Alternative IPM Methods for *Varroa destructor* (Parasitiformes: Varroidae) (Anderson and Trueman) Mite Control in East Texas Apiaries

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Abstract: The parasitic mite *Varroa destructor* (Anderson and Trueman) is a major pest of the honey bee (*Apis mellifera* (L.)), because large quantities of the mites can cause colony collapse disorder. Over the last two decades, prophylactic treatment of *V. destructor* using pyrethroid and organophosphate chemicals has caused increased resistance, requiring higher maintenance and treatment costs for beekeepers. This study examines the therapeutic effects of three different IPM methods for the control of *V. destructor* in two apiaries in East Texas. Twenty four colonies were separated into four different groups: untreated control colonies, colonies in which the queens were caged, colonies treated with powdered sugar using the "Dustructor" apparatus (Brushy Mountain Bee Farm, Moravian Falls, NC), or colonies treated with thymol (active ingredient ApiLife Var® (Mann Lake Ltd., Hackensack, MN)). *V. destructo*r populations in each colony were monitored for 54 days using the powdered sugar shake method or via a sticky board hung up for 24-hours. Powdered sugar shake mite counts reflected lower populations of *V. destructor* in all colonies treated with any of the three IPM methods compared to those of the untreated controls. Conversely, sticky board mite counts showed no statistical difference in mite counts based on IPM treatments compared to untreated controls. These results suggest that the powdered sugar shake method is more accurate when monitoring *V. destructor* populations than the 24-hour sticky board method. Alternative IPM methods for *V. destructor* control are effective and serve as a promising new avenue for non-intrusive control of this major honey bee pest.

Keywords: Mite, control, apiary

Varroa destructor is an ectoparasitic mite of the honey bee (*Apis mellifera*) that feeds on the hemolymph of developing bees and adults and has been known to transmit several viruses (Cicero and Sammataro 2010). When present in high numbers, these mites can cause colonies to collapse and die (Boecking and Genersch 2008). This has led to a drop in the number of honey bee colonies available for crop pollination, causing economic issues for agriculture nationwide (Stankus 2008). Problems associated with V. destructor mites continue to be top issues for the beekeeping industry. Preventative

treatment of *V. destructor* using the pyrethroid fluvalinate (active ingredient in Apistan®) and the organophosphate coumaphos (active ingredient in Checkmite+®) over the last two decades has caused the mites to develop resistance to these chemicals (Lodesani 1995, Elzen and Westervelt 2002), causing higher maintenance and treatment costs for beekeepers. Alternative methods for *V. destructor* control need to be implemented in order to deter these problems.

This study compares the effects of three alternative integrated pest management (IPM) methods for the control of V. destructor mites in two apiaries in East Texas. The first method involves caging the queen bee so that egg-laying production is stopped, causing a break in the brood cycle which lowers the number of mites in the brood nest (Boot et al. 1995). The second IPM method consists of a pressurized powdered sugar application using the "Dustructor" bellows apparatus, which causes mites to slip off the bees and drop (Oliver 2013). The third IPM method involves the use of the herbal product thymol (active ingredient in ApiLife Var®), which is known to be of limited toxicity to bees while fatal for mites (Adamczyk et al. 2005). When comparing the effects of these treatments to the populations within the untreated control colonies, it is hypothesized that the IPM methods will cause a reduction in V. destructor levels to the point where colonies no longer need to be treated and will have a higher chance of winter survival.

Materials and Methods

Two apiaries known as the Riverside and Ash apiaries were selected, both located in College Station, Texas. 12 experimental colonies at the Riverside apiary were housed in 8-frame hives, whereas 12 experimental colonies at the Ash apiary were housed in 10-frame hives. Each colony contained one queen and approximately 10,000 – 15,000 workers. Two methods were used to monitor *V. destructor* mite levels: the powdered sugar shake test and the 24-hour sticky board test. *V. destructor* mite levels were monitored with these methods from 18 October 2013 ("experimental day 1") through 4 December 2013 ("experimental day 52").

