EVALUATING FIPRONIL RESIDUES AND RE-ESTABLISHMENT SUCCESS IN MANAGED HIVES FOLLOWING WILD EUROPEAN HONEY BEE (*APIS MELLIFERA)* ERADICATION IN NEW SOUTH WALES

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Summary

Following the incursion of Varroa mite (*Varroa destructor*) in New South Wales, Australia, in June 2022, eradication was attempted to protect the honey bee related industries. A Biosecurity Emergency Order was established to destroy managed and feral European honey bees within Emergency Eradication Zones, using specialised bait stations and fipronil. Our study aimed to identify managed hives within 1.5 km of a bait station and assess if they were impacted by fipronil application, to determine any residual fipronil concentrations within the hive and evaluate re-establishment success. We found eleven apiaries that met the study requirements, three of which reported colony loss during the eradication program. One apiary reported three destroyed hives that may be attributed to fipronil application at a bait station 3.08 km away (fipronil from closest bait station showed no impact). A very low concentration of fipronil was detected within the hive but was below the limit of quantification. Another apiary reported the loss of two hives, coinciding with the application of fipronil at a nearby bait station (387 m); the apiary has since successfully re-established hives. The third apiary experienced colony loss well after eradication efforts had ceased and, therefore, we consider the apiary was unlikely impacted from fipronil application; the colony was successfully re-established. The remaining eight sites were unimpacted and fipronil residues were not detected by HPLC. Further research is warranted to determine if re-introduced hives to eradication zones are impacted by residual fipronil in the environment.

Keywords: Varroa, emergency response, management.

INTRODUCTION

The Australian European honey bee (EHB) industry is a significant contributor to agricultural and horticultural production, and a major employer. Australian exports of agricultural commodities in 2019/2020 were valued at more than A$25 billion (Plant Health Australia 2021). Crucially, European bees (*Apis mellifera* L.) pollinate many of these crops, which were valued at A$14.2 billion in 2017 (Frost 2022; APAL 2022). Additionally, honey and beeswax production were valued at A$162 million (Plant Health Australia 2021). In 2021, the Australian honey bee industry contained approximately 25,000 registered beekeepers managing about 672,200 hives (Plant Health Australia 2021). The industry faces many endemic disorders (Bourke 2020) and must prepare for exotic incursions (Gillespie *et al*. 2003; Carnegie and Pegg 2018).

Varroa mite (*Varroa destructor* (Anderson and Trueman)) is an exotic external parasite of EHB and was first detected at the Port of Newcastle, NSW, on 22 June 2022 (McFarlane *et al.* 2024). An eradication program was initiated to protect the industries reliant on honey bees. The original 100 days of the response was described by Bourke *et al.* (2024), and Taylor *et al.* (2025) described the methods used to assess varroa populations in the response. The response plan had many components including attempting to trap swarms of feral bees, possibly infested with the mite (Nguyen *et al.* 2025), euthanasia of EHB and community awareness (Bourke *et al.* 2024). As part of the response, a *Biosecurity (Varroa Mite) Emergency Order* (*No 21*) *2022* was issued on 12 August 2022 which included specific clauses permitting their destruction by authorised personnel. An eradication program was established to destroy managed and feral honey bees within Emergency Eradication Zones (EEZs) (NSWG 2022). The Wild European Honey Bee Management (WEHBM) Program was initiated to control feral honey bees, operating from September 2022 until the Transition to Management was declared in September 2023 (Bourke *et al.* (2024).

Feral EHB colonies are more challenging to destroy than managed colonies, because their hives can be difficult to locate. Therefore, specialised bait stations (designed as detailed in the APVMA permit as a guideline) were deployed to attract local feral EHB to the bait station. A variety of species-specific food and attractants were used to actively attract and recruit foraging feral EHB to the bait station including: 1) 50-60% sugar and water (sugar syrup) solution contained within the bait station, 2) spraying an irradiated honey

and water mixture around the bait station, and 3) heating a mixture of irradiated honey and irradiated, loose beeswax in the immediate area surrounding the bait station.

When enough EHB were observed to be feeding from the bait station (typically >300 bees recruited in the first baiting cycle), the sugar syrup solution was replaced with sugar syrup solution spiked with fipronil (10 mg fipronil/L of sugar syrup), in accordance with the APVMA permit, PER84929 (this permit has expired and is no longer available online) (APVMA 2017). Bait stations were raised off the ground and had grease strips to exclude ants and other non-target insects. Fipronil was applied only after EHB were seen feeding from the sugar syrup; the feeding was monitored throughout the spiking time to mitigate against non-target poisoning. The fipronil and sugar syrup solution was made available to the EHB for 2-3 hours until no more bees were seen foraging from the station. Then, the fipronil was removed and the cycle was repeated until no more bees were attracted to the bait station.

