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ORIGINAL RESEARCH ARTICLE

An insecticidal refuge trap to control adult small hive beetle, *Aethina tumida* Murray (Coleoptera: Nitidulidae) in honey bee colonies.

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Summary

The establishment of the small hive beetle in eastern Australia and its subsequent economic impact on bee keeping prompted research into the insecticidal control of adult beetles inside the hive. A refuge trap comprising a two piece rigid plastic shell encasing a fipronil-treated corrugated cardboard insert was developed. The precise dimensions of the harbourage entrance slots allow beetles to enter but prevent bee access or contact with the insert. Mean fiprole (fipronil plus its toxic metabolites) residues in honey ripened in research hives over one month while the devices were in place did not exceed 1 µg kg^{-1} and no ill effects on the bees were observed. A field trial was conducted at three western Sydney apiaries during autumn 2007 when beetle numbers were naturally increasing. Results demonstrated that deployment of a single harbourage on the bottom board of 26 infested hives caused an estimated 62% overall beetle mortality within 6 weeks and reduced mean live adult beetle numbers by 96%.

Trampa insecticida para el control de adultos del pequeño escarabajo de las colmenas, *Aethina tumida* Murray (Coleoptera: Mitidulidae) en colmenas de abejas

Resumen
El establecimiento del pequeño escarabajo de las colmenas en el este de Australia y el consiguiente impacto en la apicultura ha promovido la investigación sobre el control insecticida de escarabajos adultos dentro de las colmenas. Una trampa consistente en una concha de dos piezas de plástico rígido recubierta de cartón ondulado tratado con fipronil ha sido desarrollada. Las dimensiones precisas de la ranura de entrada a la trampa permiten entrar a los escarabajos pero impiden el acceso y el contacto con el interior a las abejas. Los residuos promedio de fiprole (fipronil junto con los metabolitos tóxicos) en miel madura en colmenas de investigación a lo largo del mes en el que estuvieron puestos los dispositivos no excedieron 1 µg kg-1 y no se observaron efectos negativos en las abejas. Un ensayo de campo se realizó en tres apiarios al oeste de Sydney durante el otoño de 2007 cuando el número de escarabajos aumenta naturalmente. Los resultados demostraron que la colocación de un único dispositivo trampa en el fondo de 26 colmenas infestadas causó una mortalidad de escarabajos media del 62% a las 6 semanas y redujo la vida media de los escarabajos adultos en un 96%.

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

Introduction

The presence of the small hive beetle, *Aethina tumida*, was confirmed in hives at Richmond in western Sydney, Australia, late in 2002 (Fletcher and Cook, 2002). *Aethina tumida* is a native of southern Africa where it is usually considered to be only a minor pest (Lundie, 1940). Primary damage caused by small hive beetle is through the activity of the larvae that feed on brood, pollen and honey causing it to ferment (Lundie, 1940). Stored supers of honey or extracted comb are also susceptible to damage by larvae and adult beetles (Elzen *et al*., 1999). The small hive beetle has established in eastern Australia although initial foci were around Richmond in Sydney's west, Cowra and Stroud (Gillespie *et al*., 2003).

In Australia, the bee industry responded by developing a management plan for the small hive beetle (Animal Health Australia, 2003). Insecticide use was considered to be one of the strategies that could be used against adult and larval beetles. Based on experience in the USA (Hood, 2000), a Pesticide Permit allowing the use of permethrin soil drenches was quickly issued but uptake by apiarists has been poor, perhaps because residual effectiveness is variable (Levot and Haque, 2006a). To control adult beetles, American beekeepers also stapled coumaphos strips (Checkmite+™) normally used to control varroa mites, under pieces of stripped-back corrugated cardboard, inside their hives (Elzen *et al*., 1999; Neumann and Hoffmann, 2008). In Australia the use of coumaphos was considered unnecessarily hazardous to bees and honey but many still believed that an insecticide-based strategy for in-hive control of beetles was essential.

