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ORIGINAL RESEARCH ARTICLE

A six-month-long assessment of the health of bee colonies treated with *APITHOR™ hive beetle insecticide*

Garry Levot[a], Douglas Somerville[b]*, Nicholas Annand[c], Damian Collins[a] and Idris Barchia[a]

[a]*Elizabeth Macarthur Agricultural Institute, NSW Department of Primary Industries, Narellan, Australia;* [b]*NSW Department of Primary Industries, Goulburn, Australia;* [c]*NSW Department of Primary Industries, Bathurst, Australia*

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The safety of *APITHOR™ hive beetle insecticide* on the health of honey bee colonies was assessed in a field trial in which 16 bee colonies that were exposed to two consecutive treatments each of three-months duration, were compared with 10 untreated (control) hives. Measurements of brood area, available hive frames occupied by bees (hive strength) and hive weight (as an indirect indicator of honey production) were recorded pre-treatment and after three- and six-months exposure to APITHOR™ treatment. Samples of honey and wax collected from six of the treated hives at the same times were independently tested for the presence of fipronil and its metabolites, and no residues were detected in any sample at either time. Mean net increases in the weights of the APITHOR™ treated and control hives were not significantly different ($p > .05$). Similarly, neither mean brood area nor the mean proportion of available hive frames occupied by bees in the control and APITHOR™ treated hives was significantly different from each other ($p > .05$) at both the three- and six-month post-treatment assessments. Compared to the control hives, however, significantly ($p < .001$) fewer live beetles were recorded in the APITHOR™ treated hives at these times.

Seguimiento de la salud de colonias de abeja de la miel tratadas con *APITHOR*™, insecticida contra el escarabajo de las colmenas

Se evaluó la seguridad del insecticida APITHOR™ para el escarabajo de las colmenas en la salud de las colonias de abeja de la miel en un experimento de campo en el que se comparó la exposición de 16 colonias de abejas a dos tratamientos consecutivos de tres meses de duración cada uno, con diez colmenas sin tratar (control). Se tomaron medidas sobre el área de cría, la disponibilidad de celdillas ocupadas por abejas en la colmena (fuerza de la colmena) y el peso de la colmena (como un indicador indirecto de la producción de miel) antes del tratamiento y después de tres y seis meses de exposición al tratamiento de APITHOR™. Al mismo tiempo que se recogieron las muestras de miel y cera de seis de las colmenas tratadas, se evaluó independientemente la presencia de fipronil y sus metabolitos, y no se detectó ningún residuo en las muestras. La media del incremento neto del peso de las colmenas tratadas con APITHOR™ y las colmenas control no mostró diferencias significativas ($P>0.05$). De igual forma, ni la media de área de cría ni la media de la proporción de celdillas en la colmena disponibles ocupadas por abejas fueron significativamente diferentes entre las abejas control y las tratadas con APITHOR™ en las evaluaciones a los 3 y a los 6 meses post-tratamiento. Sin embargo, comparadas con las colmenas control, las colmenas tratadas con APITHOR™ registraron menos escarabajos vivos significativamente ($P<0.001$) en estos tiempos.

Keywords: fipronil; small hive beetle; *Aethina tumida*; bee health

Introduction

The small hive beetle *Aethina tumida* Murray was first found in western Sydney, Australia, in 2002 (Fletcher & Cook, 2005). No doubt assisted by the movement of hives, it quickly spread along the entire Australian eastern seaboard and inland in some areas (Gillespie, Staples, King, Fletcher, & Dominiak, 2003). As it did when accidently introduced into the USA (Elzen et al., 1999), the beetle caused significant damage to managed and feral hives, and restricted opportunities for Australian bee keepers to export bees to countries free of small hive beetles. In response to the need to control the beetle in managed hives and after recognizing a behavioral vulnerability displayed by the beetle, we investigated the feasibility of using an insecticidal refuge trap (Levot, 2008a). The design and construction of the trap was refined and successfully tested in the field (Levot, 2008b) and was later commercialized in collaboration with Ensystex Australasia Pty. Ltd under the trade name *APITHOR™ hive beetle insecticide*.

