FEASIBILITY OF IN-HIVE CONTROL OF ADULT SMALL HIVE BEETLES *AETHINA TUMIDA* MURRAY (COLEOPTERA: NITIDULIDAE) WITH AN INSECTICIDE TREATED REFUGE TRAP

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Summary

The significant impact of small hive beetle on the profitability of bee keeping in eastern Australia prompted research into the use of insecticidal traps for deployment in beetle infested hives. A prototype refuge trap comprising foil-covered fipronil-treated corrugated cardboard achieved up to 100% control of adult beetles in field efficacy trials conducted in western Sydney apiaries. Although demonstrating the feasibility of using such a refuge trap against beetle infestations damage to some traps by resident bees and resultant bee deaths highlighted the need for a more robust cover for the insecticide-treated cardboard insert.

Keywords: small hive beetle, fipronil, harbourage, refuge trap, *Aethina tumida*

INTRODUCTION

Since being detected in western Sydney in late 2002 (Fletcher and Cook 2002) small hive beetle, *Aethina tumida* Murray (Coleoptera: Nitidulidae) has established along the eastern Australian seaboard where it is a major threat to profitable bee keeping. With knowledge of the damage attributable to small hive beetle infestations that occurred when it entered North America (Hood 2000) the Australian Pesticides and Veterinary Medicines Authority (APVMA) issued a Pesticide Permit allowing the use of permethrin soil drenches around infested hives but uptake by apiarists has been poor because residual effectiveness is variable (Levot and Haque 2006a) and frequent re-treatment is required. In the United States of America permit approval was given to use the coumaphos product Checkmite+™ that is registered for the control of varroa mite *Varroa destructor* (Anderson and Trueman) (Acari: Varroidae) against adult beetles. Checkmite+™ strips were stapled under pieces of stripped-back corrugated cardboard and placed inside hives (Elzen *et al.* 1999). To reduce pesticide residues American regulators imposed tight restrictions on the frequency and duration for which coumaphos could be used in this way. In Australia, use of coumaphos strips was considered unnecessarily hazardous to bees and honey, and other options were sought.

Adult beetles harbour in cracks and crevices within the hive structure whether bees are present or not. Such behaviour might be exploited in control programs incorporating insecticide treated core-fluted cardboard refuge traps as alternative beetle harbourage sites. Moreover, effectiveness in hives might be enhanced if beetles seek refuge from the harassment of bees. Fipronil is non-repellent, possesses excellent toxicity to small hive beetles (Levot and Haque 2006b) and has the desirable physicochemical attributes of very low vapour pressure (Colliot *et al*. 1992) and low water solubility.

Here we report the results of limited field trials aimed at assessing the feasibility of using fipronil treated cardboard refuge traps to control adult small hive beetles in hives.

MATERIALS AND METHODS

Artificial harbourages based on those used in the laboratory bioassays (Levot and Haque 2006b) were made by covering fipronil (300 mg L^{-1}) treated 4 mm core-fluted cardboard (16 x 18 cm)(Australian Corrugated Box Company, Wetherill Park) with 50 µm thick adhesive-backed aluminium foil such that only the open ends of the corrugations were exposed. Based on experience gained during preliminary trials the openings were strengthened by adding triple-layer, 1 cm overhanging foil edges at each end (Figure 1).

Trials were conducted under APVMA Permit 8167. A condition of the permit was that harbourages could be installed in only 20 commercial hives. The trials took place in February and March 2005 when weather conditions favoured beetle survival, reproduction and dispersal. Trial sites were an 11 hive apiary at Wilberforce and a 67 hive apiary at Cranebrook in Sydney's west. Prior to installing the harbourages, the trial hives were opened and the numbers of live adult beetles on the bottom boards and elsewhere in the hives were recorded. In hives comprising of more than one box, the lid was removed and the super placed on top of the upturned lid. The frames were removed from the bottom box and the number of beetles seen recorded. The frames and super were replaced and the lid inspected for beetles that had moved from the super during inspection to escape the light. Most beetles were found on the bottom board of the hives. Harbourages were installed onto the bottom boards of ten hives at each site. At Wilberforce the devices were stuck using Silastic™ to the corrugated metal bottom boards of some hives to prevent beetles hiding underneath. At this site an untreated harbourage was placed into one control hive. Two control hives were used at the larger Cranebrook apiary.

Beginning 7 days after placement of the harbourages and then at 21 and 28 days after placement, hives were inspected at approximately the same time of day as the initial inspection and the numbers of live and dead beetles found in the hive recorded as before. At the completion of the trials (Day 28) the harbourages were removed, placed into individually labelled sealable plastic bags and brought back to the laboratory where they were de-constructed and the number of dead beetles inside recorded. This number was added to the progressive total number of dead beetles from the earlier inspections.

