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Commercialisation of the Small Hive Beetle Harbourage Device

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by Dr Garry Levot

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Foreword

Small hive beetle continues to impact on the profitability of honey production in Australia by destroying hives and spoiling produce. Since its discovery at Richmond in Sydney's west in 2002, small hive beetle has extended its range along the eastern seaboard and into inland areas of New South Wales and Queensland. A previous RIRDC research project conducted by the author demonstrated the feasibility of using a fipronil treated harbourage for the control of adult small hive beetles in the honeybee colonies. The project reported here built on the results of that previous research by bringing the product to market.

The small hive beetle harbourage was recognised as a product likely to be attractive to at least some companies involved in developing and registering pest control products. A commercial partner with capabilities in product manufacture, sales and distribution was needed if the device was to become available to beekeepers. Patent protection in Australia was essential if a commercial partner was to be attracted. If overseas patents could also be obtained the potential market, particularly in the United States of America, would be an additional incentive.

Through this project the harbourage, now marketed by Ensystex Pty. Ltd. under the tradename APITHOR™ has been shown to be both safe and effective when used in commercial bee colonies. Honey ripened while APITHOR™ was in place contained no detectable fipronil residues and there were no significant differences in key indicators of hive health in 'control' and APITHOR™ -treated hives. Beekeepers should feel confident that the use of APITHOR™ will not have any deleterious effects on their bees, honey quality or hive productivity but will significantly reduce adult small hive beetle populations in their hives if used as directed on the product label.

The importance of this report is that it demonstrates that the investment by RIRDC in a preliminary feasibility project was rewarded by the commercialisation of a product that is directly accessible and usable by Australian beekeepers. The risk associated with RIRDC's co-investment of $97,815 along with that provided by NSW Primary Industries ($107,451) in the current project was shared by the involvement of a commercial partner who has responsibility to manufacture and distribute the product. Moreover, a proportion of the revenue generated by sales of the product will be returned to the research partners in royalties that can be reinvested in other projects to benefit Australian beekeepers.

This report is an addition to RIRDC's diverse range of over 2000 research publications and it forms part of our Honeybee R&D program, which aims to improve the productivity and profitability of the Australian beekeeping industry through the organisation, funding and management of a research, development and extension program that is both stakeholder and market focused.

Most of RIRDC's publications are available for viewing, free downloading or purchasing online at www.rirdc.gov.au. Purchases can also be made by phoning 1300 634 313.

Craig Burns
Managing Director
Rural Industries Research and Development Corporation

Acknowledgments

I thank Dr. Doug Somerville (NSW Primary Industries) for his invaluable expertise in managing the bee hives and assistance with the safety, residue and field efficacy trials reported here. I thank Dr. Idris Barchia (NSW Primary Industries) for conducting the statistical analysis and Mr. James Caldwell (NSW Primary Industries) for assisting with field work. I thank the Wheen Foundation Directors for allowing me to conduct the efficacy trial at their facility at Richmond and the Australian Queen Bee Exporters company for donating the package bees to stock the hives for the efficacy study. I acknowledge the support and encouragement provided by Dr. David Dall and Ms. Margie Thompson (both formerly of RIRDC). I thank Mr. Russell Martin (NSW Primary Industries) for his assistance handling commercial and patent agreements and Mr. Steve Broadbent (Ensystex Pty. Ltd.) for his wisdom in seeing the commercial potential of my device.

Abbreviations

APVMA	Australian Pesticides and Veterinary Medicines Authority

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Executive Summary

What the report is about

This report describes the commercialisation of the APITHOR™ small hive beetle harbourage and the results of bee safety, honey residue and field efficacy trials conducted to support full product registration of the device by the Australian Pesticides and Veterinary Medicines Authority (APVMA).

Who is the report targeted at?

The report is written for beekeepers and advisors to the honeybee industry.

Where are the relevant industries located in Australia?

Small hive beetle is currently found in New South Wales, Queensland and Western Australia but may exist, or from time to time be inadvertently taken into, Victoria or South Australia in hives. The current APVMA Minor Use Permit (PER12007) for APITHOR™ small hive beetle harbourage allows general use in New South Wales, the Australian Capital Territory, Queensland, Western Australia, South Australia and Victoria. Beekeepers from these States or Territory whose enterprise is impacted by small hive beetle will benefit from the outcome of this project by having access to a safe and effective device for the control of beetles in their hives.

Background

Previous RIRDC research, reported in *Insecticidal control of small hive beetle* (Levot 2007), developed and successfully field trialled a small hive beetle harbourage that comprised a two piece, tamperproof plastic housing for a fipronil-treated corrugated cardboard insert. The device needed to be patented, commercialised, registered with the APVMA and available to Australian beekeepers at a reasonable price.

Aims/objectives

This project aimed to bring to market the small hive beetle harbourage device developed during the feasibility project, *Insecticidal control of small hive beetle* (Levot 2007) through establishment of a team that included a commercial manufacturer and an experienced regulatory affairs consultant. The aim was to market the device under permit whilst collecting additional residue, safety and efficacy data to satisfy registration requirements as set out by the APVMA.

Methods used

Opportunities to commercialise the device were pursued by Expression of Interest. After the Exclusive Licence Agreement with the commercial partner was in place Ensystex Pty. Ltd. began manufacture of the small hive beetle harbourage in Thailand. Bee safety, honey residue and field efficacy trials using harbourages containing cardboard inserts treated with Ultrathor Water-based Termiticide (100g fipronil L^{-1} Ensystex Pty. Ltd.; APVMA Registration No. 64449; Batch no. J-140-2; Date of Manufacture - July 2010, Ensystex Pty. Ltd.) were conducted in accordance with APVMA Guidelines (if they existed, e.g. APVMA ***Guideline 28 Residues in Honey***) or according to protocols developed in consultation with industry specialists.

Results/key findings

Research into the insecticidal control of adult small hive beetles culminated in the development of an insecticidal refuge trap for deployment inside commercial bee colonies. The device (APITHOR™) is comprised of a two piece rigid plastic shell encasing a fipronil-treated corrugated cardboard insert.

Comparison of key hive health parameters (frames of bees, area of brood and weight of honey produced) between 'control' and APITHOR™-treated hives demonstrated no significant differences over a six week trial interval. Mean fiprole (fipronil plus its toxic metabolites) residues in honey ripened while the devices were in place did not exceed the limit of quantification (1 $\mu g\ kg^{-1}$). In a 36 day long field trial conducted in a beetle infested apiary at Richmond in Sydney's west, live adult beetles were eliminated from hives containing APITHOR™ while beetle numbers increased by approximately 20% in co-located control hives. With this level of effectiveness and with no apparent adverse effects on bees and no detectable residues in honey arising from the deployment of APITHOR™ harbourages in bee colonies, beekeepers should feel confident that use of this new device as directed on the product label to control small hive beetle in their hives will not compromise their produce or threaten the health of their bees.

Implications for relevant stakeholders for:

The project has succeeded in bringing the small hive beetle harbourage to market. Ensystex Pty. Ltd. was enlisted as the preferred commercial partner and is manufacturing APITHOR™ in its Thailand facility and selling via a dedicated website (https://apithor.com.au) or by telephone order (133536). Since 29th September 2010 (and until 30th June 2012) APITHOR™ has been available for general use by beekeepers under APVMA Minor Use Permit (PER12007) but it is expected that the additional data on the safety to bees, honey and wax and effectiveness in field trials generated during this project will be adequate to satisfy APVMA registration requirements. An application to register APITHOR™ was submitted to the APVMA on the 18th July 2011 (Application Number: 54227; Product Number: 66708) and at time of writing (11th August 2011) was undergoing preliminary assessment. Australian beekeepers now have a safe and highly effective tool to control small hive beetles in their hives. A condition of the Exclusive Licensing Agreement with Ensystex Pty. Ltd. is that a royalty will be returned to RIRDC and NSW Primary Industries from sales of APITHOR™. Royalty funds will be available for reinvestment in bee related research. This condition ensures that the project will contribute an on-going benefit to the honey bee industry.

Recommendations

In APITHOR™ Australian beekeepers now have a legal, affordable, safe and effective product available to them to control small hive beetles in their hives.

