Recent Varroa Projects at Fera Science

Ben Jones, Senior Scientist Bee Health, outlines some of the latest investigations into varroa control

Varroa infestations can have devastating effects on honey bee colonies if left untreated. Varroa populations increase exponentially through the beekeeping season (Kraus & Page, 1995) and remain a major issue for beekeepers in the United Kingdom (UK). Varroa mites vector and increase levels of deformed wing virus (DWV) in honey bees (Martin et al, 2012; Posada-florez et al, 2019; Stainton, 2022), which is associated with a significant reduction in the population size of honey bees in colonies (Budge et al, 2015) and can lead to colony death (Dainat et al, 2012). Varroa treatments are therefore a vital part of routine beekeeping in order to maintain the health of honey bee colonies.

There have been two large-scale Defra-funded varroa projects performed at Fera Science in recent years. Between 2019 and 2021, a large scale 'Varroa Management' field study was conducted to investigate the efficacy of different treatment regimens on varroa loads and colony health. In 2021, a two-year laboratory and field study was initiated to investigate the effects of temperature on thymol-based varroa treatments.

Varroa Management Project (2019–2021)

A field study was performed to assess the effectiveness of different treatment regimens for *Varroa destructor* between 2019 and 2021. The treatment regimens were designed to accommodate different beekeeping groups: small-scale beekeepers, bee farmers and beekeepers who prefer chemical-free, biotechnical methods. Colonies in three of Fera's experimental apiaries were set up at the end of 2019. The colonies were then subject to one of four treatment regimens outlined below and monitored throughout 2020.

Treatment Regimens

Colonies in three experimental apiaries were initially set up in October 2019 to include 20 colonies per apiary headed by sister queens. Colonies were pre-treated with amitraz and frames of bees and varroa drops were counted to ensure colonies were standardised. Within each apiary, the colonies were split into four groups of five colonies: three treatment groups and a control group comprising a total of 15 colonies each of:

1. Control group: no-treatment group. No varroa treatments for the duration of the experiment.
2. Beekeeper group: treatment group for small-scale beekeepers. Two oxalic acid trickle treatments (Api-Bioxal®) in January and February 2020 and formic acid treatment (MAQS®) in August 2020.
3. Bee farmer group: treatment group for large-scale beekeepers. Two oxalic acid fumigation treatments in January and February 2020 and oxalic acid fumigation (Api-Bioxal®) three times in August/September 2020.
4. Chemical-free group: chemical-free treatments. Queen caging at end of April for 21 days. Drone brood removal three times through June and July (two frames per colony for 21 days). However, drone combs were sealed in only five of the 15 colonies across the three apiary sites. This group was therefore dropped from the analysis.

Colony Monitoring and Varroa Loads

Colony assessments were performed each month and floor debris samples were collected. In order to track the varroa populations during the beekeeping season, the natural mite drops in the months between the treatments were compared. In addition, DWV levels were monitored from adult bees between the initial treatments of oxalic acid in 2020, and prior to the final treatments in 2020. Finally, in October 2020 and February 2021, all colonies were treated with Apivar® and oxalic acid in order to compare the remaining mites in colonies across the different treatment regimens.

Results

Natural varroa drops between April and June 2020 were significantly lower in the beekeeper-treated colonies compared to the control and bee farmer colonies, indicating that the initial oxalic acid trickle treatment reduced the mite populations more compared to oxalic acid fumigation and no treatment (Figure 1). After the final treatments in 2020, there were more colonies that contained at least 1,000 varroa mites in the control group compared with the treatment colonies but this difference was not significant (Table 1). The colony sizes, DWV levels or mortality of colonies were not significantly different between the untreated and treated colonies.