In 2006 we reported results of laboratory bioassays that aimed to identify suitable insecticides for use in a refuge trap (Levot and Haque, 2006b). Fipronil was considered to be superior to several alternative insecticides by having the desirable physicochemical characteristics of extremely low vapour pressure (Colliot *et al*., 1992) and low water solubility as well as excellent contact efficacy against adult small hive beetles (Levot and Haque, 2006b) and no repellent effects. The behaviour of the beetles in laboratory culture (Haque and Levot, 2005) suggested that a refuge trap incorporating corrugated cardboard might be devised for in-hive use. Prototype harbourages comprised of fipronil-treated corrugated cardboard covered with adhesive-backed 50µm thick aluminium foil were tested in the laboratory (Levot and Haque, 2006b) and in the field (Levot, 2008) and were found to be effective in killing adult beetles. In this study the final design of the harbourage is described together with data suggesting the safety and efficacy of the device in field trials conducted in commercial apiaries.

Materials and Methods

Harbourage design

The harbourage is comprised of two black, rigid moulded plastic shells (180 x 150mm) that hold a fipronil-treated 4mm corrugated knife-cut cardboard insert (165 x 130mm) (Australian Corrugated Box Company, Wetherill Park) 10mm back from the 3mm wide entrance slots (Fig. 1). Locating pins and catches in the paired mouldings allow the shells to snap together. They can be permanently joined with glue or ultra-sonic welds to produce a tamperproof device that can be safely handled without fear of contacting the insecticide (Fig. 2). Size differences between the beetles and bees and the precise dimensions of the harbourage entrances prevent bees from contacting the cardboard insert but allow beetles easy access.

For the trials described below the cardboard inserts were dipped in fipronil solution (300mg L^{-1}; 1.5mL of REGENT® 200SC L^{-1} of water) or left untreated as controls. Each card retained approximately 16mL of solution. They were air-dried before being sandwiched between the plastic shells of the harbourages. One hundred harbourages were assembled using plumbers' PVC plastic pressure pipe cement (Type P Plumbers Mate™ PVC-U Pipe Cement) to permanently bond the plastic shells. A 50cm long retrieval wire was attached via one of two small holes near the edge of the plastic housing.

Fig 1. The components of the small hive beetle harbourage prior to assembly.

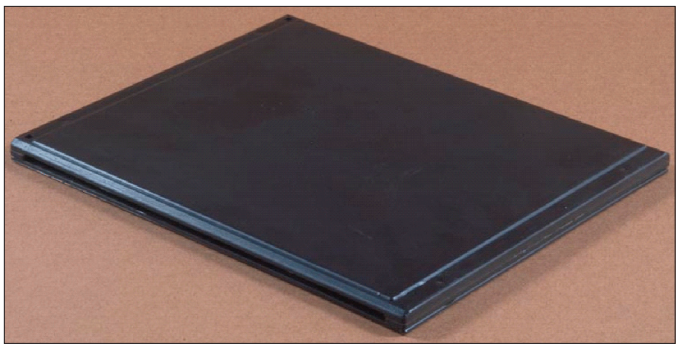

Fig 2. The assembled small hive beetle harbourage.

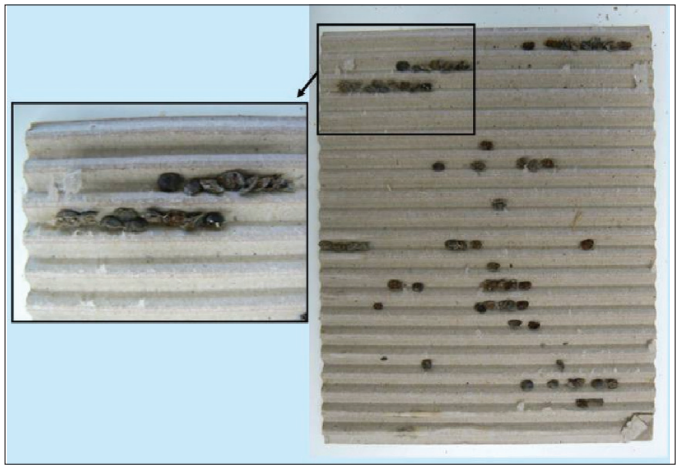

Fig 3. Dead beetles inside a deconstructed harbourage removed from a hive at the completion of the field trial.

