughly crushed using a rolling pin. Approximately 0.05 mL of the mixed sample was placed

in each well of the hemocytometer (Reichert Bright-Line, Improved Neubauer, 0.1 mm depth) and a compound microscope under 400x magnification was used to count spores in five of the twenty-five grid squares (four outside corner grid squares and one centre grid square) in both wells. The total number of spores was recorded. The well average was then multiplied by 50,000 to find the average spore load per bee. Although there is currently no globally recognized agreement on economic thresholds for *Nosema* spp. (Holt & Grozinger, 2016), the widely used economic treatment threshold of a million spores per bee for *N. apis* was used for this study, but this threshold has not yet been updated to reflect *N. ceranae* (Williams et al., 2011).

Once *Nosema* spp. levels in each sample were quantified (spores/bee), a subset of each bee sample (10 bees per sample) was sent to the

Table 2.

List of primers used to identify *Nosema* species

Target	Primer	Sequence (5'-3')	Amplicon size (bp)
RPS5	RPS5-F	AATTATTTGGTCGCTGGAATTG	105
	RPS5-R	TAACGTCCAGCAGAATGTGGTA	
	Ncpol-F	TGG GTT CCC TAA ACC TGG TGG TTT	662
RNA polymerase	Ncpol-R	TCA CAT GAC CTG GTG CTC CTT CT	297
	Napol-F	AGC AAG AGA CGT TTC TGG TAC CTC A	
	Napol-R	CCT TCA CGA CCA CCC ATG GCA	

National Bee Diagnostic Centre in Beaverlodge, Alberta for identification of *Nosema* species. Samples were pooled among all three yards to create a composite sample for each treatment group (Fumagilin-B® and control) across seventeen collection periods, for a total of thirty-four pooled samples.

Diagnosis of *Nosema* species

Nosema spp. identification performed by the National Bee Diagnostic Centre followed protocols developed by Hamiduzzaman, Guzman-Novoa, & Goodwin (2010) and Gisder & Genersch (2013). Species were determined by a conventional polymerase chain reaction (PCR) using a Multiplex Supermix (Qiagen). Positive controls were included in each PCR. Positive controls for *N. apis* and *N. ceranae* consisted of PCR product which had been cloned into plasmid using PGEM T-easy vector and JM109 competence cells. The DNA inserts were sent for sequencing to confirm *Nosema* species. Amplification assays were performed by adding 60 ng of genomic DNA and 0.4 nM of each primer (Tab. 2) in a Veriti thermal cycler (Applied Bioscience Technologies). RPS5 was chosen as a reference housekeeping gene. PCR conditions were five minutes at 95°C for initial denaturation and enzyme activation, followed by thirty-five cycles of one minute at 94°C, one minute at 58°C, one minute at 72°C and a final elongation at 72°C for seven minutes. Amplification products were separated by 1% agarose gel, stained with SYBR Safe (Invitrogen) and visualized under UV and

blue-light illumination.

Statistical analysis

This study was designed as completely randomized. Due to the nature of our count data and individual colony spore values containing many zeros, the model assumptions of normal distribution and constant variance of the residuals could not be met for the original data or through transformation. Therefore, non-parametric Kruskal-Wallis analyses were conducted in Minitab (2018) with the unit of replication being individual colonies. Independence was assumed through randomization. The level of significance was set at 5% ($\alpha = 0.05$).

RESULTS

Spring Fumagilin-B® treatment

Before the application of the spring Fumagilin-B® treatment on 23 April 2018, there was no significant difference in *Nosema* spp. spore loads among hives in the control or treatment groups ($\chi^2 = 1.22$, $df = 1$, $P = 0.27$). Before treatment, control colonies had on average ($\pm SE$) $7.4 \pm 3.3 \times 10^5$ spores per bee while Fumagilin-B® colonies had on average $4.3 \pm 2.5 \times 10^5$ spores per bee. Fumagilin-B® colonies had significantly fewer *Nosema* spp. spores than control colonies after treatment (29 May 2018 compared to 23 April 2018) ($\chi^2 = 4.88$, $df = 1$, $P = 0.03$). By 29 May 2018, Fumagilin-B® colonies had on average $4.7 \pm 2.5 \times 10^5$ spores per bee while control colonies had on average $10.4 \pm 3.3 \times 10^5$ spores per bee.