Discussion

Natural varroa mite drop was reduced from April to June following the oxalic acid trickle treatments in January and February 2020. High efficacy of oxalic

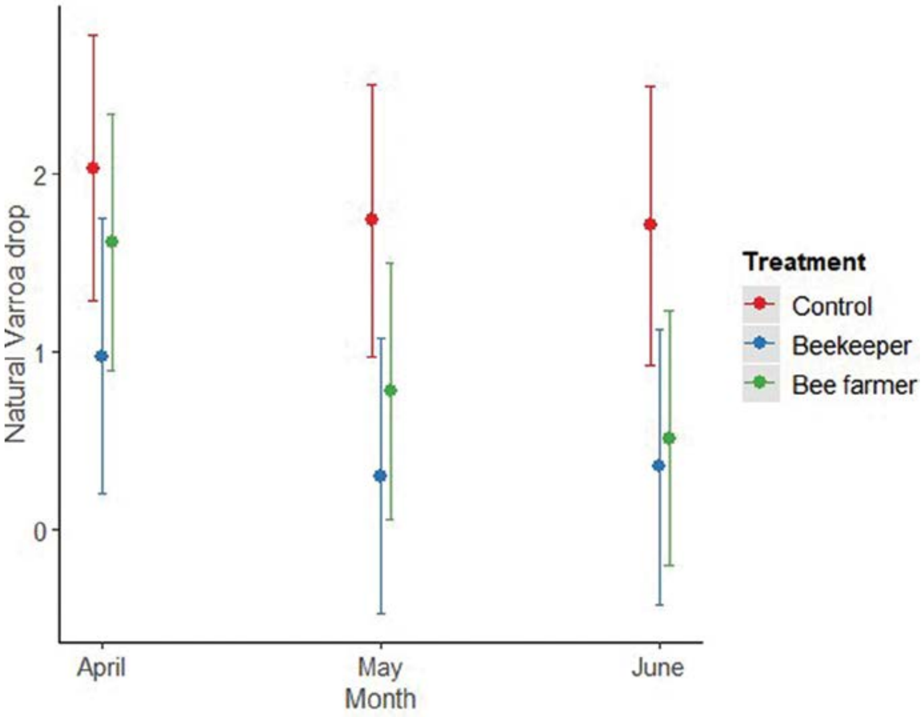

Figure 1. Natural daily varroa mite drops from April to June 2020 following the oxalic acid treatments. Error bars represent 95 per cent confidence intervals

acid trickle and fumigation treatments have been previously demonstrated in the UK (Al Toufailia et al, 2015). However, our results show that natural daily mite drops were reduced by an average of only 1.25 mites in the oxalic acid trickle-treated beekeeper colonies and were not significantly reduced in the oxalic acid fumigation bee farmer colonies, indicating that the varroa mite populations in colonies were generally low overall. Daily natural mite drop remained consistently low from the end of March until the end of June 2020 and well below treatment thresholds (Beebase varroa calculator, www.nationalbeeunit.com). Likewise, there were no differences in DWV levels between controls and treated colonies in our study. DWV levels have been shown to increase with varroa infestation (Castelli et al, 2021, Martin et al, 2012) and although mite numbers increased dramatically by the end of our study, it is possible that varroa loads remained low

Table 1. The percentage of surviving colonies containing at least 1,000 mites following the treatment regimen

Treatment regimen	Percentage of colonies with at least 1000 mites
Bee farmer	43
Beekeeper	40
Control	67

enough for long enough to explain the lack of difference in DWV levels between treated and control colonies. Low overall DWV levels until June may also explain the lack of mortality and lack of reductions in colony sizes that have been previously associated with DWV (Kevill et al, 2019; Budge et al, 2015).

There was also a counter-intuitive lack of difference between the number of varroa mites remaining in colonies between the control and treated colonies after the final treatments in 2020. At the end of the study, all colonies – including the controls – were treated in order to count the remaining mites in the colonies after the treatment regimens. By this time, mite numbers were extremely high, in line with an exponential increase. An upper limit of 5,000 mites was therefore applied when performing these final counts. A biologically relevant mite population of 1,000 mites (*Managing Varroa*, The Animal and Plant Health Agency, 2020) was therefore chosen for comparisons between the groups. However, by using a binary indicator of at least 1,000 mites, the analysis inevitably suffered from a reduced resolution for detecting differences in mite numbers between colonies. In addition, we cannot rule out the possibility that mites equalised between control and treated colonies by robbing bees after the final treatments. This, and the very low mite numbers detected in all colonies until the end of the season, may explain the lack of treatment effects observed in our study. Although our results are surprising, our results reflect the unfortunate risk of field studies where the researcher must trade off obtaining field realistic results with a lack of experimental control.