Data analysis took account of the considerable migration of beetles into the hives during the trial period. Over time, the numbers of live and dead beetles increased as immigrant beetles entered the hives where they may, or may not have been killed in the refuge trap. This made it difficult to measure the level of control achieved if the number of dead beetles was to be considered in the estimate. To address this difficulty, beetle mortality after 28 days is presented as a proportion of the total beetle population present at this time. A generalised linear model with errors assumed to follow a binomial distribution was fitted to the mortality data (McCullagh and Nelder 1989). A logit link function was used to relate the observed mortalities to the harbourage effect. As well, the actual numbers of live beetles present throughout the trial are presented to demonstrate the levels of infestation likely to be present after deployment of the device. The percentage reduction in live beetles was calculated as: 100 x (B-A/B) where B was the mean 'pre-treatment' live beetle count and A was the mean 'post-treatment' live beetle count. If the numbers of dead beetles present are considered together with the numbers of live beetles remaining after the traps had been in use for 28 days, an impression of the potential infestation risk becomes evident.

RESULTS

All trial hives contained beetles prior to placement of the harbourage on the bottom board but only low numbers were present in some hives. The adult beetle population present in a hive at any time comprised both live and dead beetles. In this regard, the mean beetle mortality at the Wilberforce apiary was estimated to be 98% (range 97-99%) after 28 days. By this time the total number of beetles found dead on the bottom board or dead inside the harbourages was 1285. Compared to the pre-treatment counts, the mean number of live beetles in the Wilberforce hives was reduced by about 60% after 7 days, about 80% after 21 days and by 93% at the conclusion of the trial (Table 1). The number of beetles in the control hive remained high throughout the trial interval. No bee deaths or other ill effects were observed.

At the Cranebrook apiary the level of infestation

Figure 1. Prototype cardboard harbourage used in the field trial.

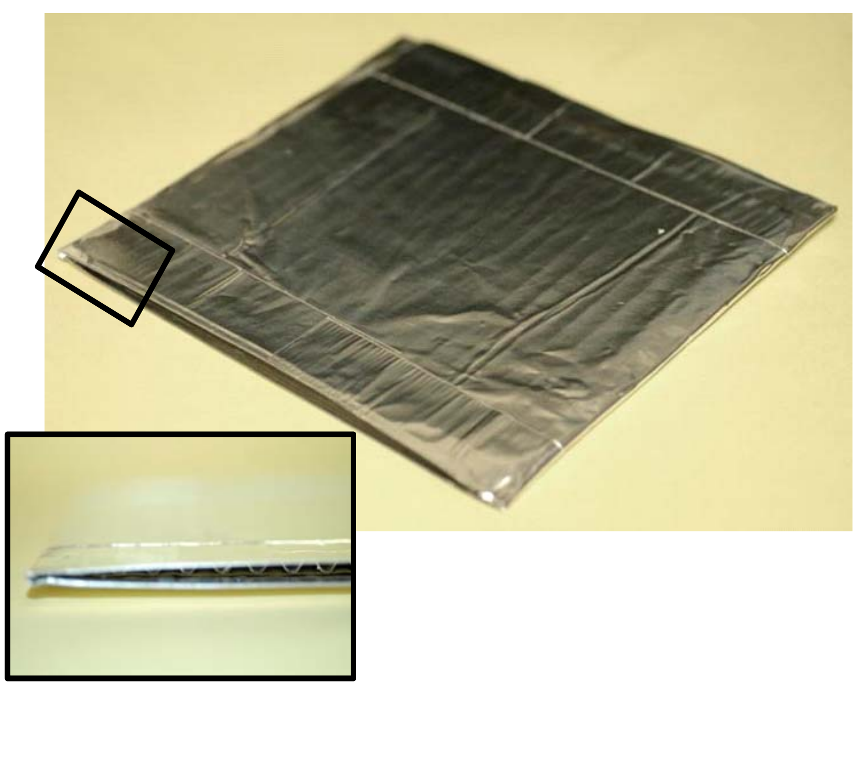


Table 1. Reduction in live adult small hive beetle numbers in commercial hives achieved using the prototype harbourage.

