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PP-101 [Bee Health]

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# Evaluation of Promising Novel Acaricide Against *Varroa destructor*

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## Introduction

Infestation of honey bees by *Varroa destructor* is a significant cause of colony loss, particularly during winter months in Canada[1]. Currently, beekeepers have limited options available to treat bee colonies for varroosis. Varroa mites have quickly developed resistance to a number of registered compounds (i.e. fluvalinate, coumaphos, amitraz)[2-6], while remaining treatment options often pose health hazards to beekeepers[7,8] or have variable efficacy[9,10]. As such, alternative control strategies are urgently required, particularly those that involve new active compounds. Based on previous laboratory screening assays, we discovered a new acaricidal substance, 1-allyloxy-4-propoxybenzene, referred to as 3c{3,6}, which causes disruption of host-finding behaviour, paralysis and death of mites[11-13].

## Objectives

A) To determine the efficacy of 3c{3,6} against *Varroa destructor* using full-sized honey bee colonies in the spring and in the fall.

B) To determine the effects of 3c{3,6} on colony productivity metrics.

## Materials and Methods

Miticidal efficacy of 3c{3,6} was evaluated for colonies in single Langstroth hive boxes during the fall of 2022 and the spring of 2023, during which applicator design and dose of 3c{3,6} were varied. Experiments were conducted at Agriculture and Agri-Food Canda's Research Farm in Beaverlodge, Alberta, Canada.

**Experimental Set-Up:**

***2022:*** Colonies were founded using 1-kg New Zealand package bees in the spring of 2022, with the original queens replaced with New World Carniolan stock. As the newly established colonies did not initially contain detectable varroa loads, they were inoculated with mites from local colonies. The average varroa infestation level on adult bees was 4.1 ± 0.7 % at the start of the experiment on 8 Sep 2022.

The 2022 experiment used 40 colonies equally distributed into five treatment groups. Two 3c{3,6} applicator materials were tested: wood and cardboard (Fig. 1). Treatment groups included 3c{3,6} impregnated on either wooden or cardboard applicators, negative solvent controls on wooden or cardboard applicators, and a positive control treatment of Thymovar®. A total of 8 g of 3c{3,6} was applied to treated colonies, with the active compound equally divided across 3 applicators per colony.

***2023:*** Colonies were sourced locally, being selected from surviving overwintered hives, but did not include those used in the previous 2022 experiment. Queen sources consisted of locally bred of Italian and New World Carniolan stock. The starting size of colonies was 5 frame sides of adult worker bees and 3 frame sides of combined open and capped brood, with remaining frames consisting of food stores. The average varroa infestation level on adult bees was 2.5 ± 0.4 % at the start of the experiment on 11 May 2023.

In 2023, the same wooden applicator design was as used as in 2022 (Fig. 1). A total of 20 colonies were stratified into two equal treatment groups: 3c{3,6} (wood) and a negative solvent control (wood). Due to a lack of additional surviving colonies with mite loads after the winter of 2022, no positive control was included. A total of 10 g of 3c{3,6} was applied to treated colonies.

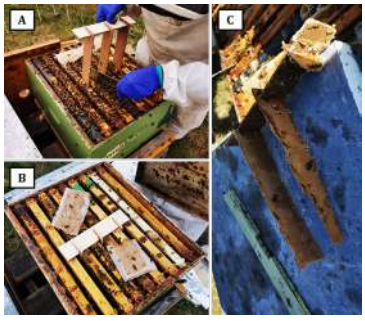


**Figure 1.** Applicators used in the fall 2022 and spring 2023 experiments. Three strips (24.0cm × 5.0cm × 0.5cm wood, or 24.0cm × 5.0cm × 0.3cm cardboard) were impregnated with a total of 8 g (2022) or 10 g (2023) of 3c{3,6} and suspended between frames. [A] A wooden 3c{3,6} applicator being inserted into an experimental colony. [B] Placement and positioning of a fully-inserted wooden 3c{3,6} applicator. [C] A heavily-degraded cardboard 3c{3,6} applicator, having been removed for examination following 21 days within colonies.

**Efficacy Evaluation:**

The exposure period for treatments was 42 days, followed by a subsequent "clean-up" phase of 42 days, during which treatments were removed and alternative miticides applied to kill any mites remaining in colonies. In 2022, Apivar® was employed as a clean-up treatment, while in 2023, multiple applications of 65% liquid formic acid were used. Efficacy was evaluated by counting the varroa mites falling onto adhesive boards placed beneath colonies during the treatment versus the clean-up phases, and by performing alcohol washes of adult bees at selected intervals. Mortality-based efficacies were calculated for each treatment group using Dieteman's formula. A GLS approach was used to model daily mite mortality per treatment over each measurement interval, to determine interactive effects between treatment and time[12].

**Effects on Colonies:**

Immediately prior to the application of treatments and following their removal, areas of brood, pollen and honey as well as adult worker populations were visually quantified using plexiglass grids. The infestation levels of mites in sealed brood were also counted from 100 sealed brood cells[14,15].