Autumn Fumagilin-B® Treatment

Nosema spp. spore loads between control and Fumagilin-B® colonies did not differ significantly before the application of autumn Fumagilin-B® treatment on 3 October 2018 ($\chi^2 = 0.06$, $df = 1$, $P = 0.80$). Before treatment, control colonies had on average ($\pm$ SE) $5 \pm 0.5 \times 10^5$ spores per bee, while Fumagilin-B® colonies had on average $0.015 \pm 0.015 \times 10^5$ spores per bee. The treatment did not significantly affect *Nosema* spp. spore counts in autumn ($\chi^2 = 0.83$, $df = 1$, $P = 0.36$) (3 October 2018 compared to 29 October 2018). During this period, Fumagilin-B® colonies maintained lower levels of *Nosema* spp. spores than control colonies, but the difference was not significant. The control colonies had average spore counts of $3.3 \pm 1.4 \times 10^5$ spores per bee, and treatment colonies had an average spore count of $1.1 \pm 0.6 \times 10^5$ spores per bee. Neither control colonies nor Fumagilin-B® colonies met or exceeded the economic treatment threshold during the autumn treatment period.

Seasonal *Nosema* spp. infection trends

The seasonal trend of *Nosema* spp. infection for our study on control colonies showed fluctuations in *Nosema* spp. spore levels between 28 February 2018 and 1 May 2018. During this period, *Nosema* spp. spore levels were below the economic treatment threshold of 1 million spores per bee (Fig. 1). However, we detected a sharp increase in *Nosema* spp. spore levels as May progressed, reaching a level of 1.7 million spores per bee in control colonies by the end of the month, which was the highest spore count during the study. By the June observation period, *Nosema* spp. spore levels in control colonies had decreased below the treatment threshold once again and remained at low levels through the summer months (Fig. 1). By October, *Nosema* spp. spore levels increased again but remained well below the economic treatment threshold. Colonies that were treated with Fumagilin-B® displayed similar trends in spore counts with a few notable exceptions. Fumagilin-B® colonies had similar *Nosema* spp. spore level trends to control colonies between 28 February 2018

Fig. 1. Mean *Nosema ceranae* spore loads (number of spores per bee $\times 10^6$) $\pm$ SE in honey bee colonies in Nova Scotia ($n = 72$) from February 2018 to March 2019 after spring and autumn applications of Fumagilin-B® following label rate. The first arrow labelled '1' indicates the spring application of Fumagilin-B® to treatment colonies, while the second arrow labelled '2' indicates the autumn application of Fumagilin-B® to treatment colonies. Note: x axis for sampling date is not to scale.

and 23 April 2018. After the application of Fumagilin-B® on 25 April 2018, *Nosema* spp. spore levels reduced in comparison to control colonies (Fig. 1). The most notable difference between the two treatment groups was observed in May, when colonies treated with Fumagilin-B® maintained *Nosema* spp. spore loads below 0.5 million spores per bee and never increased above the economic threshold unlike the control colonies. Similar to the control colonies, *Nosema* spp. spore levels decreased in treated colonies by the end of June and stayed at low levels throughout the summer. *Nosema* spp. spore levels increased slightly in the autumn by 10 October 2018, but spore levels never reached the economic threshold. By the end of the trial in March 2019, both the Fumagilin-B® and control colonies had low spore counts (Fig. 1).

Identification of *Nosema* species

Of the samples pooled by collection period, 74% (25/34) tested positive for *N. ceranae*, while 26% (9/34) contained no *Nosema* spp. spores. No *N. apis* was detected during the trial.

DISCUSSION

We found that Fumagilin-B® suppressed *N. ceranae* but does not completely eradicate it, supporting what others have observed (e.g. Williams et al., 2011). Fumagilin-B®'s mode of action against *Nosema* spp. is the inhibition of the enzyme methionine aminopeptidase2 (MetAP2), which is required for normal cell functioning (Zhang et al., 2006; Huang et al., 2013; Johnson et al., 2013; van den Heever et al., 2016). The inhibition of MetAP2 leads to cell growth arrest (Frottin et al., 2016), meaning that Fumagilin-B® does not kill spores but instead suppresses the reproduction of *Nosema* spp. spores (Williams et al., 2008; Huang et al., 2013). Although a suppressive effect was not detected for the autumn application, the spore loads in both control colonies and Fumagilin-B® colonies never met the economic treatment threshold to warrant a treatment, and there was no clear trend in *Nosema* spp. spore levels. Optimal *Nosema* spp. management for beekeepers may

be a single spring application of Fumagilin-B®, but colonies should still be monitored in the autumn. Based on the economic threshold of 1 million spores/bee, feeding Fumagilin-B® as per label directions in the spring can suppress *N. ceranae* significantly and keep spore levels below the treatment threshold for the spring build-up phase. This requires further investigation however, as it is not known if the current recommended treatment threshold is also applicable to *N. ceranae* (Williams et al., 2011). Honey bees are in their linear growth phase of development in May in NS, and the rapid growth and expansion of colonies is important in the preparation of the first nectar flow and for commercial crop pollination. If colony growth is impeded during the month of May, beekeepers may have fewer hives available for pollination, and colonies may not produce a significant honey crop. Therefore, managing *N. ceranae*, particularly during the month of May, gives colonies the opportunity to maximize population growth.

