RESULTS FROM A LASI TRIAL

# How Effective is Apistan® at Killing Varroa?

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**The mite *Varroa destructor* was first detected in Britain, in Devon, in 1992. For the first decade or more it could be controlled effectively by inserting Apistan* strips into hives. Apistan contains a synthetic chemical, fluvalinate, which is highly toxic to varroa but has low toxicity to honey bees and humans. However, when pests are controlled with a specific synthetic chemical, resistance usually develops.**

Fluvalinate-resistant varroa mites were first reported in 1992 in Italy (Lodesani et al, 1995) and in 2001 in Britain (Thompson et al, 2002). When varroa mites are not resistant to fluvalinate, colony treatment with Apistan can kill almost 100% of the mites (Borneck and Merle, 1990; Ferrer-Dufol et al, 1991). However, when they are resistant, the kill is only approximately 30% (Faucon et al, 1995).

Laboratory studies have shown that resistant varroa can withstand 10–20 times more fluvalinate than non-resistant ones (Thompson et al, 2002). The occurrence of resistant varroa can lead to rapid increases in colony mortality (Milani, 1999). This is because varroa can spread virus diseases, such as deformed wing virus, that kill colonies (Dainat et al, 2012; Francis et al, 2013).

## Determining the Effectiveness of Apistan


At the Laboratory of Apiculture and Social Insects (LASI), we have not used Apistan to control varroa for more than five years. Nowadays, we mainly use oxalic acid and bee hygienic behaviour.

Our research shows that a single sublimation treatment of 2.25 grams of oxalic acid to a broodless colony in winter, when all the varroa are phoretic, ie, carried on adult bees, kills 97% of the mites without causing any harm to the bees or colonies (Al Toufailia et al, 2015). We have also shown that varroa populations in hygienic colonies increase by 60% less than in non-hygienic colonies over one year (Al Toufailia et al, 2014).

In the summer of 2014, we decided to use Apistan on some colonies that appeared to have large varroa populations and were producing worker bees with deformed wings, a sign of deformed wing virus and a possible harbinger of colony death (Dainat and Neumann, 2013). We decided against using oxalic acid as it is not very effective at killing varroa when colonies have brood, as in the summer (Gregorc and Poklukar, 2003).

In colonies with brood, most of the mites are in brood cells where they will not be contacted by the oxalic acid. Apistan treatment lasts six weeks and so contacts all the varroa, even those temporarily in brood cells. As the hives had not been treated with Apistan for five years, we thought that any resistance might have reduced.

We applied Apistan in the recommended manner, placing two strips into each hive on 12 August 2014 and removing them

**Summary**

This research determined the effectiveness of Apistan®, two strips per hive, at killing varroa in broodless hives in winter in Sussex, England. Two groups, each of 20 hives, were studied. In one group, Apistan had not been used for five years. In the other, one treatment of Apistan had been made four months previously. The proportions of varroa killed were 58% and 33%, with the 33% kill being significantly lower statistically than the 58% kill (P <0.01).

The results show that Apistan is not very effective at killing varroa, presumably because of resistance. They also show that a single Apistan treatment resulted in the next treatment being significantly less effective, indicating strong selection for resistance.

on 22 September 2014, 41 days treatment in total.

We then decided to measure the effectiveness of Apistan in killing varroa in our colonies, both for our own future reference and because we thought it would be of interest to beekeepers. We decided to make the application in winter to broodless colonies, so that we could make an accurate determination of the proportion of varroa killed.

## Taking the Samples

On 6 December 2014, we took samples of approximately 300 worker bees (mean ± standard deviation: 310.3 ± 40.4 bees per hive) from 20 of the colonies that we had treated with Apistan four months previously and from another 20 hives that had not been treated.

The colonies had an average of 6.4 frames of worker bees, range 5–8.5 frames, with no significant difference between the two groups of hives. All were queenright, had been prepared for winter with adequate honey stores, and were in hives consisting of two medium-depth Langstroth boxes, each with ten frames, floor, inner cover and telescopic lid.

## Two Apiaries

The hives were in two apiaries, one on the University of Sussex campus (colonies not treated with Apistan in the summer) and the other 3 km to the east at Ashcombe Farm (colonies treated with Apistan in the summer). The two apiaries were under very similar conditions in the South Downs.

At the same time as we collected the worker bees, we treated each colony with two Apistan strips.