In March 2023, the option was made available to small apiaries (ie. apiaries with less than 10 hives) within EEZs to either euthanise their hives themselves without assistance from New South Wales Department of Primary Industries Surveillance and Destruction staff, or to leave their hives in situ. Hives left in situ were potentially exposed to fipronil bait stations deployed within the EEZ and had the potential to be impacted by fipronil.

The impacts of baiting and attempts to re-establish these hives is largely unknown, particularly those hives that were not destroyed, or disposed of. Also, the published literature on the impacts of fipronil residues and persistence is limited, particularly in the context of EHB (APVMA 2010). Therefore, our study aimed to identify those managed hives that were left in situ (within 1.5 km of a bait station) and determine concentrations of residual fipronil within managed hives following fipronil application.

MATERIALS AND METHODS

We used historical information obtained from the Varroa Mite and Wild Bee Management, MAX Plant Biosecurity records. MAX is a software program that contained data on registered beekeepers with hives in eradication zones (Figs. 1 and 2) that elected not to have their hives destroyed, either by DPI or themselves. We identified and contacted those beekeepers. If any hives were located within 1.5 km of a bait station and exposed to fipronil application, then we obtained permission from the beekeeper to sample the hive for nectar/honey, then a hive health assessment was conducted and the beekeeper was asked to complete a short survey. In addition to the survey, we asked beekeepers if any treatments were used in the hives to control pests (eg. fipronil used to treat small hive beetle (*Aethina tumida*)). We visited eleven apiaries and collected samples from November 2023 to January 2024 (Table 1).

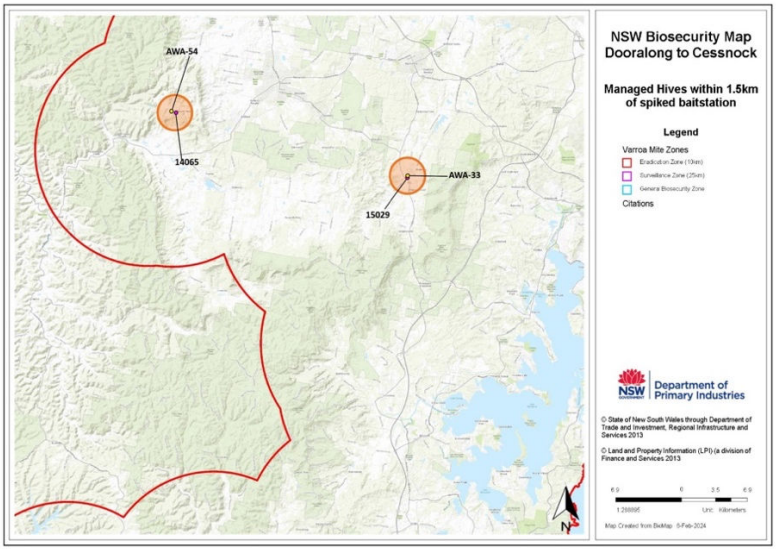

Figure. 1. Managed hives (pink) and bait station (yellow) locations, Northern Sector, Dooralong to Cessnock

Table 1. Study cases (hives) with MAX case IDs, nearest bait station/s within 1.5 km, distance from bait station/s, fipronil application (spike) dates, shake sampling date, beekeeper survey results, fipronil concentrations in shake/nectar samples and general hive health.

