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## Short Communication

Does fumagillin control the recently detected invasive parasite *Nosema ceranae* in western honey bees (*Apis mellifera*)? ☆Geoffrey R. Williams<sup>a</sup>, Michelle A. Sampson<sup>a</sup>, Dave Shutler<sup>a,\*</sup>, Richard E.L. Rogers<sup>b</sup><sup>a</sup> Department of Biology, Acadia University, Wolfville, NS, Canada B4P 2R6<sup>b</sup> Wildwood Labs Inc., 53 Blossom Drive, Kentville, NS, Canada B4N 3Z1

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## ABSTRACT

Western honey bee (*Apis mellifera*) colonies in Nova Scotia, Canada were sampled in spring and late summer 2007 to evaluate efficacy of fumagillin dicyclohexylammonium (hereafter, fumagillin) against *Nosema ceranae*. Colonies treated with fumagillin in September 2006 ( $n = 94$ ) had significantly lower *Nosema* intensity in spring 2007 than did colonies that received no treatment ( $n = 51$ ), but by late summer 2007 no difference existed between groups. Molecular sequencing of 15 infected colonies identified *N. ceranae* in 93.3% of cases, suggesting that fumagillin is successful at temporarily reducing this recent invasive parasite in western honey bees.

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Nosemosis of western honey bees (*Apis mellifera*) is caused by two different microsporidians, *Nosema apis* and *Nosema ceranae*. Infection occurs in adult midgut epithelial cells after spores are ingested during trophallaxis or cleaning of contaminated comb (Bailey, 1981; Webster, 1993). Pathology associated with *N. apis*, the historical *Nosema* parasite of western honey bees, is well-described, and includes dysentery, reduced honey yield, increased winter mortality, and poor spring build-up of surviving colonies (Fries, 1993). First detected in western honey bees in 2005 (Huang et al., 2007), *N. ceranae* likely jumped from the Asian honey bee (*Apis cerana*) over 10 years ago (Klee et al., 2007; Paxton et al., 2007; Chen et al., 2008), so its pathology is not as well understood. In Spain, *N. ceranae* was associated with reduced honey production and increased colony mortality (Higes et al., 2006a), and was highly pathogenic when inoculated experimentally (Higes et al., 2007; Paxton et al., 2007).

To combat *N. apis*, apiculturists recommend the use of the antibiotic fumagillin dicyclohexylammonium (hereafter, fumagillin), which disrupts this parasite's DNA replication (Katznelson and Jamieson, 1952; Hartwig and Przelecka, 1971; Webster, 1994). In Canada, Fumagilin-B® (Medivet Pharmaceuticals Ltd.) is the only commercially registered product containing fumagillin available

to beekeepers for *Nosema* treatment. Chemotherapy typically occurs during fall syrup-feeding of hives (Gochner and Furgala, 1969), before peak infection during winter and early spring (Picard and El-Shemy, 1989). Fall and spring chemotherapy is often recommended for severe infections, but this may not reduce *N. apis* below damaging levels (Wyborn and McCutcheon, 1987). It is not known if fumagillin is effective against *N. ceranae*, in part, because fumagillin was ineffective against the closely related *Nosema bombi* in the bumble bee *Bombus occidentalis* (Whittington and Winston, 2003). Because *N. ceranae* may be more virulent than *N. apis*, and because the former has only recently spread from Eurasia to become a global concern, data on the efficacy of fumagillin against this parasite are of significant interest. Here we present evidence that fumagillin is effective at managing *N. ceranae* in western honey bees.

Eight different beekeeping operations from 5 counties in Nova Scotia, Canada, volunteered their colonies for this study; 94 (5 beekeeping operations) and 51 (3 beekeeping operations) colonies had been treated or not treated with Fumagilin-B®, respectively, in September 2006 according to label instructions (Table 1). We collected bees in both spring (20 April–4 May) and late summer (20–26 August) 2007 ( $n = 15$ –21 colonies per operation) from each of these 145 colonies. Workers were collected from the hive entrance using a portable vacuum device, and kept at  $-20$  °C until spore suspensions for each colony from each sampling period were created by adding 15 ml of distilled water to crushed abdomens of 15 randomly selected individuals (Cantwell, 1970; Rogers and Williams, 2007a). Estimation of *Nosema* intensity per colony (mean spores

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**Table 1**

Median intensity (number of spores/bee) and prevalence (percent of colonies) of *Nosema* in spring (20 April–4 May) and late summer (20–26 August) 2007 in western honey bee (*Apis mellifera*) colonies (*n*) from 8 beekeeping operations in Nova Scotia, Canada that had been treated or untreated with Fumagilin-B® in September 2006

Operation	<i>n</i>	Spring		Late summer	
		Median intensity	Prevalence (%)	Median intensity	Prevalence (%)
<i>Untreated</i>					
1	15	10,725,000	100	1,425,000	80
2	19	2,725,000	74	1,625,000	89
3	17	1,475,000	82	1,875,000	71
<i>Treated</i>					
4	16	0	31	0	38
5	21	0	29	0	33
6	17	0	6	2,625,000	88
7	20	700,000	70	2,925,000	90
8	20	2,375,000	90	2,687,500	95

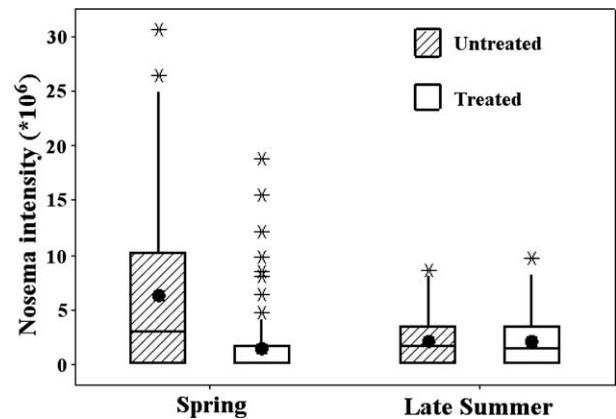
per bee) was accomplished using light microscopy and a hemacytometer (Cantwell, 1970; Rogers and Williams, 2007a). For each spore suspension, averages of 2 estimates of intensity were used.