Patent protection of APITHOR™ small hive beetle harbourage in the United States of America potentially opens up additional markets for the device. When issues relating to the international patents for fipronil and its production are resolved, consideration should be given to registering APITHOR™ in the USA and in other countries where small hive beetle is a pest.

Introduction

Previous RIRDC research reported in *Insecticidal control of small hive beetle* (Levot 2007) developed and successfully field trialled a small hive beetle harbourage that comprised a two piece, tamperproof plastic housing for a fipronil-treated corrugated cardboard insert. Early field testing of the harbourage in naturally infested commercial hives was very encouraging. 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. In trials conducted in three western Sydney apiaries, compared to control hives, the number of live small hive beetles was reduced by up to 96% in hives in which a single harbourage had been placed on the bottom board. 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 (Levot 2008a).

In 2008 the device was granted patent protection in Australia and New Zealand. It was considered that the domestic market alone would be attractive to a commercial partner but, in addition, in 2010 patent protection in the United States of America was granted as well.

Following advertisement in the Sydney press (26th May 2009) and email or postal contact with potential partners for Expressions of Interest in commercialising the small hive beetle harbourage Ensystex Pty. Ltd. Australasia was contracted to commercialise the product. Subsequently Ensystex and the project partner organisations entered into an Exclusive Marketing Rights Agreement. Ensystex was well advanced in development of other fipronil based products and has extensive experience in insecticide product manufacture, marketing and distribution. Ensystex has an international profile with manufacturing capability in Thailand.

Considerably more data was needed to support development of the product. Advice from the APVMA was that new safety, residue and efficacy data was needed and that the trials needed to be conducted with the final End-Use-Product. NSW Primary Industries was granted an APVMA Research Permit (PER11184, Appendix 1) until 30th June 2011 allowing specified staff to use Ultrathor Water-based Termiticide (100g fipronil L^{-1}; Ensystex Pty. Ltd.) treated harbourages in a total of 100 hives at up to five sites in NSW and Queensland.

This report details the commercialisation of the small hive beetle harbourage named APITHOR™ by Ensystex Pty. Ltd. and includes information on product manufacture as well as the results of residue, safety and field efficacy trials that are included in the submission to APVMA to register APITHOR™.

Objectives

This project aimed to bring to market the small hive beetle harbourage device developed during the feasibility project, *Insecticidal control of small hive beetle* (Levot 2007), by establishing a team that included a commercial manufacturer and an experienced regulatory affairs consultant. The aim was to market the device under permit whilst collecting additional residue, safety and efficacy data to satisfy registration requirements as set out by the APVMA.

Methodology

Commercial partner and manufacture

To be attractive to any potential commercial partner patent protection of the small hive beetle harbourage was essential. With the assistance of FB Rice and Co. applications for patent protection in Australia, New Zealand, the United States of America and Canada were submitted to the appropriate patent offices.

A manufacturer for the device was sought by Expression of Interest. Newspaper advertisement was supplemented by direct email or postal contact with prospective partners. Several key attributes were identified as being essential requirements in the successful partner. They needed to have demonstrated their capacity to register pest control products and have access to fipronil. The latter had proved problematic due to patent restrictions. The successful applicant would produce, package and sell the harbourages with the project assisting where appropriate.

An experienced regulatory affairs consultant was enlisted to the project team. It was agreed that familiarity with the requirements and processes of the APVMA would facilitate the issuing of permits and later, the preparation of a registration dossier for the product.

Field residue, safety and efficacy trials

Residue, safety and field efficacy trials were conducted according to published Guidelines if they existed, or in carefully designed controlled experiments conducted in accordance with the conditions of APVMA Research Permit PER11184 (Appendix 1).

For each of the trials APITHOR™ small hive beetle harbourages (Figures 1 and 2) were manufactured in the Ensystex Pty. Ltd. facilities in Thailand. This included the treatment of batches of cardboard inserts by immersion in an aqueous fipronil solution (300 mg L^{-1}) (Ultrathor Water-based Termiticide, 100g fipronil L^{-1} Ensystex Pty. Ltd.; APVMA Registration No. 64449; Batch no. J-140-2; Date of Manufacture - July 2010, Ensystex Pty. Ltd.). Quality control checks performed by an independent laboratory confirmed that the fipronil content of the cardboard inserts fell within specification. Boxes of cellophane wrapped harbourages (Batch no. ENS001-0810; Date of Manufacture - August 2010) were transported to Menangle where wire lanyards were attached to individual harbourages in preparation for deployment in the hives.

Figure 1 APITHOR™ small hive beetle harbourage.

Figure 2 APITHOR™ small hive beetle harbourage prior to final assembly and sealing.

Residue trial

This trial was conducted at an apiary (Figure 3) near Cootamundra, NSW beginning in November 2010 and running for six weeks. The apiary had a low and variable natural infestation of small hive beetles that was supplemented by introducing 50 adult beetles to each trial hive on day -1. The trial was conducted according to the principles outlined in the APMVA Residue Guideline No. 28

Residues in Honey (APVMA 2001). Six hives selected on the basis of their similar health status and strength, were assigned to the trial. Each was assessed for the number of frames of bees, hive weight and the area of brood. To assess brood area a frame containing a 5 x 5 cm grid was overlain on the individual frames containing brood and the number of squares with brood recorded (Figure 4). This number was converted to square centimetres of brood by multiplying by 25. Before weighing the hives, a single APITHOR™ harbourage was installed on the bottom board of each hive (Figure 5) and two central frames were removed from the super and replaced by new foundation. This ensured that honey subsequently removed from these frames had been collected and ripened while the harbourage was in place. Each hive was moved on a trolley to a mobile weighing platform that comprised a pair of Ruddweigh™ load bars and a digital display (Figure 6). After weighing each hive was moved back to its respective position within the apiary.

Figure 3 The Cootamundra apiary where the residue and safety trials were conducted.

Figure 4 **Estimating the brood area on a hive frame.**

Figure 5 **APITHOR™ installed on the bottom board of one of the hives used in the residue trial.**

Figure 6 Weighing the hives.

In accordance with the APVMA Guidelines (APVMA 2001) the trial was conducted during a rich honey flow. Paterson's curse was flowering prolifically in the Cootamundra area and bees remained active throughout the six weeks long trial. After this time the hives were re-assessed for the same indicators of hive health (frames of bees, hive weight and area of brood) as before. During the trial period when honey productivity was very high, some hives needed to be re-supered to accommodate the stores of honey. Supers of known weight were used so that comparison of the pre- and post-treatment weights of the hives (which approximated the yield of honey) could include a correction for the weight of the added empty supers. At the completion of the trial the same two central frames that had been installed in each super but now with drawn comb and full of honey, were removed from the six hives, uncapped and extracted using a manually driven, three-frame rotary extractor (Figure 7).

Figure 7 Extracting the honey for residue analysis.

In accordance with the APVMA Guidelines (APVMA 2001) the honey from the twelve frames was pooled. Subsequently six sub-samples were poured into clean, labelled glass jars and frozen. Wax from the cappings that floated on top of the honey was also collected into sample jars. The jars containing the honey and wax were then frozen prior to despatch to AgriSolutions Pty. Ltd. at Deception Bay, Queensland as coded samples. AgriSolutions conducted fiprole extractions of sub-samples from each jar by dissolution in hot water followed by liquid/liquid partitioning with dichloromethane after cooling. The extracted liquids were passed through a 0.45 µm PTFE filter prior to analyses for total fiprole (fipronil and toxic metabolites) via GC/MS/MS.

Safety trial

This trial was conducted concurrently with the residue trial above and at the same apiary. Twenty hives assessed as being similar in terms of health status and strength as the six hives in the residue trial (described above) were selected. Ten were randomly allocated to the untreated control group and ten to the APITHOR™ treatment group. Fifty adult beetles were introduced to these hives to supplement the low-level natural infestation. The six residue trial hives were also included making a total of 16 APITHOR™ treated hives. As before, detailed measurements of the number of frames of bees, brood area and hive weight were recorded for each hive. A single APITHOR™ harbourage was placed onto the bottom board of the treated hives. A harbourage containing an untreated cardboard insert was installed on the bottom boards of the control hives. Six weeks later the same hive parameters were re-assessed. Again, some of the 'safety' trial hives needed to be re-supered to accommodate the stores of honey. As before, supers of known weight were used so that comparison of the pre- and post-treatment weights of the hives could include a correction for the weight of the added empty supers.