The powdered sugar shake test required the addition of one and one half cups of bees (approximately 250 workers) from a colony to two or three tablespoons of powdered sugar. The sugared bees are placed into a mason jar with a wired lid, then lightly shaken for several seconds. This motion allows the mites to lose their grip and dislodge off the bees. When the bees are removed from the jar, it is possible to count the numbers of *V. destructor* mites present. This method is also a humane treatment for the bees because they simply lick off the remaining powdered sugar left on them.

The 24-hour sticky board test required a gridded plastic board (Dadant & Sons Inc., Hamilton, IL) sprayed with cooking spray (ConAgra Foods, Omaha, NE). These boards, which fit the length and width of the hive frames, were placed under the frames for a 24-hour period. When mites drop to the bottom of the hive, they stick to the board, preventing them from seeking out a bee.

These sticky boards do not harm the bees (Jevrosima et al. 2011). *V. destructor* mite levels were monitored with this method from 17 October 2013 ("experimental day 2") through 4 December 2013 ("experimental day 45").

Prior to treatment, baseline data of *V. destructor* mite population levels from all experimental colonies were obtained. A total of 12 colonies were used in each apiary, with three colonies serving as untreated controls to compare with the treated colonies to determine if *V. destructor* levels were affected by natural causes.

The first treatment involved the caging of queens for two weeks to break the brood cycle. By breaking the brood cycle, no eggs were laid and thus no developing larvae pupating in their cells to attract *V. destructor* mites (Boot et al. 1995). Queens were caged at both apiaries on 1 October 2013 and released on 14 October 2013.

The second IPM treatment used was the "Dustructor" pressurized powdered sugar method (Brushy Mountain Bee Farm, Moravian Falls, NC). The pressurized application of powdered sugar dislodges mites off bees and causes them to drop. Colonies were treated with the "Dustructor" once on 14 October, 21 October, 28 October, 4 November, 11 November, and 22 November 2013.

The last method used was the herbal product thymol (active ingredient in ApiLife Var®). These thyme-based wafers were administered by breaking them into four equal pieces and placing each piece over the brood nests. This allowed the evaporation of the active ingredients, which are toxic to the *V. destructor* mites, to be released in the hives. Each colony was treated with thymol twice a day on 14 October, 21 October, 28 October, 4 November, 11 November, and 22 November 2013.

A repeated measures analysis of variance for each apiary and each *V. destructor* monitoring system was performed, as data from the same colonies were used. The colony numbers were randomized and nested into the IPM treatments. A full factorial ANOVA was conducted, testing for the effects of IPM treatment, experimental day, and their impacts on the number of mites counted by each monitoring method. The level of analysis for all tests was set at $\alpha = 0.05$. All descriptive data are reported as means ± s.e.m.

Results

Statistically significant lower V. destructor levels over time were found for all colonies monitored at the Riverside apiary regardless of treatment (Figures 1 and 2; Table 1). Statistically lower V. destructor levels over time were found between colonies belonging to the untreated controls and colonies that had caged queens, or those that were treated with powdered sugar or thymol when monitoring mite levels using the powdered sugar shake (Figure 1; Table 1). However, there were no significant statistical differences between untreated controls and the three IPM treatments when monitoring mite levels using the 24-hour sticky board test (Figure 2; Table 1).

Statistically significant lower V. destructor levels over time were observed for all

colonies monitored at the Ash apiary regardless of treatment (Figures 3 and 4; Table 2). Statistically lower V. destructor levels over time were found between colonies belonging to the untreated controls and colonies that had either caged queens, or that were treated with powdered sugar or thymol when monitoring mite levels using the powdered sugar shake (Figure 3; Table 2). However, there were no significant statistical differences between untreated controls and the three IPM treatments when monitoring mite levels using the 24-hour sticky board test (Figure 4; Table 2).