The Effects of Temperature on Thymol-based Varroa Treatments (2022–2023)

Thymol-based veterinary medicines for the treatment of varroa are commercially available to beekeepers, although large ranges in efficacies are reported: Apiguard®, 36–96 per cent; ApiLife Var®, 31–98 per cent; Thymovar® 39–93 per cent (Apiguard® FAQ's, 2021; Coffey & Breen, 2013; Floris et al, 2004; Stainton, 2022; Salord et al, 2015). A decrease in the efficacy of powdered thymol is correlated with a decrease in ambient temperature (Chiesa and D'agaro, 1991) and a previous study reported lower efficacies of Apiguard® and Thymovar® in northern Italy when temperatures were lower, compared to central and southern regions, although no direct correlation with temperature was found (Baggio et al, 2004). Beekeepers in England and Wales have also reported inefficiencies of thymol-based products in cooler locations. In the case of Apiguard®, the situation can be further complicated as extra information for use in different temperatures is given on the manufacturer's website, rather than on the treatment packaging.

The primary aim of this project is to determine the effects of ambient temperature on thymol-based varroa treatment products for beekeepers. The second aim of this project is to provide advice to beekeepers on the efficacy of thymol-based veterinary medicines across locations with different temperatures in England and Wales. In order to achieve these goals, the effect of temperature on thymol-based treatment products was investigated in a laboratory study using adapted 'mini colonies'. The results of this laboratory phase will be used to identify field sites representative of the different temperatures in England and Wales (Figure 2) to 'ground truth' the laboratory results in 2023.

In 2022, the laboratory phase of this study was completed by monitoring the varroa drops from colonies in adapted mini-mating nuclei placed in controlled environment (CE) rooms at Fera Science. As the mini colonies contained only ~400 bees held in a small, confined space, a dose response study was initially carried out to calculate the correct dose of treatment

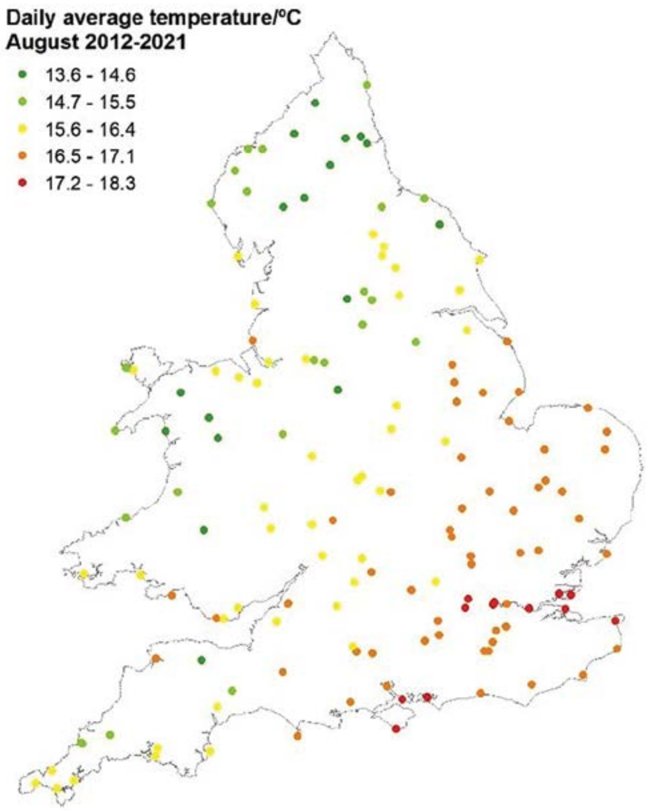

Figure 2. Average August temperatures (°C) across England and Wales from 2012–2021 (Met Office data)

that would not kill the bees, as a pilot study revealed high honey bee mortality when doses were simply calculated from the internal area of the mini colony. The mini colonies were placed within one of three CE rooms held at 19°C, 20°C or 25°C and had their entrances closed. Each CE room contained 40 colonies that contained either Apiguard® (n=10), Thymovar® (n=10), ApiLife Var®(n=10), or were left untreated (n=10). Learning from the field experiment in 2019–2021, the colonies were populated with bees from an apiary maintained with high varroa loads in order to give our experimental system the best chance of detecting differences in efficacy across the different treatments and temperature ranges. Colonies were placed on mini varroa inserts and varroa drops were monitored over 48 hours. All colonies were then treated with Apivar® in order to calculate efficacies.