APITHOR™ comprises a fipronil (.48 g kg^{-1})-treated corrugated card permanently enclosed within a specially designed plastic shell that prevents bees accessing or contacting the cardboard insert (Levot & Somerville, 2012). Deployment of a single APITHOR™ significantly and quickly reduces adult small hive beetle numbers in hives (Levot & Somerville, 2012) and is capable of eliminating beetles from individual hives in some

*Corresponding author. Email: doug.somerville@dpi.nsw.gov.au

circumstances (Levot, 2008b). In 2011, in recognition of the need for an effective treatment for the small hive beetle and of the effectiveness and safety of APITHOR™ demonstrated in short-term trials run over six weeks (Levot & Somerville, 2012), the Australian Pesticides and Veterinary Medicines Authority (APVMA) made APITHOR™ available to Australian bee keepers under a temporary permit. Bee keeper confidence in the device is reflected in the sale of 54,000 APITHOR™ by Ensystex Australasia Pty. Ltd between July 2011 and June 2012 (S. Broadbent, personal communication). A condition of the permit was that further data on the long-term safety of APITHOR™ to bees would be collected.

In Australia, label directions allow the deployment of APITHOR™ for up to three months in hives with no restrictions preventing immediate repeat treatment if deemed necessary. To determine whether these directions were safe for bees, the current study on bee colony health was conducted continuously over six months as two consecutive three-month-long treatments. It is considered unlikely that treatment for longer than three months would be required to control beetles in hives under most circumstances, so the deployment interval used here represents a higher than usual, albeit allowable, use pattern.

Bee colony health can be estimated using several qualitative and quantitative parameters; however, to assess the health impact, if any, of a hive treatment, it is important that only things that can be measured objectively be included. For example, hive health is reflected in the number of adult bees in the hive (hive strength), the presence and amount of brood, and honey production. Each of these parameters can be quantified with reasonable accuracy (Delaplane, Van Der Steen, & Guzman, 2013). Change in hive weight over time approximately equates to the weight of honey produced and is more convenient to measure over time than honey yield per se. In the current study, each of these parameters was measured in beetle infested APITHOR™-treated, or untreated hives typical of those used in Australia, which were managed according to normal commercial beekeeping practices.

Materials and methods

With the exception of one APITHOR™-treated hive which had a full-depth (244 mm) super, the trial hives comprised eight-frame, Langstroth boxes (500 mm (l) × 347 mm (w) × 244 mm (d)) with WSP supers (500 mm (l) × 347 mm (w) × 193 mm (d)). Twenty-six, "double" (two box high) hives that were similar in strength (number of hive frames occupied by bees) were allocated a unique identifier number which was marked on the outside of the hive. These hives were randomly allocated to either the APITHOR™-treated group or to the untreated control group and remained co-located for the duration of the trial. There were 16 hives in the APITHOR™ treatment group and 10 in the control group.

Six of the hives in the APITHOR™ treatment group were randomly chosen for the "residue" component of the study. A clean spoon was used to remove a section of comb containing honey from an outer frame from the brood box of each of these hives. The brood box honey samples were transferred into clean, labeled glass jars that were transferred onto ice within four hours of collection. Two central frames from the supers of these hives were marked with the hive number before being removed and replaced by brand new foundation frames that were also marked with the hive number. The use of new foundation ensured that after three months, these frames could be removed knowing that the honey and wax collected had been entirely formed during the period that APITHOR™ had been in place. The original marked frames were taken back to the laboratory where honey was extracted from the pairs of frames as separate pre-treatment samples. The extractor was thoroughly washed with hot water to remove all traces of honey and dried with paper towel in between extraction of the frames from each hive. Duplicate pre-treatment honey samples from the six individual "residue" hives were transferred into clean, labeled glass jars and immediately placed into a freezer.

The 26 hives were individually weighed on a set of platform scales that were set up on site. Each was then systematically dismantled. Both sides of individual frames in the brood box were inspected and by superimposing an empty hive frame containing a string grid (50 × 50 mm) onto each frame, the brood area of each hive was estimated (Figure 1). As a further measure of colony health, the number of hive frames occupied by bees was recorded. During the dismantling process, the number of live adult beetles seen was recorded. Although perhaps not as accurate as collecting beetles in repeated inspections as advocated in Neumann et al. (2013), our intention was mainly to demonstrate that the trial hives were beetle infested. The efficacy of

Figure 1. Using the grid to estimate the area of a hive frame occupied by brood.

