PFR SPTS No. 21075

Apitraz® varroa mite kill efficacy at 6, 8 and 10 weeks' post-application

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1 Background

The parasitic mite *Varroa destructor* is an unwanted organism in New Zealand that infests honey bee (*Apis mellifera*) colonies. Thirty percent of New Zealand overwintering colony losses in the 2020 NZ Colony Loss Survey (COLOSS) were attributed to varroa (Stahlmann-Brown et al. 2021). The New Zealand beekeeping industry relies heavily on synthetic chemical impregnated plastic strips for varroa control – primarily flumethrin (marketed as Bayvarol®) and amitraz (marketed as Apivar® and Apitraz®). A full-length Apitraz treatment is 10 weeks (Appendix 1), meaning the miticide strips are in a honey bee colony for 70 days, after which time they must be removed for regulatory compliance. Within this 70-day treatment period there are anecdotal reports from beekeepers of reductions in the efficacy Apitraz strips as they age. This study was conducted to assess the efficacy of Apitraz throughout the treatment period.

2 Approach

An adaptation of the Pettis test described by Rinkevich (2020); Appendix 2) was used to assess the efficacy of Apitraz after strips had been aged in naturally infested honey bee colonies for 6, 8 and 10 weeks. Efficacy was determined by *% mite death* over an 8-h assay period by comparing the mite fall from the strip to the number of mites recovered after the bees were washed in ethanol.

There were five treatment groups in this trial (Table 1). To age the miticide strips for Treatments 1–3, two colonies (Appendix 4) were randomly allocated to each group and strips were applied consistent with the manufacturer's instructions (Appendix 1) and according to the trial schedule (Appendix 3). At completion of the miticide application period, the 8-h mite fall assay was performed in triplicate on all treatment groups (1–5) using bees pooled from colonies with high varroa loads (>10 varroa mites/300 bees).

Table 1. Varroa treatment groups.

Treatment	Miticide	Application period
1	Apitraz®	6 weeks
2	Apitraz	8 weeks
3	Apitraz	10 weeks
4	Apitraz	0 weeks
5	No miticide	NA

3 Results & discussion

The Rinkevich mite drop assay was successfully completed in triplicate on all five treatments.

Beekeepers regularly express concern that Apitraz strips that have been present in a colony for 6+ weeks (still within the treatment period) are less effective than strips that have just been applied. To test this idea the data were analysed to see if there was a difference between synthetic miticide strips that had been present in a colony for 6, 8 or 10 weeks compared with strips that had immediately been removed from a sealed packet.

Apitraz remained effective in removing varroa mites from honey bees, even when the strips had been aging in colonies for 10 weeks (i.e. at the end of the maximum treatment period; Figure 1), as determined by a binomial generalised linear mixed model with a fixed-effect term for the interaction between elapsed trial time and a random effect for unique replicate within treatment. The proportion of dead mites at 8 h varied between strip age, but this was not statistically significant and no trends were observed. In all instances, the Apitraz strips were significantly more effective at removing varroa mites from bees than the no-treatment control (Table 2).