Our results suggest that the *Nosema* species profile is shifting to be predominantly *N. ceranae* in NS, and in our test colonies, the species profile has been exclusively *N. ceranae*, an observation that has been noted elsewhere (Chen et al., 2008; Stevanovic et al., 2013). *Nosema apis* is considered a spring and autumn disease, and *N. ceranae* a disease that impacts honey bees throughout the year (Higes, Martín-Hernández, & Meana, 2010; Copley et al., 2012; Martín-Hernández et al., 2012). We found levels of *N. ceranae* well above the economic threshold in May, decreased levels during the summer months from July to September and increased levels in October. A study conducted in the United States observed a similar trend in which an increased spore load was detected in April and May, followed by a decline in the summer months and a small increase once again in the autumn months (Traver, Williams, & Fell, 2012). Increased *Nosema* spp. spore levels in both Fumagilin-B® and control colonies in October could be explained by limited flying days for bees to perform cleansing flights or by an autumn infection. Favourable weather conditions allow honey bees to take cleansing flights to defecate

outside the hive, potentially removing *Nosema* spp. spores (Retschnig et al., 2017). Possibly we would have found *N. apis* in additional beekeeping operations, but by focusing on one commercial operation we were able to reduce variability caused by different management practices and design a more controlled study. Further province-wide testing is needed to better understand if the *Nosema* spp. profile in NS honey bee colonies has completely shifted to *N. ceranae*.

We may have detected a decrease in *Nosema* spp. spore quantity in June, with or without application of Fumagilin-B®, due to the natural life cycle of *Nosema* spp. (Emsen et al., 2016). In *N. apis*, there is a seasonal trend of increased spore loads in the spring, followed by a decline in the summer and an increase once again in autumn (Bailey, 1955). However, a clear trend is not always observed with *N. ceranae* (Fries, 2010; Higes et al., 2013). The seasonal trend of *Nosema* spp. in colonies in both treatment groups displayed a dramatic spike in spore quantity in May followed by a decrease in June. Spore levels were much lower overall from July to September in both groups.

Determining the seasonal trends and presence of one or both *Nosema* spp. is important to beekeepers for a number of reasons. When both species are present, synergistic effects may occur. In laboratory experiments, Milbrath et al. (2015) found that honey bees with mixed *Nosema* spp. infections had higher mortality rates compared to bees with single-species infections, although Williams et al. (2014) found significantly higher honey-bee mortality in sole *N. ceranae* infections compared to mixed or sole *N. apis* infections. Furthermore, previous studies have shown higher spore counts in bees infected with *N. ceranae* compared to bees infected with *N. apis* (Paxton et al., 2007; Milbrath et al., 2015; Emsen et al., 2016), which suggests that the treatment threshold for *N. ceranae* needs to be adjusted for Fumagilin-B®. With the *Nosema* spp. profile shifting to be predominantly *N. ceranae*, beekeepers must adapt to the characteristics of this species and be prepared to manage it effectively.

Our study provides assurance that Fumagilin-B® suppresses *Nosema* spp. spores effectively in the spring in NS when applied at the label rate, which may be the optimal management strategy for beekeepers seeking to suppress *Nosema* spp. spore loads to optimize spring build-up. Even though our data suggest a single spring treatment effectively manages *Nosema* spp., beekeepers should still monitor their colonies in the autumn. A relatively low proportion of NS beekeepers treat with Fumagilin-B® in the spring (20%) compared to 30% in the autumn (Canadian National Bee Health Survey, 2017), and our results demonstrate that beekeepers should consider a spring treatment to maximize spring build-up. Future research should examine the treatment threshold for *N. ceranae* to determine if the current threshold of 1 million spores per bee causes economic damage to colonies infected with *N. ceranae*. Additionally, a broader analysis of colonies in the Maritimes is needed to determine if *N. ceranae* is dominating the *Nosema* spp. profile elsewhere.

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