Statistical analysis: Data (increase in the number of frames of bees, changes to brood area and hive weight increase) were analysed using the conventional analysis of variance. The F probability was used to determine whether there were significant differences between treatment means. Data (live beetle number and dead beetle number) were fitted with a generalized linear model and the square root was used as the link function to relate the observed values and the treatment effects. F values were calculated to compare treatment effects.

Efficacy trial

This trial was conducted at an apiary (Figure 8) at Richmond, NSW. In 2002 Richmond was the site of the initial discovery of small hive beetle in Australia (Fletcher and Cook 2002) and has maintained a high endemic population of beetles ever since. Thirty new, lightly beetle infested, single box hives with sister queens and similar worker bee numbers were transported to Richmond two weeks prior to the commencement of the trial. The bottom boards had been painted white to facilitate the counting of beetles. Seasonal conditions were not ideal for the bees with few nearby plants flowering during the trial interval. For the duration of the trial each hive contained a syrup (100 g L^{-1} sucrose solution) feeder in place of the terminal frame in the brood box to provide supplementary nutrition for the bees. The hives were arranged in a single line and oriented to face north. One week before the trial commenced the hives were checked and bee numbers manipulated to make the hives as similar as possible in terms of strength. During this preparatory phase, beetle numbers within the hives increased by immigration from the immediate vicinity.

Figure 8 The Richmond apiary where the field efficacy trial was conducted.

On 23rd March 2011 beetle numbers in the hives were deemed adequate (13- 41 per hive) and, based on experience from earlier years, likely to increase over the next couple of months. Each individually numbered hive was weighed on a mobile weighing platform supported by a pair of Ruddweigh™ load bars attached to a digital display. After weighing each hive was returned to its respective position within the apiary. At this time initial beetle counts were conducted. This entailed a systematic inspection of each hive. The number of beetles was determined by opening the hives and counting the numbers of live adult beetles on the bottom boards, frames and lid. After smoking the hive entrance the lid was removed for inspection and placed upturned on the ground (Figure 9). The frames were

smoked prior to their individual removal from the brood box. They were briefly inspected and placed into a spare hive box (Figure 10). The beetles remaining in the brood box were counted by drawing a 75mm wide metal spatula slowly across the bottom board and walls to move bees and disturb beetles that were harbouring within the hive box. Meanwhile the combination of smoke and light drove beetles from the frames in the second hive box onto the bottom board where they were counted and recorded (Figure 10). The new hive box containing the frames was then placed back onto the original bottom board and the lid replaced.

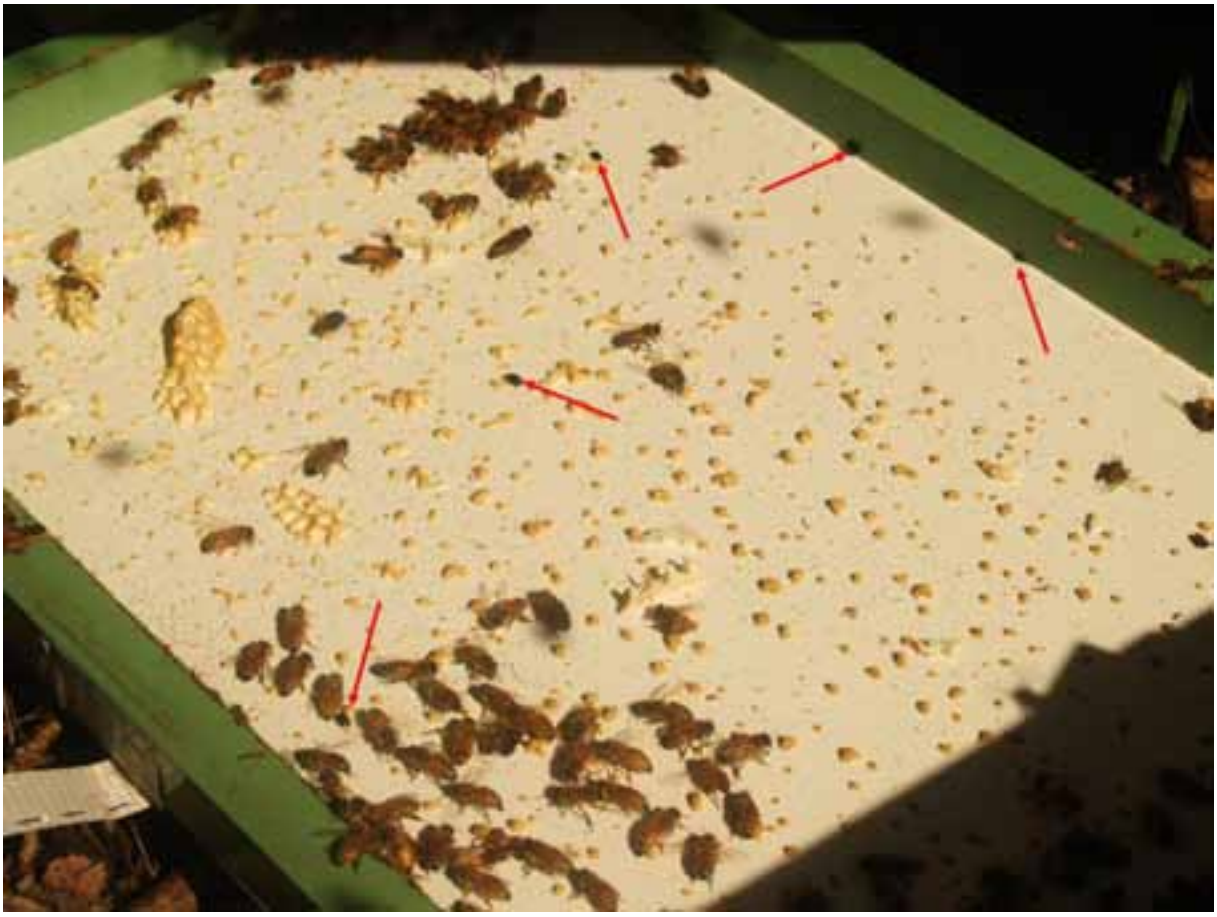


Figure 9 Several beetles (indicated by arrows) on the upturned lid of a 'control' hive.

Overwhelmingly, most beetles were found on the bottom board of the hives. On Day 0 beetle numbers were only low to moderate and the author was confident that quite accurate counts were obtained without the need to remove and replace beetles during this process. Hives were ranked in order of ascending beetle numbers, grouped in pairs and alternately allocated to either the APITHOR™ or 'control' treatment groups. A single APITHOR™ harbourage was placed on the bottom board of each 'treatment' hive. A harbourage containing an untreated cardboard insert was placed on the bottom board of each 'control' hive.

Sixteen and 36 days after harbourage placement the numbers of live beetles seen in the hives were recorded as before. At the same time the numbers of dead beetles seen in the hives were recorded and all dead beetles removed. The Day 16 live beetle counts could not include any live beetles in the harbourage and so is likely to have underestimated the live beetle count at least in the 'controls'. Immediately prior to the Day 36 inspections the hives were re-weighed. During this inspection the number of frames of bees was also recorded. After the Day 36 inspections the harbourages were removed from the hives, 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 live and dead beetles inside counted. The aggregate numbers of dead beetles removed during the two inspections

together with the numbers dead 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 hives.

Figure 10 Inspecting hive frames for small hive beetles and recording results.

Statistical analysis: Beetle counts (live or dead) were analysed using a generalised linear mixed model with errors assumed to follow Poisson distributions.

The method used to calculate efficacy made allowance for the changes in live beetle numbers in the control hives that reflected the naturally expanding population. As such, percentage reductions in the mean number of live beetles present in the hives at the Day 16 and Day 36 inspections were calculated using the formula recommended by Henderson and Tilton (1955) namely,

$$\% \text{ reduction} = 100 \times (1 - ((T_0/C_1) \times (C_0/T_1))$$

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 Day 16 or Day 36 live beetle counts in the control and treated hives respectively.