Hive location	Mean no. live beetles in hive pre-treatment[1] (range)	Mean no. live beetles in hive 7 days after placement of the harbourage (range)	Mean no. live beetles in hive 21 days after placement of the harbourage (range)	Mean no. live beetles in hive 28 days after placement of the harbourage (range)
WILBERFORCE				
Control (n=1)	>100	>100	>100	>100
Treated (n=10)	23.2 (8-50)	9.4 (3-22)	4.9 (0-11)	1.7 (0-4)
% reduction[2]	-	59.5	78.9	92.7
CRANEBROOK				
Control (n=2)	15.5 (11-20)	31 (20-42)	37.5 (35-40)	68 (56-80)
Treated (n=10)	7.6 (2-11)	2.8 (0-5)	8.8 (1-33)	4.4 (0-12)
% reduction	-	63.2	-15.8	42.1

1. No. of live adult beetles seen on bottom boards and elsewhere in hive during hive inspection.
2. % reduction in live beetles for this site was calculated as: 100 x (B-A/B) where B is the mean 'pre-treatment' live beetle count and A is the mean 'post-treatment' live beetle count.

increased by more than four fold in the untreated hives during the trial interval. In absolute terms, compared to the mean pre-treatment live beetle count in the treated hives, the mean percentage reduction in live beetles was 63% after 7 days, -16% after 21 days and 42% at the conclusion of the trial (Table 1). At the same time mean beetle mortality across the ten hives was calculated to be 96% but ranged from 84 to 100%. After 28 days the total number of dead beetles seen on the bottom board or found inside the harbourages used in the ten hives was 1212. Several hundred dead bees were observed in and around one treated hive at this site. It became apparent that contact with, and damage done to the cardboard (Figure 2) by bees forcing open the harbourage ends had led to the death of the colony.

DISCUSSION

Estimated mean beetle mortality caused by the deployment of a single treated harbourage on the bottom boards of infested hives exceeded 95% at each apiary. Considerable beetle immigration occurred throughout the trial interval and, typical of small hive beetle infestations, was not uniform across the hives. Consequently, in most hives the total number of dead beetles at the conclusion of the trial far exceeded the pre-treatment counts and varied widely. Almost 2500 dead beetles were removed from the twenty treated hives. More than half of these beetles were found inside the deconstructed harbourages (Figure 3) at the end of the trial. The maximum number of dead beetles in a single harbourage was 215. Although likely to be impressed by seeing large numbers of dead beetles, bee keepers are more prone to deem control acceptable if live beetle numbers were reduced, or remained low, after installation of the harbourages. Live beetle counts at the Wilberforce apiary decreased throughout the trial such that by 28 days there was a 93% reduction in the number of live beetles in the monitored hives (Table 1).

Figure 2. Damage to cardboard insert after bees forced their way into the end of a harbourage.

