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Evaluation of the effectiveness of fumagillin and alternative therapies for the control of *Nosema ceranae*

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Introduction

Nosema is one of the most prevalent diseases of adult honey bees (*Apis mellifera*)[1]. It is caused by two species of single-cellular microsporidian parasites, *Nosema apis* and *Nosema ceranae*. *N. ceranae* is a recently-discovered pathogen[2] which has spread to populations of *A. mellifera* in Europe[3] and throughout the world[4].

Much of our present knowledge of the effects of nosema disease is based on the pathology of *N. apis* while substantially less is known about the differential pathology, epidemiology and chemotherapeutic responses of *N. ceranae*. This is of some consequence as recent surveys demonstrate the relative prevalence of *N. ceranae* in the U.S.[5] and the fact that both *Nosema* species are found to be more commonly associated with colonies suffering colony collapse disorder (CCD)[6,7]. Our research team has also discovered the presence of *N. ceranae* in Canada and found both *Nosema* species occur with similar frequency. Beekeepers in Canada and the U.S. have suffered devastating overwintering colony losses in recent years, with many of the reported symptoms being consistent with nosema infection[8,9]. Better nosema management may be a key to ensuring the long term sustainability of the North American honey bee industry and the prevention of future widespread colony losses.

Objectives

- To evaluate acetic acid fumigation, heat treatment and irradiation as methods for disinfecting *N. ceranae* spores on comb.
- To evaluate different formulations of spring-applied fumagillin treatments for suppressing *N. ceranae* infections in overwintered colonies.

Materials and Methods

Comb Disinfection Experiment: Full-depth Langstroth frames containing honey comb were sprayed with an aqueous suspension of *N. ceranae* spores. Each inoculated brood chamber in the experiment had four of these frames placed in its centre, surrounded by five additional non-inoculated frames. Each colony received a spore inoculation dose of 4.51 × 10^8 *N. ceranae* spores. Confirmation of *Nosema* spp. was performed by PCR[4].

Five treatments were employed, each with 12 replicate colonies:

Acetic Acid: Vertical stacks of four inoculated brood chambers were fumigated with 480 mL of 80% (v/v) acetic acid in an insulated, outdoor chamber (4.52 × 1.78 × 2.42 m high) (Fig. 1A). Two electric heaters were set to maintain a temperature of 30°C over the fumigation period of 7 d.

Heat: Inoculated brood chambers placed in constant temperature ovens (49 ±0.1°C) for 24h (Fig. 1B).

Irradiation: Inoculated brood chambers irradiated with an electron linear accelerator operated by Iotron Industries Canada Inc. Brood chambers passed through the accelerator in vertical orientation to receive 10 kGy to their top surfaces and were then inverted to receive 10 kGy to their bottom surfaces.

Inoculated: Inoculated brood chambers that received no disinfection procedure.

Non-Inoculated: Brood chambers containing honey comb receiving neither inoculation nor disinfection.

Sixty 1-kg package honey bee colonies were imported from New Zealand and were hived on the brood chambers described previously, on 2 May 09. Total honey production per colony was determined by establishing the net weight gain for each constituent super.

Colonies were sampled for *N. ceranae* spores by collecting approximately 100 adult bees from the underside of the lid or the peripheral frames of the colony, on a weekly basis from 2 May to 4 June 09, after which time sampling occurred biweekly. Adult bee samples were mechanically homogenized and aliquots from these suspensions were microscopically counted for spores at 400× magnification[10].

Fumagillin Treatment Experiment: Sixty single brood chamber colonies from a commercial honey bee operation in Girouxville, Alberta, Canada were identified as having infections of *N. ceranae* in February and March of 2009. These colonies were hived in a common yard and randomized into five treatment groups, each with twelve replicates. Four treatments contained fumagillin (Fumagilin-B, Medivet Pharmaceuticals, High River, AB) applied to colonies at a rate of 50 mg a.i. per application, but each formulated differently. Drench treatments consisted of 250 mL sucrose syrup (1:1 v/v), dust treatments consisted of 20 g of icing sugar, patty treatments consisted of 100 g pollen patties while syrup treatments consisted of 2 L of sucrose syrup. The control treatment consisted of 2 L of unmedicated syrup per colony. All treatments were applied in two successive applications on 22 April and 6 May 09, so that each medicated colony received a cumulative dose of 100 mg a.i. fumagillin.

Colonies were managed, sampled and evaluated in a manner consistent with the previous experiment. Adult bee and brood areas were visually measured on 30 June and 26 August 09.