On 10 January 2015, 35 days later, we took a second sample of worker bees from each hive (mean ± standard deviation: 495.3 ± 53.7 bees per hive) and removed the Apistan strips. At this time all colonies had small patches of brood. Therefore, a few days before collecting the worker bee samples, any capped brood cells were uncapped and other brood removed to allow any mother varroa to leave their cells, thereby ensuring that all varroa were phoretic on adult bees.

## Counting the Mites

The worker bee samples were frozen after collection. Later, we extracted and counted the varroa mites from each sample using a jet of warm water and a strainer to catch the mites, as used in previous research (Dietemann et al, 2013; Al Toufailia et al, 2014; Al Toufailia et al, 2015).

In this way we were able to determine the proportion of varroa killed by the Apistan. For example, if the first sample had ten mites per 100 bees and the second had six mites per 100 bees, then the mortality was (10 - 6) ÷ 10 = 0.4 or 40%.

## Results

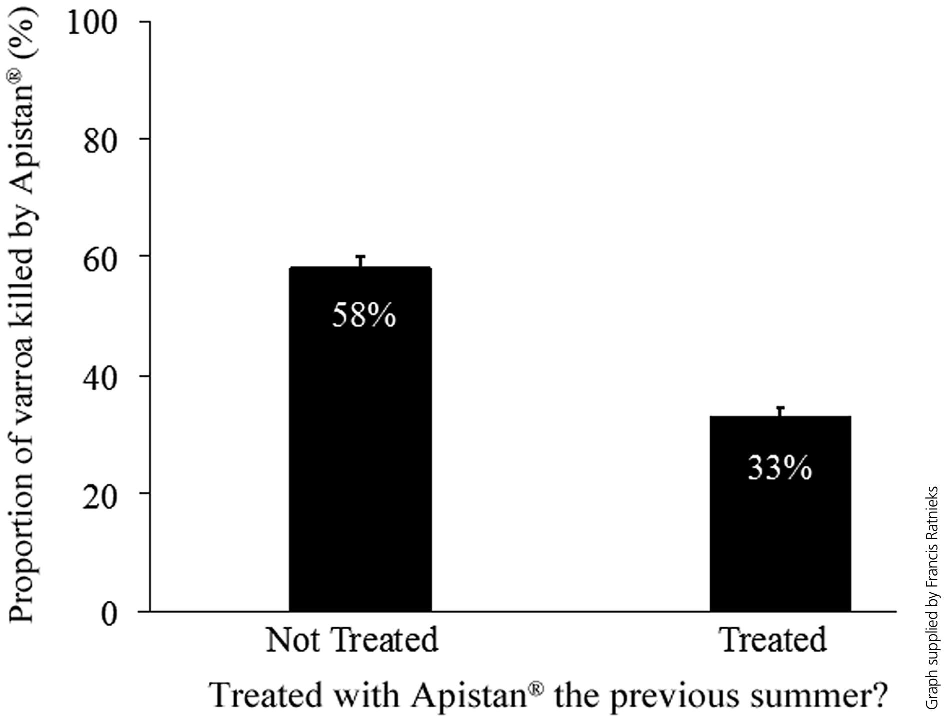
Our first winter samples of worker bees contained many varroa mites. In those taken from colonies that had not been treated with Apistan that summer, the average was 17.1 mites per 100 bees (standard error (SE): 1.6 mites per 100 bees), versus 8.8 mites per 100 bees (SE: 0.6) in the colonies that had been treated.

In the second sample, the proportion of varroa was reduced to 7.3 per 100 bees (SE: 0.8) in the colonies previously not treated versus 5.9 per 100 bees (SE: 0.4) in the previously treated colonies.

We also determined the proportion of mites killed in each colony and from these worked out that, on average, Apistan killed 58.1% (range 40–69%; SE ± 2.1%) of the varroa in the previously untreated colonies versus 33.0% (range 20–46%; SE ±1.8%) in the colonies treated with Apistan the previous summer. This difference is highly significant (F = 82.8; P < 0.01 where F = distribution variable and P = probability). In other words, Apistan killed a lower proportion of varroa in colonies that had been treated with Apistan four months previously. Colony strength had no effect on the proportion of varroa killed by Apistan (F= 0.3; P=0.6).