Changes in hive weights were analysed using the Student' t-test. Changes to the number of frames of bees in the treatments were analysed using a generalised linear model with errors assumed to follow a multinomial distribution.

Results

Commercial partner and manufacture

The small hive beetle harbourage is now patented in Australia, New Zealand and the United States of America.

Following advertisement in the Sydney press (26th May 2009) and email or postal contact with potential partners for Expressions of Interest in commercialising the small hive beetle harbourage Ensystex Pty. Ltd. Australasia was contracted to commercialise the product. Subsequently Ensystex and the project partner organisations (NSW Industry and Investment and RIRDC) entered into an Exclusive Marketing Rights Agreement. Ensystex was well advanced in development of other fipronil based products and has extensive experience in insecticide product manufacture, marketing and distribution. Ensystex has an international profile with manufacturing capability in Thailand.

Mr. Gavin Hall was appointed as Regulatory Affairs Consultant to the project. Gavin brought a wealth of knowledge and experience gained over several years' employment with the APVMA. Gavin was primarily responsible for submitting the permit applications and assembly of the registration dossier. APITHOR™ is currently (August 2011) commercially available to beekeepers under APVMA Minor Use Permit 12007 (Appendix 2).

Ensystex named the product APITHOR and registered the tradename. APITHOR™ is manufactured in Thailand using the injection moulds provided by the Principal Investigator. Suitable corrugated cardboard was sourced in Australia and cut in Thailand. Ensystex developed apparatus and standard operating procedures for the bulk treatment and drying of cards and the assembly of the harbourages. Ultrathor Water-based termiticide (100g fipronil L^{-1} Ensystex Pty. Ltd.; APVMA Registration No. 64449; Batch no. J-140-2; Date of Manufacture - July 2010, Ensystex Pty. Ltd.) was diluted to the appropriate concentration and batches of cards were immersed several times in the solution to ensure adequate wetting. Cards sampled at the beginning and end of a production run were shown to be within specification. The data on the method of treatment and fipronil content of the cards is considered commercial-in-confidence but was submitted to the APVMA to support product registration. Nine treated cards were exposed to either ambient (21^{o}C) or elevated (54^{o}C) temperature storage conditions for 14 days in an accelerated stability trial conducted by AgriSolutions Pty. Ltd. At the end of the trial the cards were analysed for fipronil (and its toxic metabolites) according to standard protocols by AgriSolutions Pty. Ltd. Comparison of the levels of fipronil found in the cards stored at 21^{o}C with those in the cards stored at 54^{o}C (data not shown) indicated that both batches remained within specification (0.36-0.60 g kg^{-1} as fipronil) thereby demonstrating a high degree of stability of fipronil in the cardboard. On the basis of these results we anticipate that APVMA will grant a minimum shelf/service life of two years.

The devices used in the field trials described below were assembled manually and glued to prevent tampering. Ensystex has indicated that APITHOR™ will be ultra-sonically welded in all future production runs. The original mouldings were modified by Ensystex such that the upper shell now has the product name APITHOR™ impressed into it (Figure 1). A product label (Appendix 3) and brochure (Appendix 4) were produced by Ensystex Pty. Ltd. and are available in hard copy or from the APITHOR™ website (https://apithor.com.au). APITHOR™ is sold in packets of twenty for $99 (August 2011 price) and is available on-line (https://apithor.com.au) or by telephone (133536) purchase from Ensystex.

Field residue, safety and efficacy trials

Residue trial

The six trial hives increased in weight by a mean of 46.2 kg (se 5.6 kg). The mean number of frames of bees increased from 17.2 (s.e. 1.2) to 29.5 (s.e. 3.0) over the six weeks interval. One hive had swarmed during the trial leaving the hive queenless and consequently without brood at the second assessment. Two other hives had less area of brood but overall, if allowance was made for the variation between hives, there was no significant difference in mean brood area throughout the trial (Table 1). The bees stored approximately 18.4 kg of honey and 2.95 kg of wax in the twelve new frames during the six weeks of the trial.

Table 2 is a key to the blinded samples submitted to AgriSolutions Pty. Ltd. for analysis. No fipronil (or metabolite) residues were detected in any of the pre-treatment honey samples. None of the bulked honey samples ripened while APITHOR™ harbourages were in place in the hives contained any detectable residues of fipronil or any of its metabolites. Two of the three wax samples contained no detectable fiprole residues. The third sample contained metabolite MB46136 at the LOQ (1 $\mu g\ kg^{-1}$) but no other residues (Figure 11).

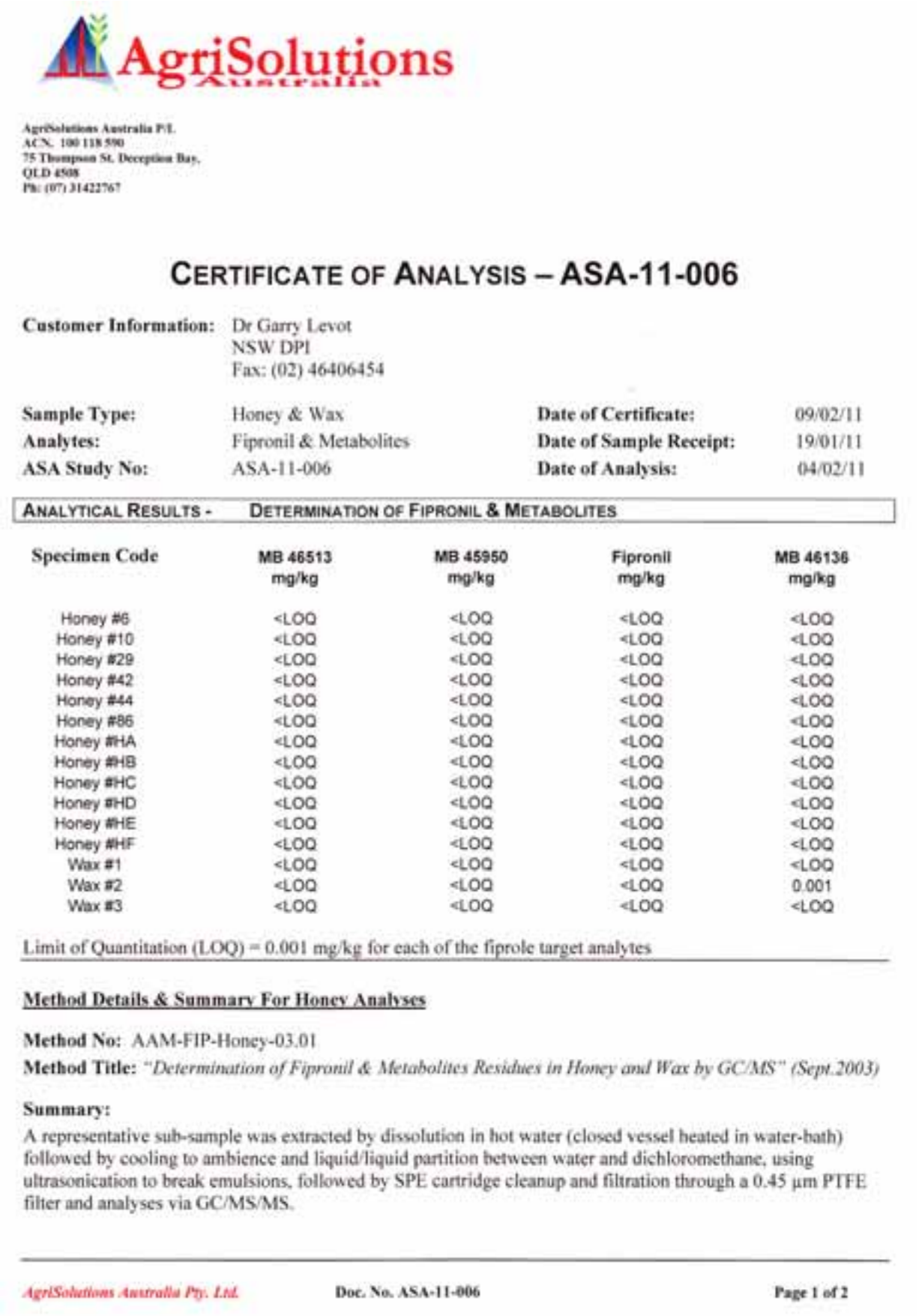
Table 1. Changes in key parameters of hive health in the APITHOR™-treated bee colonies used in the residue study.