When combining the V. destructor mite counts from both apiaries, a significant effect of treatment (DFnom=3, DFden=20, F-ratio = 3.15, P-value = 0.04), and time (DFnom=6, DFden=120, F-ratio = 65.95, P-value < 0.0001) was observed. No significant interaction effect was observed (DFnom=18, DFden=120, F-ratio = 0.41, P-value 0.98) on V. destructor levels between the untreated controls and the three IPM treatments when using the powdered sugar shake monitoring method (Figure 5).

When compared to the results from the 24-hour sticky board tests, no significant effect of treatment on V. destructor levels was observed (DFnom=3, DFden=20, F-ratio = 1.26, P-value = 0.32). However, time had a significant effect on V. destructor levels (DFnom=5, DFden=100, F-ratio = 85.66, P-value < 0.0001), but a significant interaction effect was not observed (DFnom=15, DFden=100, F-ratio = 1.27, P-value 0.24) in V. destructor counts between the untreated controls and the 3 IPM treatments (Figure 6).

Table 2: Weather data during period of study observation. Collection dates highlighted in yellow.

Table 2. Statistical values for the effects of IPM treatment for Varroa mite control in Fall 2013 at the Ash Apiary in College Station, TX. A total of 12 honey bee colonies were used, each belonging to one of four treatments: untreated controls, caged queens, powder sugar (aka "Dustructor") and thymol Varroa mite levels were monitored using the "powder sugar shake" and the "24-hour sticky board" method. See "Methods" for details. Statistical significance was set at $\alpha < 0.05$. Statistically significant values are highlighted in gray.

Effect	DF numerator	DF denominator	F value	*P* - value	DF numerator	DF denominator	F value	*P* - value
	Powder sugar shake, control vs. caged queens				*24-hour sticky board, control vs. caged queens*			
IPM treatment	1	4	13.46	0.02*	1	4	0.43	0.55
Experimental day	6	24	20.19	< 0.0001***	5	20	23.81	< 0.0001***
IPM treatment * Experimental day	6	24	0.32	0.92	5	20	1.58	0.21
	Powder sugar shake, control vs. "dustructor"				*24-hour sticky board, control vs."dustructor"*			
IPM treatment	1	4	4.11	0.11	1	4	0.09	0.78
Experimental day	6	24	22.62	< 0.0001***	5	20	24.30	< 0.0001***
IPM treatment * Experimental day	6	24	0.60	0.73	5	20	1.81	0.16
	Powder sugar shake, control vs. thymol				*24-hour sticky board, control vs. thymol*			
IPM treatment	1	4	1.17	0.34	1	4	4.04	0.11
Experimental day	6	24	8.03	< 0.0001***	5	20	41.38	< 0.0001***
IPM treatment * Experimental day	6	24	0.20	0.98	5	20	2.09	0.11