Safety to bees and honey

Five research hives located at Belgenny Farm, Camden in Sydney's south west were used in a honey residue trial. The trial began on 12 September (early spring) 2006 when ambient temperatures and flowering favoured bee activity and honey production. In accordance with Australian Pesticides and Veterinary Medicines Authority (APVMA) Guideline 28 Residues in honey (APVMA, 2001) two central frames from the middle super of each hive were removed, the cells on each frame uncapped and the honey extracted using a manual bench-top extractor. Honey from each hive was bulked into a 10 L plastic drum and homogenised by vigorous shaking before 200 mL sub-samples were poured into labelled clean glass jars and placed into frozen storage. Drawing pins were pushed into the frames so that they could be easily identified later and the frames were then placed back into the hives. At the same time a single harbourage was placed on the bottom board of each hive. One month later, the same frames were again removed. In accordance with the Guideline the honey extracted from the ten frames was bulked together and mixed as before. Five sub-samples of this honey were transferred into labelled clean glass jars which were then placed into a freezer. The frozen sample jars were sent to AgriSolutions Australia Pty. Ltd. (Deception Bay, Queensland 4508) as coded samples for total fiprole analysis.

Field efficacy trial

On the basis of favourable honey residue results a Category 23 (Research Permit) permit (PER9732) was granted by the APVMA in February 2007 with no restriction on the number of hives that could be used. Field trials began in March (early autumn) at apiaries located at South Maroota, North Richmond and Wilberforce in Sydney's western suburbs. These apiaries comprised 35, 44 and 10 hives respectively. Pre-treatment hive inspections were conducted at each apiary. At the South Maroota and North Richmond sites, 12 hives with suitable numbers of beetles were selected to be monitored and beetle counts recorded. Pre-treatment beetle counts were recorded in all 10 hives at the Wilberforce apiary but during the trial period three treated hives were lost to American Foul Brood and were not considered in the analysis.

The number of beetles was determined by opening the hives and counting the numbers of live adult beetles on the bottom boards, frames of bees and lid. In hives consisting of more than one box, the lid was removed and placed upturned on the ground. The super was placed on top of the upturned lid. The frames were individually removed from the bottom box, thoroughly inspected and placed beside the bottom box. A hive tool or 75mm wide metal spatula was drawn slowly across the bottom board to move bees and disturb beetles that were harbouring within the bottom space. The frames and super were replaced and the lid inspected for beetles that had moved from the super during inspection to escape the light. Two people counted beetles. Overwhelmingly, most beetles were found on the bottom board of the hives. Beetle numbers were only low to moderate and we were confident that quite accurate counts were obtained without the need to remove and replace beetles during this process. After the pre-treatment inspection, only the monitored hives were disturbed in this way. For the remaining (non-monitored) hives interference amounted to no more than the use of smoke to calm the bees when inserting or retrieving the harbourage via the hive entrance.

A single harbourage was placed on the bottom board of each of the hives at the three apiaries. The retrieval wire was left protruding from the hive entrance to facilitate recovery of the harbourages. Two of the monitored hives at South Maroota and another two at North Richmond acted as controls. A single hive at the small Wilberforce apiary was a control. A harbourage containing an untreated cardboard insert was placed on the bottom board of each control hive. Control hives were located near the outermost ends of each apiary in an attempt to reduce the opportunity for beetles to move from untreated hives into neighbouring treated hives, but this precaution could not mitigate that likelihood, or of possible immigration of beetles from outside of the apiaries.

Four and six weeks after harbourage placement, the numbers of live beetles were recorded as before. At the same time the numbers of dead beetles seen in the hives were recorded and all dead beetles removed. Immediately following the six week inspections the harbourages in the monitored hives were removed, placed into individual labelled sealable plastic bags and brought back to the laboratory. Here they were broken open, the cardboard peeled back and the number of dead beetles inside (Fig. 3) counted. The aggregate numbers of dead beetles removed during the four and six week inspections together with the numbers of dead beetles inside the harbourages were recorded. These figures may not represent the total number of beetles killed by the treatments as bees may have removed some dead beetles from the hive.