APITHOR™ in killing beetles has been proven previously (Levot, 2008a, 2008b; Levot & Somerville, 2012). The data collected (frames of bees, area of brood, hive weight, and number of live beetles) represented the "pre-treatment" hive assessments.

A single APITHOR™ was deployed on the bottom board of each hive in the APITHOR™ treatment group as directed on the product label. Seasonal conditions early in the trial were conducive to swarming. This threatened to interfere with the hives as the loss of the queen and many of her cohorts would compromise the performance of the hive and mask any potential treatment effects. To minimize the likelihood of swarming, all hives were re-queened with young sister queens during the first few weeks of the trial.

During the first three-month treatment interval, in line with normal beekeeping practice, additional supers needed to be placed onto many, but not all, hives to accommodate the honey productivity of the bees. To manage this, pre-weighed supers were added to hives as required. Later, when calculating the increase in hive weight attributable to the bees (mainly honey production), post-treatment hive weights were corrected for the weight of the empty supers that had been added to them during the treatment interval.

The trial commenced in spring 2012, when average maximum daily temperatures ranged from 13 to 31 °C and minimums ranged between −1.7 and 9.1 °C. The hives were moved several times during the trial as floral resources were exhausted. Initially (early October 2012), the hives were located within a cherry (*Prunus avium*) orchard in the southwest slopes of New South Wales to coincide with the major annual flowering. When the cherry flowering had finished, the hives were moved (21 October 2012) about 60 km southwest in anticipation of flowering of Paterson's curse (*Echium plantagineum*); however, within a few weeks, it became apparent that floral prospects were poor and an inspection of the hives suggested that most hives were struggling. On 21 December 2012, the hives were transported about 300 km southeast to a State forest site about 15 km south of Bermagui on the New South Wales south coast. Here, conditions remained dry and daily maximum temperatures ranged from 18.8 to 41.3 °C until late January when approximately 40 mm of rain fell over four days, followed by regular rainfall during February. From the time, the hives were located on the coast until early April 2013 when the trial concluded, various eucalypt species flowered prolifically and the bees thrived. By April, it was again very dry, day length was shortening and average maximum daily temperatures had dropped to the low to mid 20 °Cs.

Three months after initial placement of APITHOR™ in the hives, the systematic dismantling, measurement, and weighing of both the treated and control hives were repeated to measure the colony health. As previously described, records of the number of live and dead beetles observed in each hive were also made during these hive inspections. After removal from the treated hives, each APITHOR™ was placed individually into a ziplock plastic bag marked with the hive number. Later, they were broken open in the laboratory and the number of dead beetles inside recorded. These numbers were added to the number of dead beetles seen during the field inspections and represented the total dead beetle counts used in the statistical comparison of the treatment data.

The full supers were removed and new empty supers placed on top of the brood boxes following the three months post-treatment hive inspections and after the hives had been weighed. The six pairs of marked frames were removed from the "residue" hives and transported to a convenient location where the frames were extracted using a three-frame manual honey extractor. The honey from the six hives was collected as a single commodity in a 12.5-l plastic container in accordance with Australian regulatory requirements for honey residue trials (APVMA, 2001). The bulked honey was passed through a stainless steel kitchen sieve (which retained the wax cappings) into another 12.5-l plastic container, where it was mixed with a ladle. The weights of the honey and wax cappings were recorded separately. Ten sub-samples of the bulked honey were poured into clean, labeled glass jars and placed into a freezer within 12 h of extraction. Similarly, six samples of wax from the sieve were spooned into clean, labeled glass jars and frozen. After honey extraction, the marked "sticky" comb frames were replaced into their respective hives. At this time, a new APITHOR™ was placed on the bottom board of the treatment hives as a back-to-back re-treatment, that is, six months continuous exposure of the bees to APITHOR™. For the "residue" hives, this meant that the wax comb and any residual honey it contained from the first three-months treatment was subjected to a second three-month-long exposure.