* P < 0.05; ** P < 0.01; *** P < 0.001

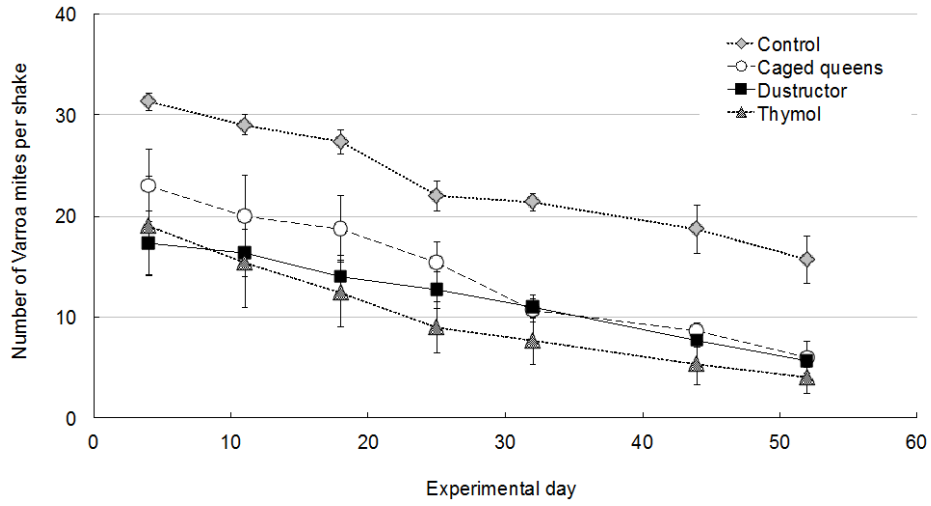

Figure 1. Number of Varroa destructor mites counted in each powdered sugar shake performed over time from 12 honey bee colonies located at the Riverside apiary from 17 October 2013 (experimental day 1) through 4 December 2013 (experimental day 52).

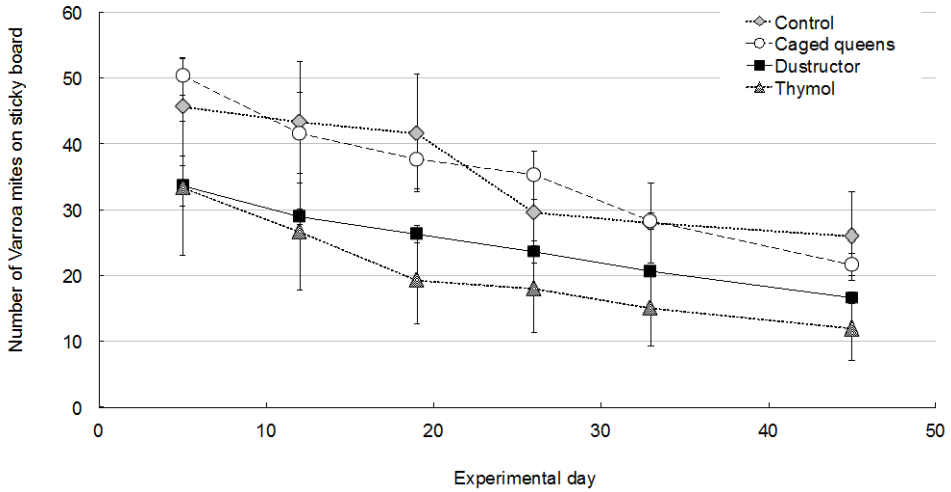

Figure 2. Number of *Varroa destructor* mites counted on 24-hour sticky board tests performed over time from 12 honey bee colonies located at the Riverside apiary from 18 October 2013 (experimental day 2) through 27 November 2013 (experimental day 45).