Statistical analysis

The pre-treatment live beetle counts represented the starting populations in each hive. Beetle immigration estimated from the mean increase of live beetles in the control hives after six weeks was used to calculate the potential target beetle population present in each hive. The aggregate dead beetle counts were compared with the calculated number of beetles estimated to be present at this time. If the aggregate dead beetle count was higher than the calculated number of beetles present, they were considered equal. 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 effects.

Percentage reductions in the mean number of live beetles present in the hives at the four and six week inspections were calculated using the formula recommended by Henderson and Tilton (1955) namely: % reduction = $100 \times (1 - ((C_0/T_1) \times (C_1/T_0))$ where C_0 and T_0 are the mean pre-treatment live beetle counts in the Control and Treated hives and C_1 and T_1 are the mean post-treatment live beetle counts in the control and treated hives respectively.

Results

Honey residue trial

The bees stored approximately 9.4 kg of honey in the ten 'trial frames' during the month. The mean total fiprole content (i.e. the sum of fipronil and its three toxic metabolites) in the bulked samples was below the Limit of Quantification (LOQ - 1.0 µg kg^{-1}). No residue of either fipronil or any of its metabolites was detected in three of the sub-samples from the treated hives. Two sub-samples were reported to contain 1.1µg kg^{-1} of metabolite MB 46136. The total fiprole content in each of the five pre-treatment sub-samples was below the LOQ.

Field efficacy trial

The mean pre-treatment beetle count in the hives assigned to the control group was 15.4 ± s.e. 7.7 (n=5) but ranged from 2 to 45. Beetle numbers increased in the control hives at each site such that at the 4 and 6 week's inspections the mean number of live beetles present were 56 and 50 respectively. Infestation levels between hives remained variable with counts ranging from 3 to 133. No beetle mortality was recorded in the control hives (Table 1).

The mean pre-treatment beetle count in the hives assigned to the treatment group was 25.9 ± s.e. 4.3 (n=26) but like the controls, ranged from 2 to 100. The mean number of live beetles present in the treated hives at the four and six week inspections

Table 1. Live and dead beetle counts and estimated percentage small hive beetle mortalities in 26 western Sydney hives containing fipronil-treated harbourages.

Hive	Pre-treatment live beetle count	Live beetle count at the 4 week's	Live beetle count at the 6 week's inspection	No. dead beetles inside harbourage after 6 weeks	Aggregate[1] dead beetle count	Estimated percentage mortality[2]
Control 1	6	4	3	0	0	0_{k}
Control 2	8	36	27	0	0	0_{k}
Control 3	16	37	52	0	0	0_{k}
Control 4	45	133	129	0	0	0_{k}
Control 5	2	70	41	0	0	0_{k}
Treated 1	5	0	0	0	0	0_{k}
Treated 2	12	1	0	2	2	4.3_{j}
Treated 3	8	0	0	2	2	4.7_{ij}
Treated 4	31	2	2	7	14	21.2_{hij}
Treated 5	5	4	6	9	10	25.0_{ghij}
Treated 6	24	14	1	6	15	25.4_{ghi}
Treated 7	23	1	1	7	15	25.9_{ghi}
Treated 8	48	4	0	4	25	30.1_{gh}
Treated 9	33	6	4	4	25	36.8_{fgh}
Treated 10	22	13	9	17	22	38.5_{efgh}
Treated 11	30	0	0	23	25	38.6_{efgh}
Treated 12	8	0	1	2	18	41.8_{defgh}
Treated 13	16	0	5	18	26	51.0_{cdefg}
Treated 14	2	21	6	13	22	56.0_{cdef}
Treated 15	65	30	9	29	56	59.5_{cdefg}
Treated 16	38	4	5	34	45	61.6_{cde}
Treated 17	47	7	7	32	53	64.6_{cd}
Treated 18	24	7	3	38	40	67.8_{cd}
Treated 19	8	1	2	30	33	76.7_{bc}
Treated 20	29	1	4	27	57	89.1_{b}
Treated 21	5	3	1	38	46	100_{a}
Treated 22	36	4	19	89	144	100_{a}
Treated 23	23	0	0	38	90	100_{a}
Treated 24	28	9	3	20	70	100_{a}
Treated 25	3	2	2	12	59	100_{a}
Treated 26	100	28	7	164	171	100_{a}
Overall				**665**	**1085**	**62**

[1] No. dead beetles removed from hives throughout the trial period plus the no. of dead beetles found inside the harbourages at the end of the trial.