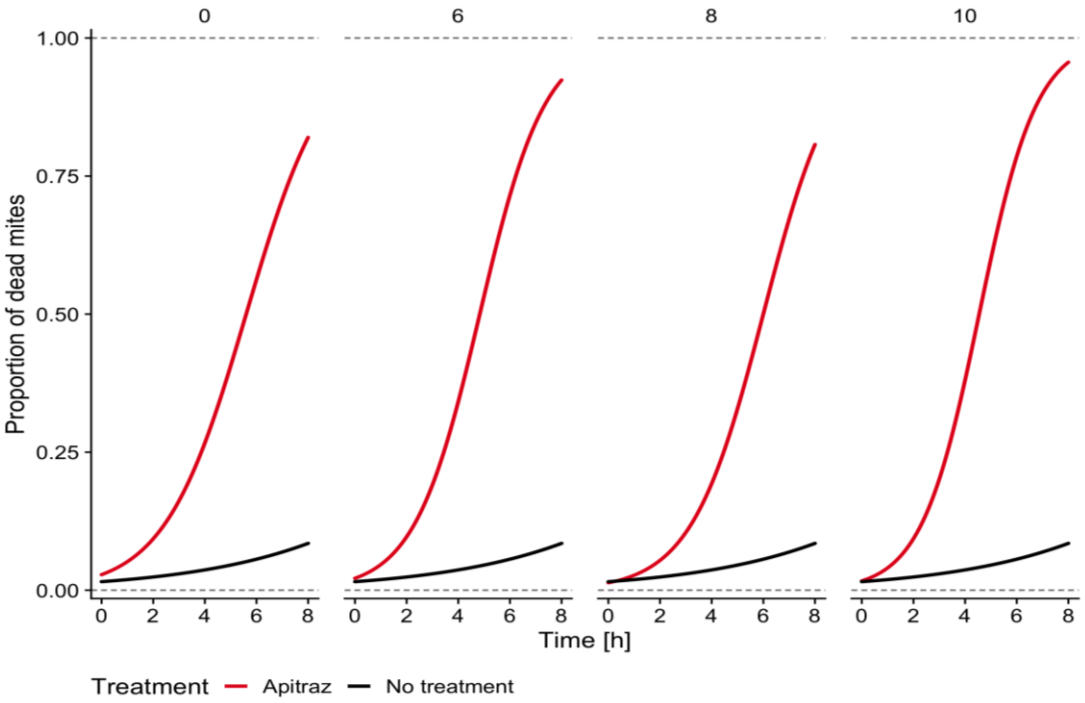


Figure 1. Varroa mite drop over an 8-h period. Apitraz® strips were aged in honey bee colonies for 0, 6, 8 and 10 weeks. All assays were run in triplicate. Apitraz was effective at rapidly removing varroa irrespective of strip age.

Table 2. Summary of pairwise tests (binomial generalised linear mixed model with a random effect for colony) for treatment and strip age combinations against 'No treatment' control. DF: degrees of freedom. Statistical significance established with a likelihood ratio test.

Treatment	Strip age	χ^2	DF	*p*-value
Apitraz®	0 weeks	61.64	1	< 0.001
Apitraz	6 weeks	101.95	1	< 0.001
Apitraz	8 weeks	97.00	1	< 0.001
Apitraz	10 weeks	169.06	1	< 0.001

4 Conclusions

- Apitraz strips that have been in honey bee hives for up to 10 weeks (i.e. the full-label treatment period) remain effective at causing varroa to drop from bees when measured using the Rinkevich mite-fall assay.
- The results of the Rinkevich mite-fall assay used for this trial have not been correlated with colony-level, in-field efficacy of this miticide for varroa control.

5 References

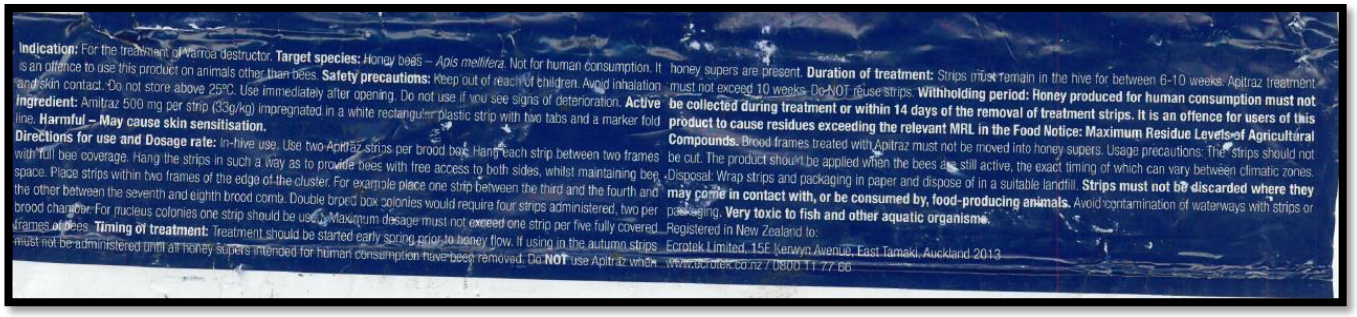
Dietemann V, Nazzi F, Martin SJ, Anderson DL, Locke B, Delaplane KS, Wauquiez Q, Tannahill C, Frey E, Ziegelmann B et al. 2013. Standard methods for varroa research. Journal of Apicultural Research 52(1): 1-54.