We performed molecular analyses of the 16S rRNA gene (Higes et al., 2006a) on a random subset (*n* = 15 infected colonies from 7 operations in the spring, 1–3 per operation) of the 145 colonies to identify species of *Nosema* that were present. PCR conditions and sequencing methods are described in Williams et al. (2008). Blastn searches compared sequence data to those of related species on GenBank. Sample representatives were deposited in GenBank (Accession Nos. EU545140, EU545141).

Fourteen of 15 (93.3%) colonies had high probability (100%) matches on GenBank to *N. ceranae*, and one colony had a high probability (100%) match to *N. apis*. Results are comparable to molecular analyses we performed on a subset (*n* = 7 infected colonies, 3 belonging to 3 operations previously sampled from the 145 colonies above and 4 belonging to 2 operations not previously sampled) of 345 colonies sampled in spring 2007, where 6 of 7 (85.7%) infected colonies had high probability matches (100%) on GenBank to *N. ceranae*, and one had a high probability match (100%) to *N. apis* (Williams, unpublished data). As has been reported from other geographic regions (Klee et al., 2007; Paxton et al., 2007; Chen et al., 2008), our data suggest that *N. ceranae* is displacing *N. apis*. Because the historical parasite *N. apis* is still present in Canada (Williams et al., 2008), as well as northern and western Europe and Australasia (Klee et al., 2007), *N. ceranae* is likely a relatively recent arrival to these regions compared to regions, such as the United States (Chen et al., 2008), where only *N. ceranae* has recently been detected.

We first used repeated-measures ANOVA to analyze effects of fumagillin treatment, using R 2.0.1. Infection intensity data were square root-transformed to improve fit to normality, but perfect fit could not be achieved due to the high frequency of uninfected colonies (Table 1). Nonetheless, our analyses are likely to be robust because of our large sample sizes.

Infection intensities were significantly different between treatment groups (repeated-measures ANOVA  $F_{1,143} = 24.6$ ,  $P < 0.001$ ). Because of the significant treatment effect, we tested for differences within sampling periods with ANOVA. *Nosema* intensity in the spring was significantly lower in colonies treated with Fumagilin-B® the previous fall than colonies that had not been treated (Fig. 1,  $F_{1,143} = 39.3$ ,  $P < 0.001$ ), but by late summer no difference existed between groups (Fig. 1,  $F_{1,143} = 0.1$ ,  $P = 0.82$ ). Given that 93.3% (14/15) of the colonies on which we did molecular work were infected with *N. ceranae*, our surveys suggest that fumagillin treatment in the fall successfully reduced intensity of this invasive parasite in the subsequent spring. Because only a single colony in-



**Fig. 1.** Comparison of western honey bee (*Apis mellifera*) colonies in Nova Scotia, Canada treated (*n* = 94, from 5 beekeepers) and untreated (*n* = 51, from 3 beekeepers) with Fumagilin-B® in September 2006 in spring (20 April–4 May) and late summer (20–26 August) 2007 (*n* = 15–21 colonies per operation). Boxplots show interquartile range (box), median (black line within interquartile range), data range (vertical lines), and outliers (asterisks). Black dots represent means.

fectured with *N. apis* was part of our statistical analyses, we were unable to test whether fumagillin was more effective against one of the *Nosema* species.

Our results could be due to differences in beekeeping management practices, rather than because of differences in fumagillin treatment. We believe this is unlikely for several reasons. First, we observed extreme differences in *Nosema* intensities (Fig. 1) that we judge would be difficult to ascribe to shared differences in beekeeping management for the eight operations we sampled. Second, geographic locations of the fumagillin-treated and untreated bee operations overlap, so that local differences in, for example, microclimates, are unlikely to be responsible for the significant differences in infection intensities. Third, bees from all of the operations are transported long distances (100s of km) through the same regions of Nova Scotia, and thus are all likely to have broadly similar exposures to *Nosema* (and many other pathogens). Fourth, our findings are supported by additional unpublished observations (Higes et al., 2006b; Pernal et al., in press). Nonetheless, future cage and field trials should be conducted to evaluate the efficacy of fumagillin against *N. ceranae*. Future studies should also investigate if fumagillin is favoring displacement of *N. apis* by *N. ceranae* because it is more effective against the former.

Differences between treated and untreated colonies disappeared approximately 1 year after treatment, suggesting that infected colonies naturally recover during the summer (Pickard and El-Shemy, 1989), that fumagillin loses its efficacy (Furgala, 1962), or that fumagillin becomes depleted from colony honey stores.

*N. ceranae* has been blamed for colony collapse of western honey bees in Spain (Martín-Hernández et al., 2007), whereas Israeli acute paralysis virus has been associated with colony collapse in the United States (Cox-Foster et al., 2007). However, many colony collapses likely result from synergistic interactions among multiple pathogens and other stressors (Rogers and Williams, 2007b). Moreover, as is the case for *N. bombi* (Tay et al., 2005), virulence may vary among *N. ceranae* haplotypes. Virulence in Spain may be higher than in other regions of the world, such as in eastern Canada and other regions of North America, that appear to be colonized by a different European haplotype (Williams et al., 2008). Investigating virulence and efficacy of fumagillin against these different haplotypes should be a priority to protect bees whose pollination services to agriculture are valued at over \$14 billion annually in the United States alone (Morse and Calderone, 2000).

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