## Results and Discussion

All 3c{3,6}-treated colonies showed significantly higher varroa mortality than their untreated counterparts. In 2022, 8 g applications of 3c{3,6} had treatment efficacies of 94.6 ± 2.6% for the wooden applicators and 94.6 ± 2.7% for the cardboard applicators, which was comparable to Thymovar® at 94.5 ± 1.6%. In 2023, the 10 g treatment of 3c{3,6} had an overall treatment efficacy of 94.6 ± 1.2%. Cardboard applicators, though as effective as wood, tended to be destroyed by worker bees within the hive (Fig. 1).

GLS models showed significant interactions between the Treatment and Day main effects in 2022 ($F$=2.49; df=72,665; $P$<0.001) and 2023 ($F$=4.81; df=1,396; $P$< 0.001). This is evident by examining the temporal patterns of mite mortality shown in Figs. 2 and 3 and the differences between treatments and controls. Noticeably, 3c{3,6}-treated colonies produced very high levels of mite fall within the first 1 - 2 weeks after application, with control treatments having high mite fall immediately after the clean-up treatments were applied. Overall, 3c{3,6} at doses of 8-10 g performed at efficacy levels comparable to most other registered varroacides used in commercially managed colonies. This offers tremendous potential for the development of a truly novel product for varroa control.

Though we found no changes in the areas of brood, pollen or honey after 3c{3,6} treatment, we did find a significant impact of the 10 g application on adult bee populations. After 6 weeks, this dose appeared to hinder the development of adult population levels compared with controls (Fig. 4). Nevertheless, we did observe this effect to be transitory with population levels becoming similar to controls later in the season. Consequently, future work should focus on effects on 3c{3,6} on bee colonies and their queens to ensure this promising miticide does not have deleterious off-target consequences.

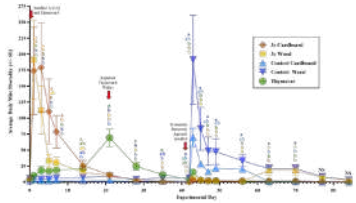

**Figure 2.** Daily varroa mortality per adhesive board interval, for each treatment group within the fall 2022 experiment, with a 3c{3,6} dose of 8 g per colony. Mortality data tracked from Day 0 (13 September 2022) to Day 84 (6 December 2022). Points represent mean ± SE error of 8 replicate colonies per treatment group. Five-way comparisons of daily mite fall across treatment groups were calculated using ANOVA and Tukey's HSD for pairwise significance (NS = not significant, pairwise significance $p < 0.05$).

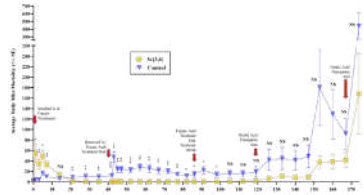

**Figure 3.** Daily varroa mortality per adhesive board interval, for each treatment group within the Spring 2023 experiment, with a 3c{3,6} dose of 10 g per colony. Mortality data were tracked from Day 0 (11 May 2023) to the experimental end on Day 85 (4 July 2023), with post-treatment mortality tracking continuing until Day 174 (1 November 2023). Points represent mean ± SE of 10 replicate colonies per treatment group. Pairwise comparisons between the 3c{3,6} and control performed using two-sample, two-tailed t-tests; (NS = not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

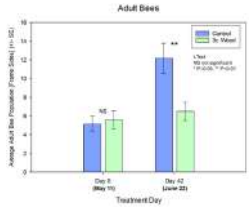


**Figure 4.** Adult bee populations (Mean frame sides ± SE) for colonies in the Spring 2023 experiment at the start of experimental treatment application (Day 0) and at the end of the application period (Day 42). Adult bee populations were significantly smaller at the end of the treatment period for the 10 g 3c{3,6} treatment.

## Conclusions

1. 3c{3,6} demonstrates significant in-hive efficacy against Varroa, in both spring and fall, at doses of 8 - 10 g per colony.
2. 3c{3,6} may have a transitory impact on colony population size at doses of 10 g per colony. Target Animal Safety studies for 3c{3,6} are required.

## Acknowledgements

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Infestation of honey bees by Varroa destructor is a significant cause of colony loss, particularly during winter months in Canada. Currently, beekeepers have limited options available to treat bee colonies for varroosis. Varroa mites have quickly developed resistance to a number of registered compounds (i.e. fluvalinate, coumaphos, amitraz), while remaining treatment options often pose health hazards to beekeepers or have variable efficacy. As such, alternative control strategies are urgently required, particularly those that involve new active compounds. Based on previous laboratory screening assays, we discovered a new acaricidal substance, 1-allyloxy-4-propoxybenzene, referred to as 3c (3,6), which causes disruption of host-finding behaviour, paralysis and death of mites. From 2019 - 2024, field trials were conducted in the Canadian provinces of British Columbia and Alberta to evaluate the effectiveness of this acaricide under a range of environmental conditions. These experiments showed efficacy of up to 94.6 ± 1.2%, using a 6-week treatment period with release devices containing 8 - 10 g of 3c (3,6) applied to wooden applicators in glycerol and isopropanol. Our findings show 3c (3,6) as being generally safe for honey bees, having limited effects on adult bee populations and no risk to the development of brood at 5 and 10 g per colony under OECD 75 testing. Nevertheless, some colony-level trials have shown elevated risks to queens at high doses. In terms of residues, fall applications of the compound were undetectable in honey harvested the following summer, while spring applications resulted in 0.060±0.004 ppm of the compound in honey extracted during same season. Overall, the data produced to date support the registration of this novel active for control of V. destructor.