Adapted 'mini colonies' on Varroa inserts within a controlled environment (CE) room at Fera

The results from this laboratory phase are intended as a proxy for what occurs in colonies in the field. For example, any differences observed in the laboratory due to temperature may simply be indicative of a slower acting treatment or may in fact be representative of what happens in treated colonies in the field. Performing the laboratory phase allows us rule out or rule in the effects of temperature and what the shape of responses are under controlled conditions. These results will therefore inform us of which locations to choose to investigate whether the laboratory results are replicated in the field in 2023. ◻

References

Al Toufailia, H, Scandian L, Ratnieks, FL (2015). Towards integrated control of Varroa: 2) comparing application methods and doses of oxalic acid on the mortality of phoretic *Varroa destructor* mites and their honey bee hosts. *Journal of Apicultural Research,* **54**(2), pp 108–120.

Animal and Plant Health Agency (2020). *Managing Varroa.*

Baggio, A, Arculeo, P, Nanetti, A, Marinelli, E, Mutinelli, F (2004). Field trials with different thymol-based products for the control of varoosis. *American Bee Journal*, **144**(5), pp 395–400.

Budge, GE, Pietravalle, S, Brown, M, Laurenson, L, Jones, B, Tomkies, V, Delaplane, KS (2015). Pathogens as predictors of honey bee colony strength in England and Wales. *PLoS One*, **10**(7), e 0133228.

Castelli, L, Genchi García, ML, Dalmon, A, Arredondo, D, Antúnez, K, Invernizzi, C, Reynaldi, FJ, Le Conte, Y, Beaurepaire, A (2021). Intra-Colonial Viral Infections in Western Honey Bees (*Apis mellifera*). *Microorganisms*, **9**(5), 1087.

Chiesa, F, D'agaro, M (1991). Effective control of Varroatosis using powdered thymol. *Apidologie*, **22**(2), pp 135–145.

Coffey, MF, Breen, J (2013). Efficacy of Apilife Var® and Thymovar® against *Varroa destructor* as an autumn treatment in a cool climate. *Journal of Apicultural Research*, **52**(5), pp 210–218.

Dainat, B, Evans, JD, Chen, YP, Gauthier, L, Neumann, P (2012). Dead or alive: deformed wing virus and *Varroa destructor* reduce the life span of winter honeybees. *Applied and Environmental Microbiology*, **78**(4), pp 981–987.

Floris, I, Satta, A, Cabras, P, Garau, VL, Angioni, A (2004). Comparison between two thymol formulations in the control of *Varroa destructor*: effectiveness, persistence, and residues. *Journal of Economic Entomology*, **97**(2), pp 187–191.

Kevill, JL, de Souza. FS, Sharples, C, Oliver, R, Schroeder, DC, Martin, SJ (2019). DWV-A Lethal to Honey Bees (*Apis mellifera*): a Colony Level Survey of DWV Variants (A, B, and C) in England, Wales, and 32 States across the US. *Viruses*, **11**(5), p 426.

Kraus, B, Page Jr, RE (1995). Population growth of *Varroa jacobsoni* Oud in Mediterranean climates of California. *Apidologie*, **26**(2), pp 149–157.

Salord, MDML, Llado, G, Miranda, M (2015). Comparison of the efficacy of Apiguard (thymol) and Apivar (amitraz) in the control of *Varroa destructor* (Acari Varroidae). *Spanish Journal of Agricultural Research*, **13**(3), p 11.

Stainton, K (2022). *Varroa Management*. Northern Bee Books.

Martin, SJ, Highfield, AC, Brettell, L, Villalobos, EM, Budge, GE, Powell, M, Nikaido, S, Schroeder, DC (2012). Global honey bee viral landscape altered by a parasitic mite. *Science*, **336**(6086), pp 1304–1306.

Posada-Florez, F, Childers, AK, Heerman, MC, Egekwu, NI, Cook, SC, Chen, Y, Evans, JD & Ryabov, EV (2019). Deformed wing virus type A, a major honey bee pathogen, is vectored by the mite *Varroa destructor* in a non-propagative manner. *Scientific Reports*, **9**(1), pp 1–10.

Vita (Europe) Limited. Apiguard FAQ's. www.vita-europe.com