Hive Identification	Pre-treatment frames of bees	Post-treatment frames of bees	Pre-treatment area of brood (cm^2)	Post-treatment area of brood (cm^2)	Net hive weight increase (kg)	No. live beetles	No. dead beetles
Hive # 6	23	38	343	0	58	0	12
Hive # 10	16	30	267	224	50.5	2	30
Hive # 29	16	31	259	300	50.5	2	42
Hive # 42	16	31	234	300	47.4	2	16
Hive # 44	16	31	197	313	51.7	0	8
Hive # 86	16	16	334	125	19	0	19

Table 2 Key to blinded samples reported on the AgriSolutions Pty. Ltd. Certificate of Analysis (Figure 11).

Pre-treatment (03 Nov. 2010) honey samples	Post-treatment (15 Dec. 2010) honey samples	Post-treatment (15 Dec. 2010) wax samples
Honey #6	Honey #HA	Wax #1
Honey #10	Honey #HB	Wax#2
Honey #29	Honey #HC	Wax #3
Honey #42	Honey #HD	
Honey #44	Honey #HE	
Honey #86	Honey #HF	

Figure 11 Certificate of Analysis issued by AgriSolutions Australia for the honey and wax samples listed in Table 2.



AgriSolutions Australia

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CERTIFICATE OF ANALYSIS – ASA-11-006

Customer Information: Dr Garry Levot
NSW DPI
Fax: (02) 46406454

Sample Type:	Honey & Wax	**Date of Certificate:**	09/02/11
Analytes:	Fipronil & Metabolites	**Date of Sample Receipt:**	19/01/11
ASA Study No:	ASA-11-006	**Date of Analysis:**	04/02/11

ANALYTICAL RESULTS - DETERMINATION OF FIPRONIL & METABOLITES

Specimen Code	MB 46513 mg/kg	MB 45950 mg/kg	Fipronil mg/kg	MB 46136 mg/kg
Honey #6	<LOQ	<LOQ	<LOQ	<LOQ
Honey #10	<LOQ	<LOQ	<LOQ	<LOQ
Honey #29	<LOQ	<LOQ	<LOQ	<LOQ
Honey #42	<LOQ	<LOQ	<LOQ	<LOQ
Honey #44	<LOQ	<LOQ	<LOQ	<LOQ
Honey #86	<LOQ	<LOQ	<LOQ	<LOQ
Honey #HA	<LOQ	<LOQ	<LOQ	<LOQ
Honey #HB	<LOQ	<LOQ	<LOQ	<LOQ
Honey #HC	<LOQ	<LOQ	<LOQ	<LOQ
Honey #HD	<LOQ	<LOQ	<LOQ	<LOQ
Honey #HE	<LOQ	<LOQ	<LOQ	<LOQ
Honey #HF	<LOQ	<LOQ	<LOQ	<LOQ
Wax #1	<LOQ	<LOQ	<LOQ	<LOQ
Wax #2	<LOQ	<LOQ	<LOQ	0.001
Wax #3	<LOQ	<LOQ	<LOQ	<LOQ

Limit of Quantitation (LOQ) = 0.001 mg/kg for each of the fiprole target analytes

Method Details & Summary For Honey Analyses

Method No: AAM-FIP-Honey-03.01

Method Title: *"Determination of Fipronil & Metabolites Residues in Honey and Wax by GC/MS" (Sept.2003)*

Summary:

A representative sub-sample was extracted by dissolution in hot water (closed vessel heated in water-bath) followed by cooling to ambience and liquid/liquid partition between water and dichloromethane, using ultrasonication to break emulsions, followed by SPE cartridge cleanup and filtration through a 0.45 µm PTFE filter and analyses via GC/MS/MS.

Certified By:

Scott Winner
Chemist - AgriSolutions Australia

Analysed By:

Andrew Keats
R&D Manager - AgriSolutions Australia

Safety trial

The records of the key indicators of hive health in the control and APITHOR™ -treated hives pre- and post-treatment, are presented in Table 3. The pre-treatment estimate of the mean number of frames of bees suggests that there were slightly (P=0.02) more bees in the APITHOR™ treated hives than in the controls at the beginning of the trial. The difference was not significant at the end of the trial. There were no significant differences between the APITHOR™ and control hives in either the pre- or post-treatment areas of brood or in the mean net increase in hive weight during the trial (Table 4). Compared to the control hives, significantly (P<0.001) fewer live beetles and significantly (P<0.001) more dead beetles were recorded for the APITHOR™ treated hives.

Table 3 Pre- and post-treatment indicators of hive health in the control and APITHOR™ -treated hives used in the bee safety study.

Hive treatment and identification	No. frames of bees pre-treatment	No. frames of bees post-treatment	Area of brood pre-treatment (cm^2)	Area of brood post-treatment (cm^2)	Net hive weight increase (kg)	No. live beetles	No. dead beetles
Control # 2	8	31	208	261	49.2	3	0
Control # 19	10	31	298	348	50.2	7	0
Control # 23	16	31	323	217	55.7	4	0
Control # 26	9	20	172	162	23.9	13	0
Control # 36	10	31	230	270	48.1	0	7
Control # 43	8	23	196	100	33.7	8	0
Control #62	12	28	220	280	43.6	8	0
Control #63	16	12	282	0	16	10	0
Control #66	8	31	206	268	53	0	0
Control #82	16	16	226	0	16.1	4	0
APITHOR # 1	16	31	207	0	43.8	0	20
APITHOR # 12	24	39	226	151	60.5	0	16
APITHOR # 28	16	31	241	223	53.9	0	26
APITHOR # 30	24	39	267	237	65	0	14
APITHOR # 31	16	28	158	300	33.4	0	5
APITHOR # 40	10	23	264	340	32.9	0	23
APITHOR # 53	12	31	210	91	12.7	0	15
APITHOR # 54	8	14	200	239	18.6	0	25
APITHOR # 60	8	16	149	227	18.5	0	11
APITHOR # 84	16	31	229	290	46.3	0	9
APITHOR #6*	23	38	343	0	58	0	12
APITHOR #10*	16	30	267	224	50.5	2	30
APITHOR #29*	16	31	259	300	50.5	2	42
APITHOR #42*	16	31	233.5	300	47.4	2	16
APITHOR #44*	16	31	197	313	51.7	0	8
APITHOR #86*	16	16	334	125	19	0	19

* indicates the six hives that were also used in the residue trial.

Table 4. Changes to key indicators of hive health in 'control' and APITHOR™-treated hives in the bee safety trial.

Treatment	Mean pre-treatment no. frames of bees	Mean increase in no. frames of bees	Mean change in area of brood (cm^2)	Mean increase in hive weight (kg)	√ Mean no. live beetles	Mean no. live beetles	√ Mean no. dead beetles	Mean no. dead beetles
Control	11.3	14.10±2.15	-45.5±41.7	38.95	2.39±0.24	5.70	0.84±0.33	0.70
APITHOR™	15.81	12.94±1.70	-26.5±33.0	41.42	0.61±0.19	0.38	4.27±0.26	18.19
Fprob	0.02	0.68	0.72	0.71	<0.001		<0.001	

Efficacy trial

The records of changes in hive weight, frames of bees and live and dead beetle numbers in the control and APITHOR™ -treated hives are shown in Tables 5 and 6 respectively. Mean hive weights and the mean number of frames of bees increased in both the control and APITHOR™ treated hives (Table 7) with no significant differences evident between the two treatments. Hive weigh largely reflected the amount of honey laid down during the trial interval though there was, on average a modest 0.3 - 0.4 frame increase in bee numbers.

On Day 0 low to moderate beetle numbers (means approximately 26 and 23 beetles) were recorded in the control and APITHOR™ -treated hives respectively (Table 7). During the trial interval beetle numbers in the control hives increased by approximately 21% indicating an expanding beetle population. At the Day 16 assessment the mean number of live beetles in the control hives was 31 (range 18-60) (Tables 5 and 7) and probably underestimated the true number as some beetles may have been inside the untreated harbourages. At the same time two live beetles were found in only one APITHOR™ treated hive (Table 6). The remaining fourteen treated hives contained no live beetles (>99% reduction). This difference was highly significant ($P<0.001$) (Table 7). At the Day 36 assessment the mean number of live beetles in the control hives was similar to that recorded on Day 16 (Table 7) but had dropped to zero in the APITHOR™ treated hives (100% reduction) (Table6). This difference was also highly significant ($P<0.001$) (Table 7).