Rinkevich FD 2020. Detection of amitraz resistance and reduced treatment efficacy in the Varroa Mite, *Varroa destructor,* within commercial beekeeping operations. PloS one https://doi.org/10.1371/journal.pone.0227264: 12.

Stahlmann-Brown P, Robertson T, Borowik O 2021. Report on the 2020 New Zealand Colony Loss Survey. New Zealand Ministry for Primary Industries Technical Paper 2021/04.

Appendix 1. Miticide manufacturer instructions

Apitraz®



Appendix 2. Rinkevich (2020) mite-fall assay

The assay was performed in round 600-mL plastic containers fixed at the bottom with a 15-cm^2 section of the allocated miticide strip with a sugar cube added. Half a cup of bees (approximately 300 bees) collected from brood frames of colonies with high varroa infestation rates were added to the container that was then inverted and placed on a mesh base. The mesh was small enough to prevent bees being able to escape the container and big enough for falling varroa mites to pass through. Beneath each container there was a plastic tray lined heavily with petroleum jelly to catch falling varroa, which were unable to escape. The varroa in each tray were counted and removed every 30 min over an 8-h period. After the 8-h monitoring period was finished, an ethanol wash was performed on each container of bees to determine the residual number of varroa.

Ethanol washes were performed as described by (Dietemann et al. 2013). Ethanol was poured into each plastic container, covering all bees to ensure they were all dead. The contents were then transferred into a sealable glass jar, sealed and shaken for approximately 60 s. The solid lid was removed and the contents poured through a pair of sieves consisting of a layer of coarse mesh (varroa can fit through but bees cannot) that was suspended over a fine mesh through which varroa cannot pass. The bees were then washed under pressure for approximately 60 s to further dislodge any varroa from the bees. The upper sieve was then lifted, and the number of varroa caught in the lower sieve was counted and the mites removed. The upper sieve was then returned to its position and the sieves were aggressively shaken. The bees were washed again for approximately 60 s and the remaining varroa counted and removed. This process was then repeated until two consecutive zero counts were observed.

Appendix 3. Trial schedule

Day	Activity
0	10-week treatments applied
14	8-week treatments applied
28	6-week treatments applied
70	All treatments removed
72	Mite fall assay on all treatment groups

Appendix 4. Beekeeping practices

All colonies were housed in single or double deep Langstroth hives and routine beekeeping took place throughout the trial, including sugar syrup supplemental feeding. To ensure that the high-varroa infestation colonies had the required varroa loading, colonies were monitored using in-field ethanol washes (Appendix 5). If varroa infestations became too high and potentially risked the success of the trial, colonies were given a 48-h "knockback" treatment of Bayvarol®. Bayvarol contains flumethrin, which has a different mode of action to amitraz, the active component of Apitraz.

Appendix 5. In-field varroa ethanol washes

Half a metric cup of bees (approximately 300) was collected from two different brood combs of each hive into a 400-mL jar containing approximately 250 mL of 95% ethanol. The jar was sealed and shaken for approximately 60 s. The lid was removed and the contents poured through a pair of sieves consisting of a layer of coarse mesh (varroa can fit through but bees cannot) that was suspended over a fine mesh through which varroa cannot pass, to complete a single wash The sieves were aggressively shaken in attempt to further dislodge any varroa from the bees. With a bucket placed under the sieves, the ethanol was caught and poured back over the bees remaining on the coarse mesh, being careful to cover all the bees. This process was repeated for a total of three washes. The upper sieve (coarse mesh) was then removed and the numbers of varroa caught in the fine mesh counted. These data are reported as mites per 300 bees.