The hives, now two box-high "doubles", were individually re-weighed as before and these weights, together with the three-month post-treatment measurements of brood area and new records of the number of hive frames occupied by bees, became the "pre-second treatment" measurements of hive health. Many hives needed to be re-supered during the period between the three- and six-month hive health measurements. To manage this, pre-weighed supers were added to hives as required and, as before, the post-treatment hive weights corrected for the weight of the empty supers.

The systematic dismantling, measurement, and weighing of the hives were again repeated six months after the initial placement of APITHOR™ into the treatment hives. Again, records of the number of dead beetles observed in each hive were made during hive inspection. At this assessment, one of the APITHOR™-treated hives was found to have no brood in the bottom box. The queen was found in the super unable to return to the bottom box due to the placement of the

queen excluder. She had produced brood in the super so, for this hive only, the brood measurement recorded was that found throughout the super. After removal from the hives, each APITHOR™ was placed individually into a ziplock plastic bag marked with the hive number. Later, they were broken open in the laboratory so that the number of dead beetles inside could be determined and added to the number of dead beetles seen during the field inspections as before.

The six pairs of marked frames were again removed from the "residue" hives and the honey extracted as before. The honey and wax samples were stored as before prior to dispatch to the analytical laboratory.

Statistical analyses

Data for pre-treatment hive weights, estimates of pre-treatment, three- and six-month post-treatment brood area, and hive weight gains after three- and six-months exposure to APITHOR™ were analyzed using conventional analyses of variance recognizing the presence (treatment) or absence (control) of APITHOR™ as the independent variable.

The numbers of live beetles recorded in the hives were analyzed using a generalized linear model with errors assumed to follow a Poisson distribution. A square root link function was used to relate the beetle numbers to treatment group. The variance ratio (F value) was used to test the treatment group effects due to over dispersion from the assumed residual distribution. A detailed method of analysis is described in McCullagh and Nelder (1989).

Similarly, the proportions of available hive frames occupied by bees pre-treatment, and after three- and six-months exposure to APITHOR™, were analyzed to test for treatment group differences using a generalized linear model with errors assumed to follow a binomial distribution. All analyses were run via GenStat 14th edition (Payne et al., 2011).

Residue analyses

Residue analyses were outsourced to Agrisearch Analytical Pty. Ltd (Rozelle, Sydney), a company accredited to conduct fipronil analyses to the standard of Good Laboratory Practice. The honey and wax samples were submitted frozen. Analyses were conducted in accordance with the validated standard operating procedure in place at Agrisearch Analytical Pty. Ltd Briefly, after the samples were brought to room temperature, residues of fipronil and its metabolites were extracted from honey by vortexing with acetonitrile: water. Magnesium sulfate and sodium chloride were added to the extract to create a partition between the acetonitrile and water layers. An aliquot of the acetonitrile layer was taken and evaporated to dryness. The residuum was reconstituted in acetonitrile: water. The final extract was analyzed using a high-pressure liquid chromatography (HPLC) coupled to a tandem mass spectrometer. Residues of fipronil and its metabolites were extracted from wax by extracting in acetonitrile: water: acetic acid and heating to melt the wax. Magnesium sulfate and sodium acetate were added to the extract to create a partition between the acetonitrile and water layers. An aliquot of the acetonitrile layer was taken and cleaned up further by adding magnesium sulfate, primary secondary amine, and C18. The extract was diluted with water and analyzed using an HPLC coupled to a tandem mass spectrometer.

The above analytical methods were validated by fortifying sub-samples of untreated control honey or wax with known amounts of the test substances fipronil and the metabolites fipronil sulfone, fipronil sulfide, and fipronil desulfinyl. The fortified samples were then analyzed using the defined method and the recovery of each test compound for each sample was determined. The limit of detection for fipronil and its metabolites in honey and wax was .0003 mg kg^{-1} in this study.

Results

Prior to treatment, mean weights of the hives allocated to the APITHOR™ and control groups were about 38 kg and not significantly different ($p > .05$) (Table 1) but ranged in weight from 32 to 47 kg. Similarly, the average proportion of hive frames occupied by bees in each treatment (approximately .95) and the number of small hive beetles in the hives were not significantly different ($p > .05$). However, mean brood area in the hives allocated to the APITHOR™ treatment group (approximately 275 ± 10 squares) was significantly ($p < .05$) less than that in the control hives (approximately 314 ± 13 squares) (Table 1) despite the randomization process.