The reduction in live beetles in the APITHOR™ treated hives was reflected in the numbers of dead beetles removed from the hives during the Day 16 and 36 inspections or retrieved from the harbourages at the completion of the trial (Tables 5 and 6). Some beetles die outside the harbourage and are removed from the hives by the bees and are lost. Therefore the numbers of dead beetles recorded in Table 6 do not match the Day 0 live beetle counts. Nevertheless, there was a significant ($P<0.001$) difference in the number of dead beetles recovered from the APITHOR™ -treated hives compared to the controls (Table 7).

Table 5. Changes in hive weight, frames of bees and live and dead beetle counts in the control hives used in the field efficacy study.

Hive treatment and identification	Hive weight (kg) Day 0	Hive weight (kg) Day 36	Net increase in hive weight (kg)	No. frames of bees Day 0	No. frames of bees Day 36	No. live Beetles Day 0	No. live Beetles Day 16	No. live Beetles Day 36	Total no. dead beetles
Control #118	15.4	21.2	5.8	6	5	17	22	26	1
Control #139	19.4	25.6	6.2	7	7	31	46	31	1
Control #134	20.8	26.8	6	7	7	20	25	21	0
Control #121	21.4	27.6	6.2	7	7	40	47	38	2
Control #131	15.4	21	5.6	4	5	20	18	25	0
Control #147	17.4	24.6	7.2	6	7	25	26	30	1
Control #128	19	26.4	7.4	6	7	26	19	34	2
Control #125	20.4	26.2	5.8	6	7	23	33	30	2
Control #123	18.2	23.2	5	6	7	13	18	25	3
Control #112	20.4	26.8	6.4	6	7	23	23	29	4
Control #144	19.4	25.8	6.4	7	7	29	60	44	3
Control #135	19.4	25.6	6.2	6	7	31	34	43	1
Control #117	16.4	21	4.6	7	5	29	41	34	2
Control #120	18.6	23.8	5.2	5	6	35	26	33	1
Control #129	16.8	23.6	6.8	5	6	27	32	30	1

Table 6. Changes in hive weight, frames of bees and live and dead beetle counts in the APITHOR™ -treated hives used in the field efficacy study.

Hive treatment and identification	Hive weight (kg) Day 0	Hive weight (kg) Day 36	Net increase in hive weight (kg)	No. frames of bees Day 0	No. frames of bees Day 36	No. live Beetles Day 0	No. live Beetles Day 16	No. live Beetles Day 36	Total no. dead beetles
APITHOR #133	18.2	24.2	6	6	7	27	0	0	8
APITHOR #141	21	28.4	7.4	7	7	22	0	0	16
APITHOR #148	18.6	23.8	5.2	6	7	17	0	0	11
APITHOR #127	17.6	22.6	5	5	6	41	0	0	24
APITHOR #136	20.2	27	6.8	6	6	17	0	0	8
APITHOR #138	16.4	21	4.6	5	5	26	0	0	29
APITHOR #145	22	26.8	4.8	7	7	18	0	0	15
APITHOR #122	18	22.6	4.6	5	6	28	0	0	14
APITHOR #126	21.2	24.8	3.6	6	6	20	0	0	2
APITHOR #111	18.8	26.8	8	7	7	31	0	0	14
APITHOR #113	19.6	26.2	6.6	5	7	34	0	0	20
APITHOR #115	18.6	24.8	6.2	7	6	16	0	0	20
APITHOR #140	16	20.6	4.6	4	4	13	0	0	4
APITHOR #124	25	28.2	3.2	6	7	21	0	0	10
APITHOR #143	18.2	23.2	5	6	5	19	2	0	15

Table 7. Comparison of changes to live beetle counts, dead beetle counts, mean hive weight increase and mean number of frames of bees in 'control' and APITHOR™-treated hives in the field efficacy study.

Treatment	Mean hive weight (kg) Day 0	Mean hive weight (kg) Day 36	Mean net increase in hive weight (kg)	Mean no. frames Day 0	Mean no. frames Day 36	Mean total dead beetle count	Mean live beetle count Day 0	Mean live beetle count Day 16	Mean live beetle count Day 36
Control	18.56	24.61	6.053	6.07	6.47	1.6	25.93	31.33	31.53
APITHOR™	19.29	24.73	5.44	5.87	6.20	14	23.33	0.13	0
p-value	0.35	0.89	0.14	0.50	0.36	<0.001	0.15	<0.001	<0.001

Discussion of Results

This project aimed to bring to market the small hive beetle harbourage device developed during the feasibility project, *Insecticidal control of small hive beetle* (Levot 2007). The outcome has been that APITHOR™ is being manufactured by Ensystex Pty. Ltd. and is available to Australia's beekeepers under APVMA General Use Permit PER12007. A comprehensive submission to register APITHOR™ with the APVMA was submitted to the APVMA by the project's Regulatory Affairs Consultant, Mr. Gavin Hall, on the 18th July 2011. The submission included reports on field trials that investigated the safety of the use of APITHOR™ to bees, the residue consequences of the deployment of APITHOR™ harbourages in hives during a honey flow and the effectiveness of APITHOR™ in controlling adult small hive beetles in honey bee colonies. The results reported here have demonstrated that the use of APITHOR™ in honey bee colonies is safe to bees, leaves no detectable residues in honey and is quick and effective in reducing adult small hive beetle infestations.

The expressed intention of this project was always to develop a ready-to-use insecticidal refuge trap that was safe for users but only if it could be achieved without compromising bee safety or the integrity of their produce. Nevertheless, the proposal to use fipronil-treated cardboard inserts in a harbourage designed to be deployed inside bee colonies has not been without controversy. The encapsulation of the insecticide treated cardboard insert within the specially designed plastic shell of the harbourage prevents bee access and the set-back from the slot entrances is sufficient that bee mouthparts cannot reach the cards. The choice of fipronil was based on the demonstrated effectiveness against adult small hive beetles (Levot 2008a, b, Levot and Haque 2006) and its physicochemical attributes. Fipronil's extremely low vapour pressure (Colliot *et al*., 1992) and low water solubility minimise the likelihood of residues in honey or wax and it's non-repellent attributes make it ideal for use in a 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 suggested as a cause of bee colony losses in France (Chauzat *et al.,* 2006).

Our results suggest that the harbourage design and label use pattern mitigate both safety and residue concerns. No residues of fipronil or its toxic metabolites were detected in any of the honey samples collected while the harbourages were in place. The hives used were moderately beetle infested at the beginning of the trial but very few live beetles remained after six weeks with APITHOR™ in place. The trial conditions provided a realistic test of the safety of APITHOR™ to bees and their produce. It was conducted during a honey flow that saw mean hive weights (i.e. honey) increase by in excess of 42 kg and frames of bees increase by more than 12. In accordance with Australian regulatory guidelines these samples were decanted from the bulked honey extracted from the six treated hives. The bulked honey had been spun, poured into a clean 20L plastic container and shaken to ensure homogeneity. Similarly, two of the three samples of wax produced by the bees during the trial interval contained no fipronil or related metabolites and the third sample was reported to contain a single metabolite at the limit of quantification i.e. 1 $\mu g\ kg^{-1}$. This level is at least an order of magnitude lower than most allowable maximum residue limits for fipronil in foods (APVMA 2011).

When allowance was made for the standard errors there were no significant differences between in the mean honey production in control and APITHOR™ treated hives (Table 4). Similarly, the differences in the mean number of frames of bees and the mean area of brood at the end of the trial period between APITHOR™ -treated and control hives, were not significant. Several hives in both the control and APITHOR™ treatment groups had swarmed and were without a queen at the final inspection. Consequently, depending on how recently swarming had occurred, there was little or no brood in these hives. Swarming occurs to a greater or lesser extent in all strains of *Apis mellifera* in response to favourable environmental conditions and abundance of nectar and pollen. With some hives in both the control and treated groups swarming, there is no reason to believe that it was associated in any way with the deployment of APITHOR™.