Max case	Nearest bait station/s (<1.5 km)	Distance from bait station/s (m)	Fipronil application dates	Bee mortality (Y/N)	Colony mortality (Y/N)	Date Colony Death	Shake Sampling Date	Re-introduced Hardware (Y/N)	Re-introduction Succesful (Y/N)	Pest Treatments (locally or in hive)	Fipronil Concentration (mg/L)	General hive health/notes at time of sampling
4088	MTA-101	1480	7/09/2023	Y (1-2 bees)	Y (colony lost due to AFB)	Sep-23	23/11/2023	Y	Y	N	ND	5 hives sampled; strong hive health (with a mixture of smaller and larger colonies); no major presence of pests.
7683	SOM-02 SOM-23	1030 1340	**SOM-02:** 21/12/2022 07/06/2023	N	N	NA	23/11/2023	NA	NA	Y (SHB treatment in hive)	ND	1 hive sampled; no impacts; good hive health; positive for varroa mite (alcohol wash) with low numbers of SHB. Low number of drone brood.
12063	MTA-104 MTA-78	894 1389	**MTA-104:** 23/08/2023 **MTA-78:** 07/09/2023 12/09/2023	N	N	NA	8/11/2023	NA	NA	Y (neighbour spraying for weeds)	ND	8 hives sampled (active hives only); signs of AFB (perforated brood, oily, smell); SHB observed; some hives with strong health.
12223	MTA-101	1078	7/09/2023	N	N	NA	11/12/2023	NA	NA	N	ND	2 hives samples; hive 1 (alive), Hive 2 (dead). Dead hive had AFB and SHB present. Hive 1 showed strong hive health.
12621	MTA-105	387	6/09/2023	N	Y	Sep-23	22/11/2023	N	NA	N	1.56	2 hives sampled; 2 hives impacted; both hives succesfully re-established with strong hive health; Indoxocarb used to treat SHB. Survey notes "DPI experience today very professional".
12630	MTA-105	826	6/09/2023	N	N	NA	19/12/2023	Y (requeening)	Y	N	ND	44 hives - 5 representative samples collected; all good hive health. Some SHB was present but manageable.
12696	MTA-103	1378	21/08/2023	N	N	NA	13/12/2023	NA	NA	N	ND	7 hives sampled; no impacts; Hives were healthy but showed early detection of varroa mite (bayvarol/sticky mats).
12800	MTA-105	487	6/09/2023	Y	Y	Dec-23 1/12/2023	13/11/2023	NA	NA	NA	ND	2 hives sampled; 1 colony impacted; loss occurred 3 months after last spike. 1 hive shows good hive health.
13958	ETT-IPU6 ETT-IPU1 ETT-IPU5	385 894 988	**ETT-IPU6:** 1/06/2023 28/07/2023 **ETT-IPU1:** 14/04/2023 22/05/2023 01/06/2023 **ETT-IPU5:** 03/05/2023 22/05/2023	Y (~1500-2000)	N	Oct-23 7/10/2023	7/11/2023	N	NA	Y (Neighbour accused of spraying poison)	ND	1 hive sampled; strong hive health at time of sampling; bee death attributed to neighbour spraying insecticide; low SHB numbers present
14065	AWA-54	370	18/05/2023	N	N	NA	20/11/2023	NA	NA	N (possible treating/spot spraying St Johns Wort)	ND	1 flow hive sampled; no impacts; moderately healthy but with high SHB numbers in honey super; no SHB traps present.
15029	AWA-33	176	09/06/2023, 1/08/2023	Y	Y	Apr-23	15/11/2023	N	NA	N	~0.25 (< LOQ)	3 flow hives sampled: hives impacted; reports weak and dead bees outside hive entrance. Colony lost before first spike. Re-establishment not attempted.

Abbreviations: SHB, Small Hive Beetle; AFB, American Foulbrood; ND, Not Detected; LOQ, Limit of Quantification; N, no; Y, yes; NA, not applicable.

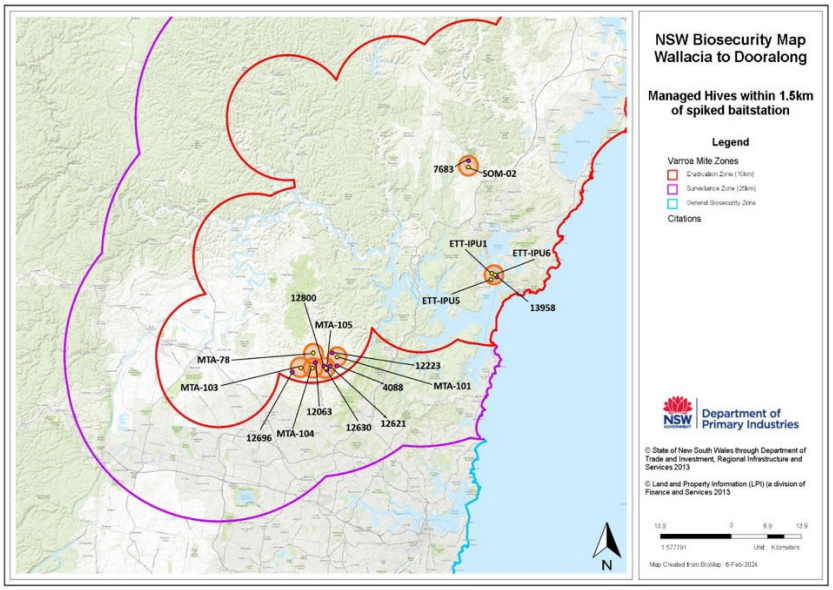

Figure. 2. Managed hives (pink) and bait stations (yellow) locations, Southern Sector, Dooralong to Wallacia.