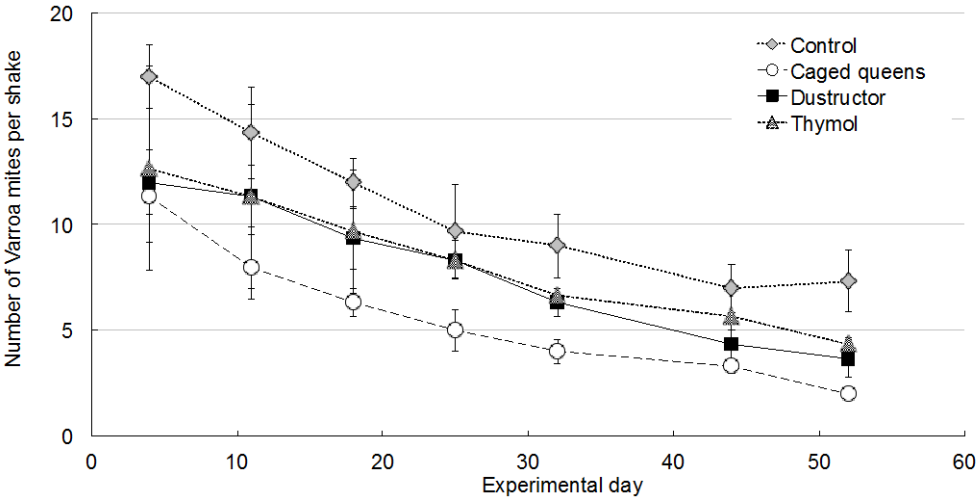

Figure 3. Number of *Varroa destructor* mites counted in each powdered sugar shake performed over time from 12 honey bee colonies located at the Ash apiary from 17 October 2013 (experimental day 1) through 4 December 2013 (experimental day 52).

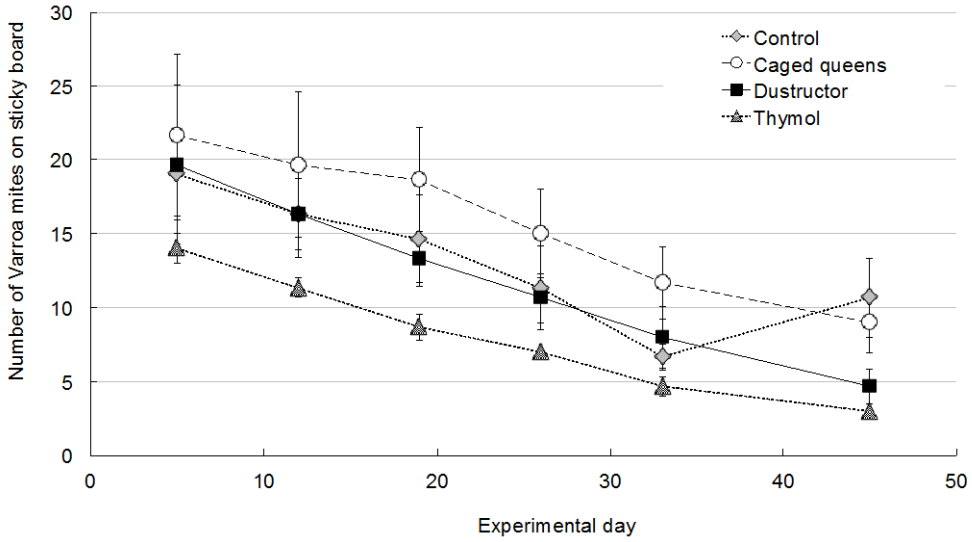

Figure 4. Number of *Varroa destructor* mites counted on 24-hour sticky board tests performed over time from 12 honey bee colonies located at the Ash apiary from 18 October 2013 (experimental day 2) through 27 November 2013 (experimental day 45).