Although not the main focus of attention in the residue and safety trials, significantly more dead beetles and significantly fewer live beetles were retrieved from the APITHOR™ treated hives (Table 3). Similarly impressive results occurred in the field efficacy trial. In the efficacy trial the pre-treatment live beetle counts represented the starting populations in each hive. There was no way of accurately measuring the number of beetles migrating into, or out of the hives but it has been shown that beetles entering hives usually stay (Annand, 2011). Similarly it was not possible to accurately measure the number of beetles killed by the treatment. This was evident by the disparity in the number of beetles recorded in the APITHOR™ treated hives on Day 0 and the total number of dead beetles recorded by Day 36 (Table 5). It is certain that an unknown number of beetles would have died outside the harbourage and been removed by the bees. For this reason alone, rather than measuring efficacy by estimating beetle mortality, the reduction in the number of live beetles in the APITHOR™ treated hives provides the best indication of the efficiency of the device. In this regard, the apparent effectiveness of APITHOR™ in reducing live beetle numbers in the safety trial (Tables 3 and 4) was confirmed by the results of the field efficacy study (Tables 5, 6 and 7). By the first (Day 16 after placement of APITHOR™) assessment of live beetle numbers in the hives, greater than 99% control had been achieved with fourteen of the fifteen hives containing no live beetles. At the final (Day 36) assessment no live beetles (100% control) were observed in any of the treated hives.

With this level of effectiveness and with no apparent adverse effects on bees and no detectable residues in honey arising from the deployment of APITHOR™ harbourages in bee colonies, beekeepers should feel confident that use of this new device as directed on the product label to control small hive beetle in their hives will not compromise their produce or threaten the health of their bees. Control will continue to rely on the skill and diligence of beekeepers to manage their hives and stored supers, with due regard for the possibility that beetle infestation could destroy their operation. APITHOR™ provides an additional tool to reduce the risk of this occurring.

Implications

The project has succeeded in bringing the small hive beetle harbourage to market. Ensystex Pty. Ltd. was enlisted as the preferred commercial partner and is manufacturing APITHOR™ in its Thailand facility and selling via a dedicated website (https://apithor.com.au) or by telephone order (133536). In collaboration with the Principal Investigator Ensystex has developed promotional and technical brochures together with product labels and material safety data sheets. Since 29th September 2010 (and until 30th June 2012) APITHOR™ has been available under APVMA Minor Use Permit (PER12007) but it is expected that the additional data on the safety to bees, honey and wax and effectiveness in field trials generated during this project will be adequate to satisfy APVMA registration requirements. The registration dossier was submitted to the APVMA by the regulatory affairs consultant on the 18th July 2011. We anticipate the APVMA will have made a judgement on the registration claim sometime during 2012 but if not, a renewal of the Minor Use Permit will be sought.

Recommendations

Patent protection of APITHOR™ small hive beetle harbourage in the United States of America potentially opens up additional markets for the device. When issues relating to the international patents for fipronil and its production are resolved, Ensystex Pty. Ltd. should consider registering APITHOR™ in other countries where small hive beetle is a pest.

Appendices

1. APVMA Research Permit PER 11184.

Australian Government
Australian Pesticides and Veterinary Medicines Authority

PERMIT TO ALLOW RESEARCH USE OF AN AGVET CHEMICAL PRODUCT

PERMIT NUMBER –PER11184

This permit is issued to the Permit Holder in response to an application granted by the APVMA under section 112 of the Agvet Codes of the jurisdictions set out below. This permit allows a person, as stipulated below, to use the product in the manner specified in this permit in the designated jurisdictions. This permit also allows any person to claim that the product can be used in the manner specified in this permit.

THIS PERMIT IS IN FORCE FROM 13 FEBRUARY 2009 TO 30 JUNE 2011.

Permit Holder:
NSW DEPARTMENT OF PRIMARY INDUSTRIES
Elizabeth Macarthur Agricultural Institute
Woodbridge Road
MENANGLE NSW 2570

Persons who can use the product under this permit:
Dr. Garry Levot, Mr. Nicholas Annand, Dr. Michael Hornitzky and employees of NSW Department of Primary Industries (NSW DPI) and persons under the supervision of the NSW DPI staff specified above.

PER11184 Permit Version 1 Page 1 of 2

CONDITIONS OF USE

Product to be used:
REGENT 200SC INSECTICIDE
Containing: 200 g/L FIPRONIL as the only active constituent.

Directions for Use:

Situation	Pest	Rate
Bee Hives	Small Hive Beetle (*Aethina tumida*)	Dilute 1.5 mL/1L water and apply approximately 20 mL/harbourage.

Critical Use Comments:
With a hive tool, paint scraper or similar implement, remove wax and debris from a sufficient area of the bottom board to accommodate the harbourage. Place harbourage, flat surface down, on the bottom board with the slot ends aligned away from the hive entrance. The harbourage must sit flat on the bottom board such that beetles cannot shelter underneath. In hives with corrugated or distorted bottom boards apply a thin bead of silicone sealant to the underside of the harbourage and press down firmly onto the bottom board. Unless stuck to the bottom board, a thin wire may be attached to the harbourage to facilitate later removal from the hive via the hive entrance thus removing the need to open the hive. Monitor harbourages for damage or for 'waxing up' of the slot entrances. Replace if damaged or when effectiveness declines. Remove harbourages after 3 months or when control has been established.

Restraints:
DO NOT use in hives with perforated bottom boards.
DO NOT use in hives subject to water inundation.

Jurisdiction:
NSW and QLD only.

Additional Conditions:
This permit provides for the use of a product in a manner other than specified on the approved label of the product. Unless otherwise stated in this permit, the use of the product must be in accordance with instructions on its label.

Persons who wish to prepare for use and/or use products for the purposes specified in this permit must read, or have read to them, the details and conditions of this permit.

Trial records
The permit holder must maintain records of the trials performed under this permit. Specifically details must include the date and location where the trials were conducted, rates and frequency of application, total amount of product used and the names and addresses of the persons conducting the trial. These details must be maintained for a minimum period of two years from the date of expiry of this permit and must be made available to the APVMA upon request.

Maximum number of hives to be treated
Total 100 Small Hive Beetle infested hives at up to 5 sites in NSW and QLD.

Issued by

Delegated Officer

2. APVMA Permit PER 12007 allowing the general use of APITHOR™.

Australian Government
Australian Pesticides and Veterinary Medicines Authority

PERMIT TO ALLOW MINOR USE AND SUPPLY OF AN UNREGISTERED AGVET CHEMICAL PRODUCT

FOR THE CONTROL OF SMALL HIVE BEETLE

PERMIT NUMBER - PER12007

This permit is issued to the Permit Holder in response to an application granted by the APVMA under section 112 of the Agvet Codes of the jurisdictions set out below. This permit allows a Supplier (as indicated) to possess the product for the purposes of supply and to supply the product to a person who can use the product under permit. This permit also allows a person, as stipulated below, to use the product in the manner specified in this permit in the designated jurisdictions. This permit also allows the Permit Holder, the Supplier (if not one and the same) and any person stipulated below to claim that the product can be used in the manner specified in this permit.

THIS PERMIT IS IN FORCE FROM 29 SEPTEMBER 2010 TO 30 JUNE 2012.

Permit Holder:
AUSTRALIAN HONEY BEE INDUSTRY COUNCIL
Level 2
105 Pitt Street
SYDNEY, NSW 2000

Supplier:
Ensystex Australasia Pty Ltd
Unit 3, The Junction Estate
4-6 Junction Street,
Auburn, NSW 2144

Persons who can use the product under this permit:
Persons Generally

CONDITIONS OF USE

Product to be used:
APITHOR HIVE BEETLE HARBOURAGE
Containing: 0.48 g/kg FIPRONIL as the only active constituent.

Directions for Use:

SITUATION	PEST	RATE
Honey Bee Hives	Small Hive Beetle (*Aethina tumida*)	1 device per hive

Critical Use Comments:
Use in accordance with the directions for use on the product label included as Attachment 1.

Jurisdiction:
ACT, NSW, QLD, VIC, WA only.