Auburn University Bees – Monitoring for varroa:
https://www.facebook.com/auburnbees/videos/200731601035729.

Appendix 6. Apitraz® varroa mite kill efficacy

Data

	AT0R1	AT0R2	AT0R3	AT6R1	AT6R2	AT6R3	AT8R1	AT8R2	AT8R3	AT10R1	AT10R2	AT10R3	NTR1	NTR2	NTR3
10:30	0	0	0	0	0	1	1	1	0	0	0	1	0	0	0
11:00	0	1	1	0	0	0	0	0	0	0	3	1	1	0	1
11:30	1	1	2	0	1	0	1	1	2	2	7	1	0	2	1
12:00	0	1	0	0	2	0	0	1	0	3	5	2	0	0	1
12:30	0	4	1	2	3	1	3	3	1	6	4	3	0	0	1
13:00	0	5	2	3	2	3	2	3	2	3	17	2	1	0	0
13:30	2	3	4	5	5	1	4	2	1	3	9	4	0	1	0
14:00	3	2	2	4	2	4	4	6	4	2	18	6	0	1	0
14:30	3	1	0	5	1	1	4	2	2	4	7	4	0	0	0
15:00	1	4	3	3	3	2	5	4	1	10	7	1	0	0	1
15:30	1	3	1	3	3	1	11	11	7	5	8	4	0	0	0
16:00	3	2	3	1	2	0	3	5	5	8	11	5	0	2	0
16:30	3	1	2	0	2	2	1	4	7	1	3	8	0	0	1
17:00	0	1	0	1	2	0	3	5	3	2	4	1	0	0	0
17:30	2	1	0	0	2	0	1	7	1	5	2	4	0	0	1
18:00	2	2	1	0	1	1	1	3	2	0	1	0	0	1	0
Residual	**8**	**4**	**13**	**3**	**3**	**7**	**13**	**16**	**19**	**1**	**1**	**11**	**64**	**69**	**56**

Treatments

Treatment code	Description		
AT0R1	Apitraz	0 weeks in colony	Replicate 1
AT0R2	Apitraz	0 weeks in colony	Replicate 2
AT0R3	Apitraz	0 weeks in colony	Replicate 3
AT6R1	Apitraz	6 weeks in colony	Replicate 1
AT6R2	Apitraz	6 weeks in colony	Replicate 2
AT6R3	Apitraz	6 weeks in colony	Replicate 3
AT8R1	Apitraz	8 weeks in colony	Replicate 1
AT8R2	Apitraz	8 weeks in colony	Replicate 2
AT8R3	Apitraz	8 weeks in colony	Replicate 3
AT10R1	Apitraz	10 weeks in colony	Replicate 1
AT10R2	Apitraz	10 weeks in colony	Replicate 2
AT10R3	Apitraz	10 weeks in colony	Replicate 3
NTR1	No treatment	NA	Replicate 1
NTR2	No treatment	NA	Replicate 2
NTR3	No treatment	NA	Replicate 3

Appendix 7. Attachment 1 — Apitraz® varroa mite original report

21143 - James Sainsbury - Apitraz®

*In Word document: Double-click on above **icon** to open embedded documents.*

*In PDF: Double-click on relevant **Appendix** in left side "Attachments" pane.*

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PUBLICATION DATA

Fale G, Cross S, Mortensen AM, Jochym M, Sainsbury JP. June 2021. Apitraz® varroa mite kill efficacy at 6, 8 and 10 weeks' post-application. A Plant & Food Research report prepared for EcroTek. Milestone No. 90251. Contract No. 39143. Job code: P/481014/01. PFR SPTS No. 21075.

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