Figure. 3. Visible nectar/honey within open cells of a frame, deposited by foraging EHB.

Figure. 4. Frame containing nectar/honey were held flat and vigorously shaken until the nectar/honey solution fell from the cells into the collection tray.

To quantify the concentration of fipronil within the hive, we targeted nectar and shake samples. EHB are highly sensitive to fipronil, with a reported LD_{50} of 4.17 ng/L (Johnson, 2015). Previous assessments of bait station efficacy found that EHB succumb to fipronil 31.6 ± 9.5 min after first forage (Nguyen et al., in prep.). Consequently, colonies affected by fipronil would likely collapse shortly after exposure, precluding the conversion of contaminated nectar or syrup into capped honey. Nectar/shake samples, therefore, represent a more reliable matrix for analysis, as they are expected to contain the highest detectable concentrations of fipronil. Therefore, we collected shake and uncapped honey (typically comprising nectar or sugar syrup) and analysed these samples using high-performance liquid chromatography (HPLC) following established methods (Tomasini et al., 2011).

To collect the shake solution, a large clean tray was set up near the hive and covered with baking paper. Frame/s with open cells containing nectar/sugar syrup were held flat and horizontally to the tray and vigorously shaken until the nectar/sugar syrup solution fell from the cells into the tray (Figs 3 and 4). If the solution was not readily shaken from the frames, then we used a syringe to extract the sample. When a representative sample was collected, the solution was

transferred to a vial, placed on ice in a dark container, immediately transported to a freezer and to the University of Newcastle laboratories for analyses. All samples were stored at -18 °C until required for analysis.

The nectar/honey samples were analysed for fipronil using a modified QuECHeRS method (Quick, Easy, Cheap, Effective, Rugged, and Safe) for determination by HPLC, as described by Tomasini *et al*. (2011). The QuEChERS method is recognised as an official method in the detection of multiple pesticide residues in fruits, vegetables and honey (Anastassiades *et al.* 2003; Lehotay 2007; Tomasini *et al*. 2011). This method is particularly useful for the determination of polar, middle polar and non-polar pesticide residues in various food matrices because it is robust, inexpensive and suitable for determination of pesticides in complex samples (Tomasini *et al.* 2011). In optimal conditions, the limits of quantification of fipronil using the QuEChERS method is 0.6 mg/kg.

Briefly, the QuECHERS procedure involves initial extraction of honey with acetonitrile, followed by liquid-liquid partitioning formed by the addition of $MgSO_4$. Nectar/honey (2.0 g) samples were weighed and placed into a 50.0 mL PTFE centrifuge tube. Deionized water (4.0 mL) was added and the mixture vortexed until it became homogenous. Then 4 mL acetonitrile was added and the sample homogenized for 1 min. After adding 1.6 g anhydrous magnesium sulphate, the mixture was vortexed again for 1 min, and then centrifuged for 5 min at 5000 rpm. Finally, 20 µL of the upper acetonitrile layer was filtered (0.45µm) into 2mL vials before injection in HPLC system for analysis.

RESULTS AND DISCUSSION

We identified eleven viable cases that met the criteria and where a beekeeper agreed to participate in the study (Table 1). Of the eleven cases, three showed signs of potential impacts from spiking as "colony loss" was experienced during the baiting program. The first case reported colony loss in three flow hives in approximately April 2023. The nearest bait station (bait station ID AWA-33, distance of 176 m) was first spiked on 9 June 2023 and subsequently on 1 August 2023. The initial spike date occurred two months after the observed colony loss date (based on the recollections of the beekeeper) although the report of hundreds of dead bees outside hives was typical of a fipronil impacted hive. We deduced that the observed colony loss was not due to fipronil application at AWA-33. However, we found that a bait station (AWA-34) 3.08 km away was spiked on 15 May 2023, closer to the reported colony loss date. Bait stations may be most effective up to 1 km. Numerous studies have shown bees can travel much longer distances when foraging for food resources (Couvillon *et al*. 2015; Beekman and Ratnieks 2000; Visscher and Seeley 1982). Therefore, the reported colony loss for Case 1 may be due to fipronil application at AWA-34, which may also explain the very low concentration of fipronil detected in the shake sample, as determined by HPLC. Re-establishment of these hives was not attempted by the beekeeper.