[2] Figures followed by the same letter were not significantly different.

were 6.2 ±1.7 and 3.7 ± s.e. 0.8 (n=26) respectively. With allowance for the increase in beetle numbers in the control hives, these represent reductions of 93 and 96% compared to the pre-treatment counts. At the completion of the trial, live beetles could not be found in six of the 26 monitored treated hives, and 19 hives contained five or fewer beetles. At the same time migration increased beetle numbers in the control hives by more than three-fold such that the mean live beetle count exceeded 50 (Table 1). For statistical purposes the mean migration rate seen in the control hives was assumed to occur uniformly across the treated hives. Comparisons between the actual numbers of dead beetles found in the hives or inside the harbourages, with the calculated potential target beetle populations suggested that use of the harbourages caused 62% overall mortality. In line with the large variation in the number of dead beetles recorded for individual hives estimates of beetle mortalities also varied widely but, with the exception of Hive 1, were significantly different to those for the control hives (Table 1).

Discussion

Beetles readily sought refuge in the harbourage and were killed by contact with the fipronil treated cardboard insert. No deleterious effects on bees were observed and the hives thrived during the time the harbourages were deployed. Compared to control hives, the installation of the harbourage onto the bottom board of bee colonies significantly reduced the number of live small hive beetles in hives by 96% across the three western Sydney apiaries. The effectiveness of the harbourages was obvious at the completion of the trial when no, or only a few live beetles remained in the hives. It was clear that mortality of beetles rather than any other influences such as beetle emigration from treated hives was responsible for this level of control. Beetle mortality was directly attributable to the deployment of the harbourages as the aggregate number of dead beetles recorded at the completion of the trial was 1085 with 665 of these found inside the deconstructed cardboard inserts removed from the trial harbourages (Table 1).

The three-fold increase in the number of small hive beetles infesting the control hives during the course of the six week study was attributed to beetle immigration and could not be ignored when estimating the effect of the harbourages on small hive beetle populations. The migration of beetles posed some challenges in estimating the beetle populations of individual hives that were potentially being targeted by the harbourage treatment. Moreover, allowance for this immigration was necessary if the numbers of dead beetles recorded in the hives were to be included in the calculations used to estimate percentage mortality. This was because many more beetles were killed during the course of the trial than were present at the pre-treatment assesments. It was assumed that the same mean rate of immigration seen in the control hives also occurred in the treated hives and that the potentially targeted population for each hive was an estimate based on this rate of immigration imposed on the pre-treatment beetle count. The assumption that beetle migration was uniform may be too simplistic. The typically uneven distribution of beetles in the hives (Neumann and Elzen, 2004) prior to placement of the harbourages (Table 1) probably reflects their susceptibility to beetles. Hives are known to vary in their attractiveness to beetles, perhaps due to odours associated with the presence of a yeast (*Kodamaea ohmeri*) (Torto *et al*., 2007; Benda *et al*., 2008) emanating from susceptible hives. As a consequence of assuming beetle immigration was uniform but knowing that hive susceptibility varies, the estimates of the potential beetle populations in individual hives may have under-, or over-estimated the true numbers. Uncertainty regarding the absolute precision of the beetle counts (Neumann and Hoffmann, 2008) further confounds the situation and moreover, it is unlikely that the sum of the dead beetles removed during the four and six week inspections and those retrieved from inside the harbourages at the end of the trial were the only beetles killed by the treatment. These numbers do not take account of any dead beetles that bees may have ejected from the hives and gone undetected. This unknown component, together with differences that might have occurred because of non-uniform beetle immigration across the treated hives, may account for the discrepancy between the estimates for overall beetle mortality (62%) and the reduction in the number of live beetles (96%). Nevertheless, by either measure, substantial control of infestations was achieved. From a practical standpoint, beekeepers are likely to be encouraged by the sight of large numbers of dead beetles, but form their opinion on the effectiveness of the harbourage by the number of live beetles remaining in their hives. In this regard the results presented here suggest that they could expect to see around 95% reduction in live beetles within four to six weeks.