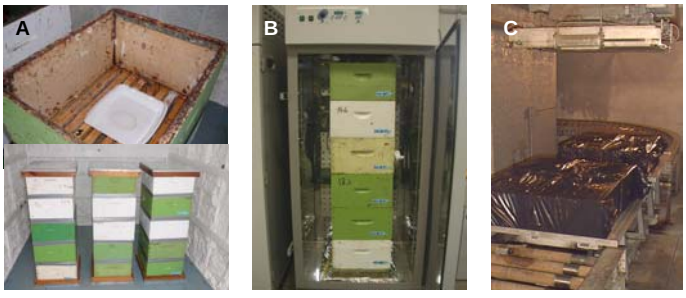

Figure 1. **A.** Acetic acid fumigation (Top, acetic acid in tray; Bottom, stacked boxes in fumigation chamber); **B.** Heat treatment in constant temperature oven; **C.** Electron beam irradiation.

Results and Discussion

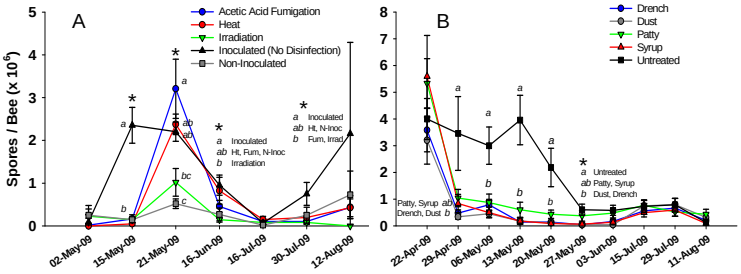

Figure 2. *N. ceranae* spore levels for: **A.** Comb disinfection; **B.** Fumagillin efficacy experiments.

Comb Disinfection Experiment: Thirteen days after hiving package bees on comb, spore levels within inoculated, untreated colonies rapidly proliferated to 2.4 ± 0.4 x 10^6 spores per bee while all other treatments remained below a maximum of 167,000 spores (Fig. 2A). Nevertheless, by 21 May spore levels in the acetic acid fumigation treatment and the heat treatment were similar to inoculated, untreated colonies whereas irradiated colonies still remained at levels similar to non-inoculated colonies. Over successive weeks, separation among treatments diminished until on 16 July spore levels in all colonies, including those inoculated and untreated, were at or below an average of 100,000 spores.

In general, the acetic acid fumigation, heat and irradiation treatments suppressed spore development in bees for a short duration of time, however only spore levels in the irradiated treatment were maintained at levels similar to non-inoculated colonies for the duration of the summer. The question of whether heat and acetic acid fumigation treatments are suitable for complete suppression of *N. ceranae* will be more comprehensively answered through analysis of fall 2009 and spring 2010 samples.

Fumgaillin Treatment Experiment: At the commencement of the spring-applied fumagillin experiment on 22 April 09, colonies had an average of 4.3 ± 0.5 x 10^6 spores per bee. After only one week of treatment application, clear effects were evident: irrespective of the formulation, 100 mg applications of fumagillin suppressed *N. ceranae* levels until 27 May (Fig. 2B). On this date, levels of spores in the untreated colonies remained below 0.6 x 10^6 spores per bee and similar to the levels in the patty and syrup treatments. From 3 June onward, spore levels in untreated colonies remained low and indistinguishable from other treatments.

The seasonal decrease in untreated spore levels during mid-summer is similar to that seen for *N. apis* in temperate climates[11] and does not mimic the absence of seasonal trends reported from infected colonies in Europe[7]. Continued sampling during the fall of 2009 and spring of 2010 will further document the temporal phenology of *N. ceranae* in northern climates.

No significant differences were detected in the areas of adult bees or sealed brood for the 30 June and 26 August assessment dates. Honey production was also similar among treatments (122.0 ± 7.9 kg). Based on the lack of response in colony level productivity parameters and the apparent natural mid-season decrease in spore levels seen in this experiment, it is possible that in northern climates control of *N. ceranae* should be more directed at fall, rather than spring infection levels. Continued sampling and analysis of these data may confirm this hypothesis.

Conclusions

1. Irradiation is the most effective method of disinfecting comb contaminated with *N. ceranae* spores.
2. Applications of 100 mg a.i. fumagillin are effective at suppressing active infections of *N. ceranae* over spring and summer months.
3. In northern Alberta, *N. ceranae* spore levels appear to naturally decline during mid-summer, similar to patterns historically seen for *N. apis*.

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