Case two reported colony loss in two hives in September 2023, coinciding with the nearest bait station (MTA-105, 387m) fipronil application on 6 September 2023. Since the time of spiking and colony loss, both hives had successfully re-established with healthy colonies and with no signs of fipronil poisoning during re-colonisation (hives re-assessed for hive health on 22 Nov 2023). HPLC results showed 1.56 mg/L of fipronil, however, the beekeeper reported use of traps containing oil and diatomaceous earth, to treat small hive beetle and other pests, along with chemicals including Indoxacarb, an oxadiazine pesticide. Indoxocarb has similar topological polar surface area to fipronil (NCBI 2024), and we suspect that the presence of indoxocarb might interfere with the detection of fipronil as determined by HPLC. EHB are very sensitive to fipronil with a reported LD_{50} of 4.17 ng/L (Johnson 2015), well below the concentration reported by HPLC. Given that the hives were healthy during the time of sampling, therefore, we think that it is unlikely the reported figure was an indication of fipronil, but more likely confounded by the presence of indoxocarb or its metabolites.

Case three reported colony loss on 1 December 2023 with the nearest bait station (MTA-105, 487m) having fipronil applied on the 6 Sep 2023. The reported date of colony loss falls well after the spike date and after eradication efforts had ceased. On September 2023, the National Management Group decided that eradication was no longer feasible (Bourke *et al.* 2024) and the eradication response program transitioned to management of the pest, following the National Transition to Management Plan which was initiated on the 19 Sep 2023. HPLC results determined no detectable concentrations of fipronil from the hive samples. Therefore, we consider it was unlikely that the fipronil application in September at MTA-105 was the cause of colony loss in December. The lost colony was successfully re-established.

We did not detect fipronil at the remaining eight sites and all sites that attempted re-establishment were

successful according to the survey results. We think that these results are not surprising given that most hives showed no impacts from fipronil during the eradication effort. Furthermore, most hive samples were taken some time after the nearest bait station/s were spiked, and the hives were healthy or active when samples were taken. If the hive survived, then there was little chance that there was fipronil within the honey, given the high sensitivity of EHB to fipronil. It should be noted that our study was not indicative of all hives that were exposed to fipronil because most of these hives were destroyed during the program. Therefore, we proposed that any fipronil that may have been present was likely either removed or degraded. Also, we think that it was unlikely that fipronil would be detected within the hive samples, considering that the hives were healthy or active at the time of sampling and that EHB are very sensitive to fipronil.

Based on our findings, we suggest that most apiaries that met the criteria for the study showed no impact from fipronil application from WEHBM bait stations. We recognise that our study had some limitations. Our study sites may not be representative of all apiaries that may have potentially been affected by the WEHBM eradication program. For instance, hives that were exposed to and impacted by fipronil during eradication were probably destroyed, owner reimbursement costs claimed, and hives subsequently disposed of. Therefore, these fipronil impacted hives were not available to be included this study, limiting the sample subset to mostly non-impacted hives. Furthermore, commercial beekeepers were asked to opt in and agree to sampling, with many declining to participate in the study. This applied especially for several commercial apiarists with large amounts of hives which may have been impacted. This opt-out option may have substantially decreased our sample size. Conversely, recreational beekeepers, with much smaller quantities of hives, were much more agreeable to participate in the study. Additionally, unregistered beekeepers were not included the study, as the keeping of bees without registration was against current NSW law.

We were heartened by the conduct of the survey and by the response from most beekeepers. We think that the beekeeper survey was effective, not only to collect qualitative information, but also to engage with beekeepers, allowing them to voice their experiences with the program. There were several comments expressing the professionalism and care taken by DPI staff.

CONCLUSION

Our study aimed to investigate the impact of residual fipronil and re-establishment success of managed hives that were exposed to fipronil application from a WEHBM bait station. This aim is distinct from the assessment of residual fipronil in the environment (ie. foraged from wild hives) and, therefore, our research does not entirely address the safety of re-introduced hives. However, we did not find much evidence to support the theory that re-established hives would be adversely impacted by the fipronil used in WEHBM bait stations. Further research is needed to determine if re-introduced hives to eradication zones are impacted by residual fipronil in the environment.

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