## Discussion

Our results confirmed what we had expected. Apistan was not effective at killing the varroa in our colonies, presumably because of high levels of resistance to fluvalinate. The kill of 33% in the colonies we had treated the previous summer is very similar to that reported previously; 30%, for resistant varroa (Faucon et al, 1995). It is likely that similar results would be found




*Proportions of* Varroa destructor *mites killed via a 35-day Apistan treatment (6 December 2014 to 10 January 2015). Treated hives (n = 20 hives) had received a previous Apistan treatment from 12 August to 22 September 2014. Other than that, hives had not been treated with Apistan for five years or more. The proportion of varroa killed in the previously non-treated hives (n = 20), 58%, is significantly greater than the proportion killed in the hives treated the previous summer with Apistan, 33%. Apistan will kill close to 100% of non-resistant varroa. The results indicate: 1) a high level of Apistan resistance; 2) that the single previous Apistan treatment a few months before caused a big drop in varroa mortality, indicating that the previous treatment had selected for resistant mites within the study hives.*

Francis Ratnieks

*How LASI counts phoretic varroa mites on worker bees*
*Top left: Worker bees are shaken into a gardening tub and a sample is taken with a home-made scoop that will hold 250–300 bees when full. The bees in the scoop are then put into a zip-lock bag and frozen. Great care is taken not to take the queen. Samples are taken from broodless hives, in December or early January, so that all varroa are phoretic. We always take bees from the centre frames*
*Top right: Varroa mites are washed off the bees and collected using a jet of warm water and a double-mesh honey strainer*
*Bottom left: Varroa mites pass through the first mesh but are trapped by the second, finer, mesh, where they are counted*
*Bottom right: After varroa mite extraction, the bees in the sample are counted to determine the number of mites per 100 bees*

by other beekeepers in Britain, given that resistant varroa have been present for 15 years.

## Extracting and Counting

Extracting and counting the numbers of varroa on workers bees is simple to do and does not require specialised equipment. Beekeepers or beekeeping associations may like to try it for themselves.

The samples of worker bees from which varroa are extracted need to be taken from a colony without brood. This can be achieved by working in the winter. We find that December is the month with the greatest proportion of broodless hives. However, brood rearing varies from year to year. In the winter of 2015–16, brood rearing in most hives continued long into December, presumably because of the mild autumn.

## How Useful is a Varroa Kill of 33% or 58%?

To make the question clearer, consider kill levels of 97%, such as from applying oxalic acid to broodless hives (Al Toufailia et al, 2015), versus 48.5%, from a less effective treatment such as applying oxalic acid to hives with brood or applying Apistan to resistant varroa. It seems that the first is twice as effective as the second, as 97% is double 48.5%. However, when we look at the surviving proportions, 3% versus 51.5%, it is clear that the first result is much more than twice as effective.

The varroa population would have to double slightly more than five times (3–6, 6–12, 12–24, 24–48, 48–96) to get back to where it was. For the second result, the varroa population has to double slightly less than once to get back to 100.

The populations of all living organisms have the ability for exponential growth, 2 – 4 – 8 – 16 – 32, etc, when not overcrowded.

## How Useful is a Method that only Kills Half the Varroa in a Colony?

We carried out a study in which we determined varroa populations in 42 colonies at an interval of one year (Al Toufailia et al, 2014). In that year, the mite populations increased, on average, by 40 times per colony, equivalent to slightly more than five doublings. This means that killing half the varroa in a colony slows population growth by the equivalent of only approximately one-fifth of a year, on average.

The range across all 42 colonies was from a 7.4- to 65.4-fold increase, equivalent to three to six doublings. The varroa population increase was significantly lower, 19.4-fold (range 7.4 to 28.2), in fully hygienic colonies (ie, colonies that removed 95% or more of a standard patch of freeze-killed brood within 48 hours), versus 45.1-fold (range 21.1 to 65.4) in colonies that were less than fully hygienic.

## Conclusion

Overall, our results show that Apistan is of limited value in controlling varroa populations in LASI honey bee colonies and probably in colonies throughout Britain. The evolution of resistance to synthetic chemicals is almost inevitable and it is unrealistic to expect that varroa can be controlled by these chemicals in the future. Alternative approaches, including the use of formic and oxalic acids and hygienic behaviour, should be used instead. ⌘

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## Statistical Analysis Methods

Data were analysed using IBM SPSS statistical program version 20. If necessary, we log or arcsine transformed the response variable to meet the assumptions of ANOVA (Grafen et al, 2002, Zuur et al, 2010). We then used ANOVA to test the effect of the Apistan in winter, when the colonies are broodless, on colonies treated and non-treated with Apistan the previous summer.

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