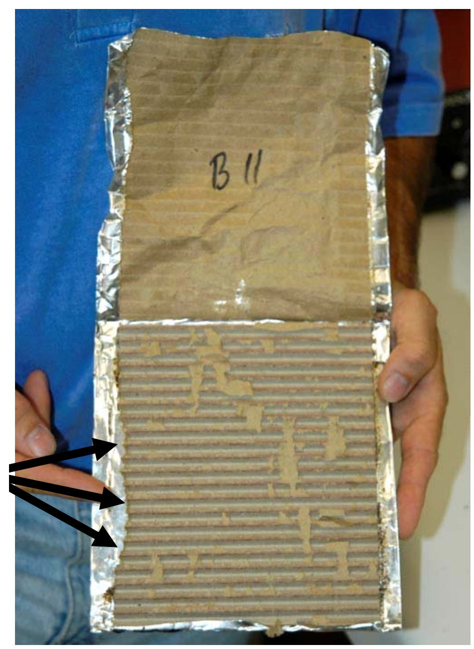
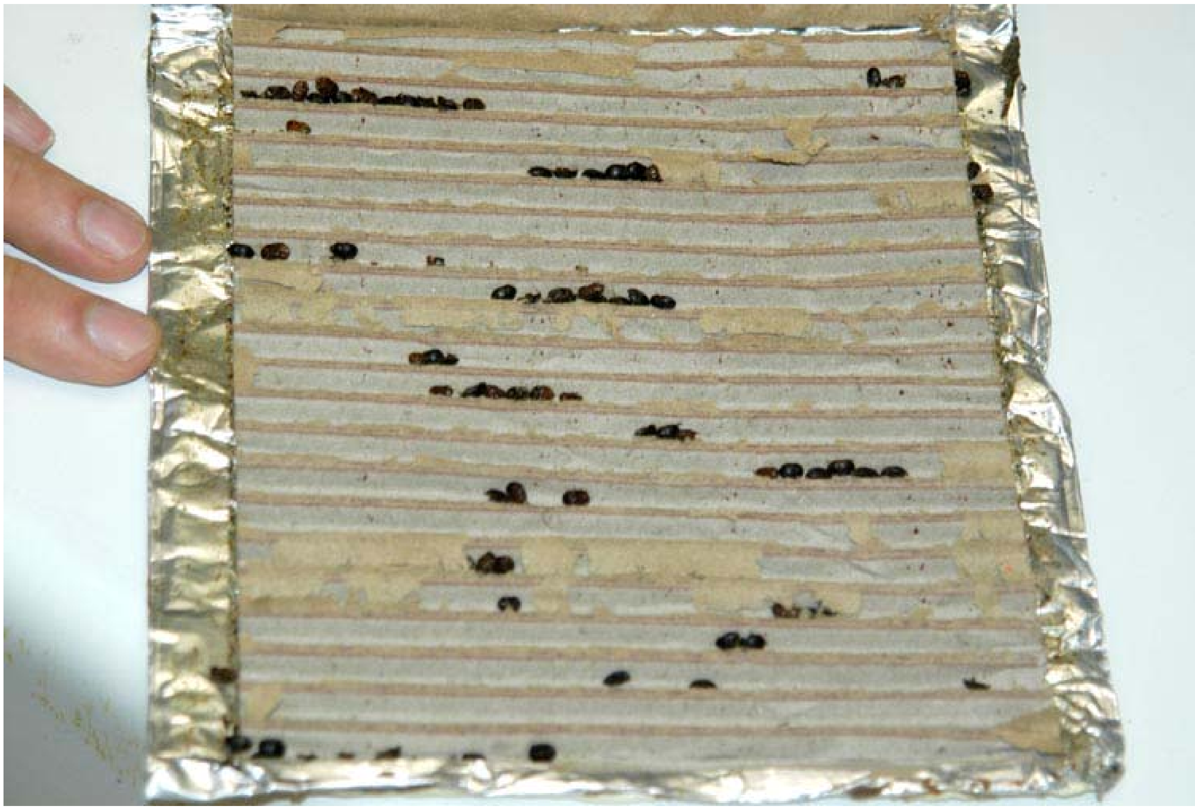


Figure 3. Dead beetles inside a deconstructed harbourage removed from a hive at the completion of the field trial.



At the Cranebrook apiary the changes in mean live beetle numbers in the treated hives fluctuated throughout the trial interval and, compared to the pre-treatment levels, were reduced by 42% after 28 days. At the same time the number of live beetles in the control hives increased by 440% (Table 1). This suggests that the potential infestation in the treated hives would have been much higher had the harbourages not been deployed. At Cranebrook the treated hives were nestled among 57 untreated hives so there was ample opportunity for reinfestation of treated hives by immigration from untreated hives. Recruitment of newly-emerged beetles and migration of beetles from untreated hives into treated hives may have masked a much greater potential reduction in the number of live beetles. This contention is supported by the large decline in beetle numbers achieved at the smaller Wilberforce apiary. Unlike the situation at Cranebrook, here treated hives outnumbered untreated hives ten to one so the impact of beetle migration is likely to have been much less.

No adverse effects were observed at the Wilberforce apiary but one Cranebrook hive was lost when bees forced apart the foil entrance slot and attacked the cardboard insert (Figure 2). Fipronil is extremely toxic to honey bees (Mayer and Lunden 1999) and, although the accusation is contentious, has been implicated as a cause of bee colony losses in France (Chauzat *et al.* 2006). In the trials reported here protection of the bees relied on the low vapour pressure of fipronil (Colliot *et al.* 1992) and on the aluminium foil covering to provide physical separation of bees from the insecticide-treated cardboard insert. The dimensions of the crimped foil harbourage slot entrances aimed to exploit the size difference between bees and small hive beetles and so exclude bees but allow beetles to enter. This approach has proven inadequate, or at least unreliable, as one colony was killed when bees damaged the trap. Failure to protect bees not only places the colony at risk but is likely to lead to contamination of honey. Neither of these things could be tolerated.

The control achieved in these trials suggests that fipronil-treated refuge traps can be effective but need to be more robust to satisfy regulatory requirements for residue free honey and safety to bees. As a consequence of the knowledge gained in these trials a two-piece rigid plastic shell with precisely sized openings that allow beetles to enter but exclude bees is being developed. The intention is that once assembled, the plastic shell will protect the corrugated cardboard insert and position it out of the reach of inquisitive bees. The aim is to produce an effective product whose use will not compromise the quality of honey or any other bee products and that will be safe in the hands of consumers.

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