Additional Conditions:
Unless otherwise stated in this permit, the use of the product must be in accordance with instructions on its label (Attachment 1).

PERSONS who wish to prepare for use and/or use products for the purposes specified in this permit must read, or have read to them, the details and conditions of this permit.

Supply:
The supplier must supply the product in a container that complies with the requirements of section 18(1) of the Agricultural and Veterinary Chemicals Code Regulations. Attached to this container must be a label which is identical in content and format to the label in Attachment 1.

Export of Produce:
Exported produce must have appropriate residue tolerance limits established in the importing countries and any residues must not exceed the tolerance limits.

Other matters:
The Permit Holder must report any adverse experiences to the APVMA immediately upon notification of any such event.

Issued by

Delegated Officer

ATTACHMENT 1

READ SAFETY DIRECTIONS BEFORE USING

Apithor

Hive Beetle Harbourage

ACTIVE CONSTITUENT: 0.48 g/kg fipronil

GROUP 2B INSECTICDE

For the control of Small Hive Beetle (*Aethina tumida*) in honey bee hives.

FOR EMERGENCY USE UNDER APVMA PERMIT

THIS PRODUCT IS NOT REGISTERED

Contains 20 baits (which it is illegal to sell separately)
NET CONTENTS: (20 x ___g) ___g

Ensystex Australasia Pty Ltd
ACN 102 221 965
Unit 3 The Junction Estate
4 - 6 Junction Street
AUBURN NSW 2144
http://www.apithor.com.au

CUSTOMER SERVICE FREECALL: 13 35 36

APVMA Permit No. PER12007

Batch Number / Date of Manufacture:

DIRECTIONS FOR USE (ACT, NSW, Qld, WA & Vic only)

Restraints:
DO NOT use in hives with perforated bottom boards.
DO NOT use in hives subject to water inundation.
DO NOT open or remove the insert from the harbourage device.

Situation	Pest	Rate
Honey bee hives	Small Hive Beetle (*Aethina tumida*)	1 device per hive
Critical Comments	With a hive tool, paint scraper or similar implement remove wax and debris from a sufficient area of the bottom board to accommodate the harbourage. Place harbourage, flat surface down, on the bottom board with the slot ends aligned away from the hive entrance. The harbourage must sit flat on the bottom board such that beetles cannot shelter underneath. In hives with corrugated or distorted bottom boards apply a thin bead of silicone sealant to the outer edge of the underside of the harbourage and press down firmly onto the bottom board. Unless stuck to the bottom board a thin wire may be attached to the harbourage to facilitate later removal from the hive via the hive entrance thus removing the need to open the hive. Monitor harbourages for damage or for 'waxing up' of the slot entrances. Replace if damaged or when effectiveness declines. Remove harbourages when control has been established or after 3 months.	

NOT TO BE USED FOR ANY PURPOSE, OR IN ANY MANNER, CONTRARY TO THIS LABEL UNLESS AUTHORISED UNDER APPROPRIATE LEGISLATION.

WITHHOLDING PERIOD
NOT REQUIRED WHEN USED AS DIRECTED

GENERAL INSTRUCTIONS
For use under APVMA permit PER12007 only.

PROTECTION OF WILDLIFE, FISH, CRUSTACEANS AND ENVIRONMENT
Dangerous to aquatic arthropods. DO NOT contaminate streams, rivers or waterways with the product or used containers.

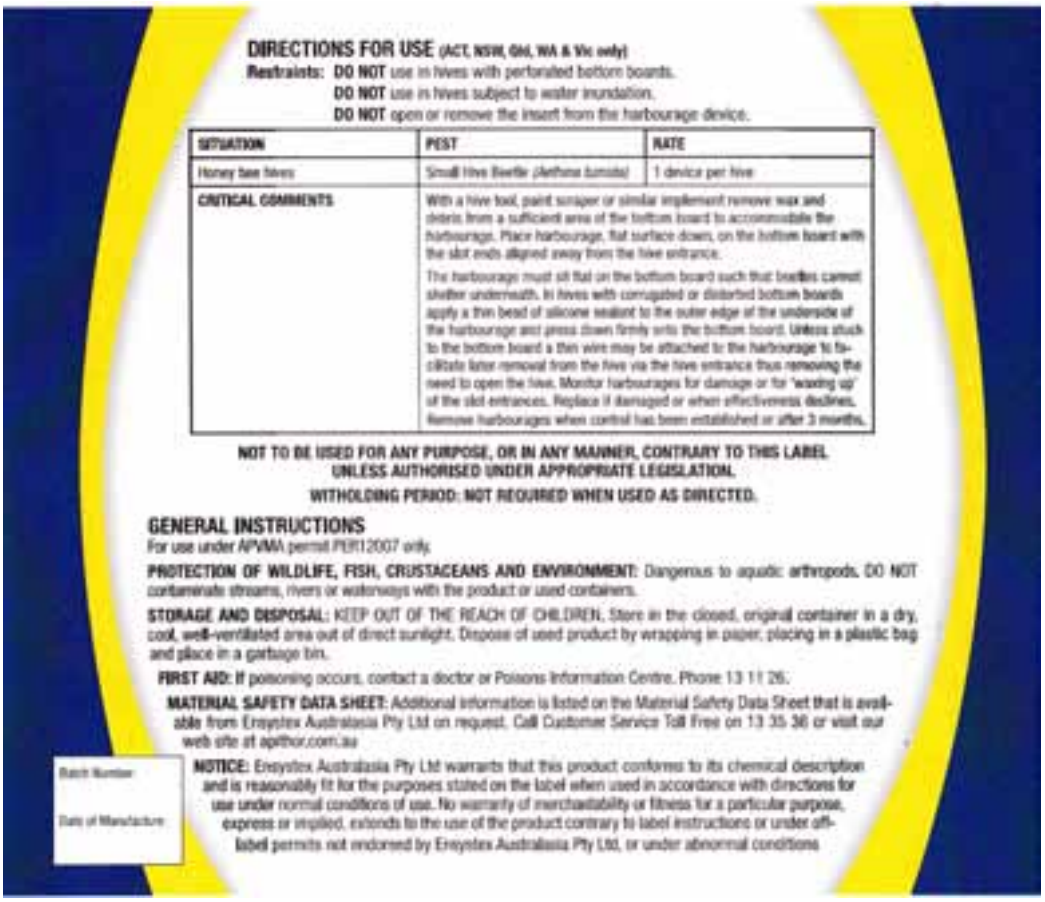
STORAGE AND DISPOSAL
KEEP OUT OF THE REACH OF CHILDREN. Store in the closed, original container in a dry, cool, well-ventilated area out of direct sunlight. Dispose of used product by wrapping in paper, placing in a plastic bag and place in a garbage bin.

FIRST AID
If poisoning occurs, contact a doctor or Poisons Information Centre. Phone 13 11 26.

MATERIAL SAFETY DATA SHEET
Additional information is listed on the Material Safety Data Sheet that is available from Ensystex Australasia Pty Ltd on request. Call Customer Service Toll Free on 13 35 36 or visit
our web site at http://www.apithor.com.au

NOTICE
Ensystex Australasia Pty Ltd warrants that this product conforms to its chemical description and is reasonably fit for the purposes stated on the label when used in accordance with directions for use under normal conditions of use. No warranty of merchantability or fitness for a particular purpose, express or implied, extends to the use of the product contrary to label instructions or under off-label permits not endorsed by Ensystex Australasia Pty Ltd, or under abnormal conditions.

3. The APITHOR™ product label.



4. Ensystex Pty. Ltd. brochure to support APITHOR™.

References

Annand N. (2011). Small Hive Beetle Biology - Providing Control Options. Final Report No. 11/044 to Rural Industries Research and Development Corporation.

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Commercialisation of the Small Hive Beetle Harbourage Device

by Dr Garry Levot

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The smal hive beetle is a major pest of honeybee hives. This report describes the commercialisation of the APITHOR™ small hive beetle harbourage and the results of bee safety, honey residue and field efficacy trials conducted to support full product registration of the device by the Australian Pesticides and Veterinary Medicines Authority (APVMA).

The small hive beetle harbourage is now patented in Australia, New Zealand and the United States of America.

The report is written for beekeepers and advisors to the honeybee industry.

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