The proposal to use fipronil in this way has not been without controversy. The encapsulation of the insecticide treated cardboard insert in the plastic shell of the harbourage means that compounds other than fipronil could be substituted should this be deemed necessary. Not all insecticides are suitable, and only those with similar efficacy and physicochemical attributes as fipronil should be considered as alternatives. As well as having excellent efficacy against small hive beetle adults in laboratory bioassays (Levot and Haque, 2006b) and field situations (Table 1) fipronil has extremely low vapour pressure (Colliot *et al*., 1992), low water solubility and non-repellent attributes that suit its use in an in-hive refuge trap. Concerns about the use of fipronil have arisen because *Apis mellifera* is extremely sensitive to fipronil (Mayer and Lunden, 1999) and because fipronil residues have been detected in pollen and nectar from sunflower and maize plants developing from treated seed (Aajoud *et al*., 2006). Fipronil residues have been implicated as a cause of bee colony losses in France (Chauzat *et al*., 2006) and sublethal doses of fipronil were reported to cause reduced foraging (Colin *et al*., 2004) and poor olfactory learning behaviour in honey bees (Decourtye *et al*., 2005; El Hassani *et al*., 2005). Not surprisingly, there is some sensitivity to the proposal to use fipronil inside hives, but our results suggest that the harbourage design and proposed use pattern mitigate residue concerns. Residues of fipronil and its toxic metabolites in honey collected while the harbourages were in place were reported as either being undetectable (less than the LOQ) or a maximum of 0.1 µg kg^{-1} above the LOQ. In accordance with Australian regulatory guidelines these samples were decanted from the bulked honey extracted from the five treated hives. The bulked honey had been spun, poured into a clean 10 L plastic container and shaken to ensure homogeneity.

There is no reason to expect that there would be residue differences between sub-samples of a homogenised commodity taken from the same vessel. Rather than true low-level contamination of the honey, the minute residue reported for two of the sub-samples is considered to be signal 'noise' at the limit of quantification of the gas chromatograph. However, even if the results were real, the level reported is at least an order of magnitude lower than most Maximum Residue Limits for fipronil in foods.

The harbourage was convenient to use. For trial purposes it was necessary to disassemble the monitored hives to obtain beetle counts but for the hives that were not monitored the harbourages were simply 'posted' through the front hive entrance slot and reclaimed by pulling the retrieval wire. To ensure the harbourage sits flat on the bottom board there would always be an advantage in opening the hive to scrape clean the bottom board and position the harbourage for optimal performance but incorporation of the retrieval wire means that there can be no excuse for not removing the harbourages when control has been established or when cool weather lessens the threat of small hive beetle infestation. Service life for the harbourage of at least twelve months is anticipated but stability trials to determine the likely shelf-life and service life of the product are not yet completed. The device was the culmination of considerable effort that aimed to achieve a bee-proof protective covering for the insecticide-treated cardboard insert. The assembled rigid acrylic plastic product resists distortion and is very robust. The design ensures bees cannot access the fipronil-treated cardboard insert and prevents user access as well. Any malicious attempt to open the assembled harbourage results in obvious damage. Beetles harbour in the device out of choice even when bees are not present but there can be little doubt that it provides refuge from bee harassment when deployed in bee colonies. Intuitively, the harbourage will be most effective when beetle numbers are highest as presumably, alternative refuge sites inside the hive will already be occupied. In this trial up to 164 beetles were recovered from inside the harbourage retrieved from the hive that was most heavily infested at the pre-treatment inspection (Table 1) and similarly 215 were removed from a prototype device installed in the most heavily infested hive in an earlier trial (Levot, 2008). With no apparent adverse effect on bees or honey arising from the deployment of the harbourages in bee colonies and given the demonstrated effectiveness of the device in killing beetles and controlling infestations, the use of this refuge trap could provide beekeepers with another strategy to manage small hive beetle.

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