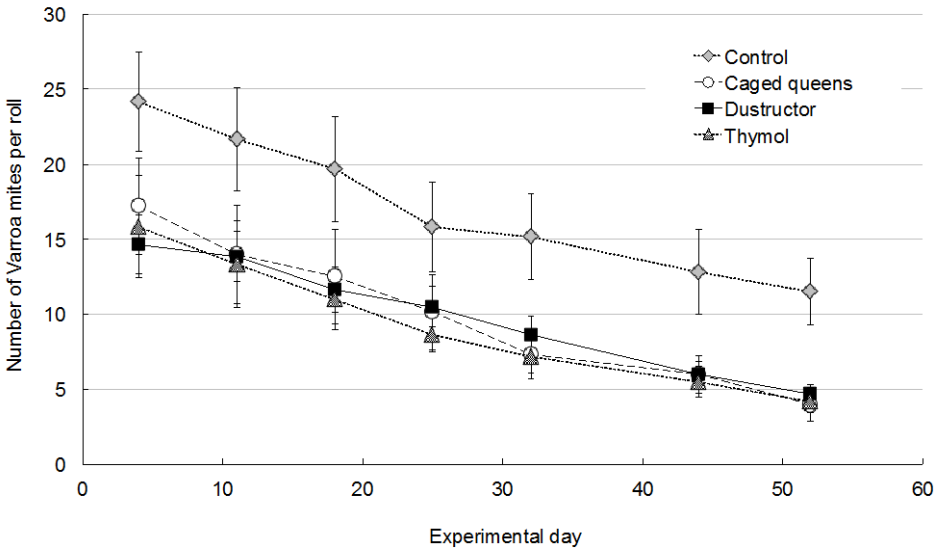

Figure 5. Number of *Varroa destructor* mites counted in each powdered sugar shake performed over time from 12 honey bee colonies located at the Riverside apiary and 12 honey bee colonies located at the Ash apiary from 17 October 2013 through 4 December 2013.

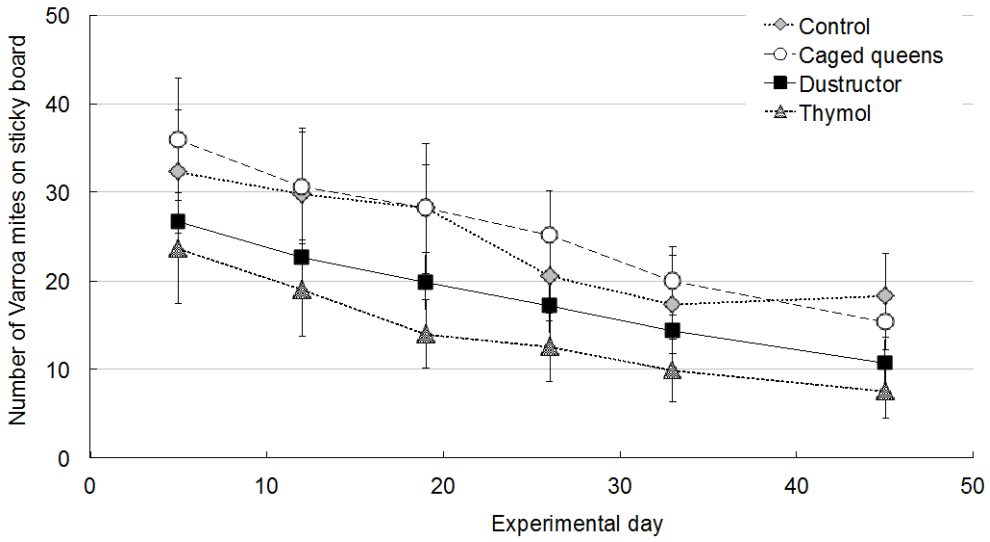

Figure 6. Number of *Varroa destructor* mites counted on 24-hour sticky board tests performed over time from 12 honey bee colonies located at the Riverside apiary and 12 honey bee colonies located at the Ash apiary from 17 October 2013 through 27 November 2013.

Discussion

The major difference between the powdered sugar and sticky board tests was the time required to become effective. The sticky boards were left unattended for 24 hours, whereas the powdered sugar provided mite counts upon application. The powdered sugar shake test was determined to be more effective for monitoring V. destructor, as the mites would remain alive and most of the bees would be protected (Oliver 2013). Conversely, with the 24-sticky board test, the mites on the board could not move, yet some had missing body parts. It is possible that some mites died in a cell from other causes rather than as a direct effect of the given treatments.

These three alternate IPM methods were determined to be effective in controlling fall populations of V. destructor. Further studies should be conducted to compare the efficacy of these treatments during the spring and summer months, as well as to compare additional treatment methods. The results of this study can be communicated to beekeepers to demonstrate how effective these treatments are in lowering V. destructor levels, hopefully preventing their colonies from being susceptible to colony collapse disorder. The powdered sugar and sticky board tests have been determined to be effective by other beekeepers (Oliver 2013), and more attention needs to be paid to the alternative methods for V. destructor control that are efficient but also less toxic to honey bees.

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