One of the APITHOR™-treated "residue" hives was found to be queenless at the three-months assessment.

Table 1. Pre-treatment comparisons of the control and APITHOR™-treated hives.

Treatment	Mean initial hive weight (kg) (S.E.)	Mean brood area (No. of 5 × 5 cm squares) (S.E.)	Mean proportion of available hive frames occupied by bees (S.E.)	Mean no. live beetles (S.E.)
Control (n = 10)	38.30 (1.28)a	313.8 (12.7)a	.94 (.02)a	35.90 (4.98)a
APITHOR™ (n = 16)	38.11 (.68)a	274.5 (10.0)b	.96 (.02)a	26.20 (4.98)a

Notes: Data in individual columns followed by the same letter were not significantly different ($p > .05$). S.E. = standard error of mean.

Consequently, this hive contained no brood and was in the process of re-queening itself. The loss of the queen is not believed to be treatment related. In recognition of this, the colony health data were analyzed without the data for that hive included. Across all hives, there was less brood at this mid-summer assessment (Table 2) than there had been at the pre-treatment assessment in spring (Table 1). There were no significant differences ($p > .05$) between the control and APITHOR™-treated hives in terms of mean net hive weight gain (approximately 30 kg), mean brood area (approximately 205 squares), or the proportion (.99) of available hive frames occupied by bees (Table 2). Compared to the control hives, significantly ($p < .001$) fewer live beetles were recorded in the APITHOR™-treated hives (Table 2), and in total, 756 (mean = 47 ± 6) dead beetles were retrieved from within the 16 APITHOR™ traps removed from the hives at this time. A further 240 dead beetles were removed from the hive bottom boards.

The queenless hive was replaced prior to the second APITHOR™ treatment. However, the marked frames from the original hive were transferred into the replacement hive so that these frames of honey were exposed to APITHOR™ for the entire six-month treatment interval. Hive health data for this hive were not included in the statistical analysis. All hives had a full complement of bees (all 16 frames occupied) prior to re-treatment (Table 3). Mean hive weight, mean brood area, and proportion of available frames occupied by bees were not significantly different ($p > .05$) in the control and APITHOR™ treatment groups (Table 3). Inevitably, however, following the first three-months treatment, the new starting populations of small hive beetles were significantly ($p < .001$) lower in the APITHOR™-treated hives than in the control hives (Table 3).

The six-month hive assessments were conducted in April (autumn) 2013. Across all hives, brood area was about half that present at the three month assessment (Table 4). Neither mean brood area nor the proportion of available hive frames occupied by bees, in the control and APITHOR™-treated hives, was significantly different from each other ($p > .05$) (Table 4). Similarly, mean net increase in weights of the APITHOR™-treated and control hives (approximately 31 kg) was not significantly different ($p > .05$). Compared to the control hives, significantly ($p < .001$) fewer live beetles were recorded in the APITHOR™-treated hives. In total, 990 (mean = 62 ± 23) dead beetles were retrieved from within the 16 APITHOR™ traps removed from the hives at the conclusion of the trial with a further 140 dead beetles removed from the hive bottom boards.

Residue analyses

The hives contained little honey at the beginning of the trial. Only two spoonfuls of comb containing honey was taken from the brood box of each "residue" hive to minimize the impact on hive health. There was adequate honey in the pairs of frames from the supers to obtain at least 400 g samples from each hive. Only small amounts of wax from the brood box honeycomb were submitted for analysis. No fiprole residues were detected in any of the pre-treatment brood box honey, brood box wax, or honey samples from the supers.

After three-months exposure to APITHOR™, 9.9 kg of honey was extracted from the 12 frames belonging to the "residue" hives and approximately 1.45 kg of sticky wax comprising the cappings of the 12 frames was collected. No fiprole residues were detected in any of these honey or wax samples.

After six-months exposure to APITHOR™, a further 11.2 kg of honey was extracted along with approximately .92 kg of sticky wax cappings from the 12 frames from the "residue" hives. No fiprole residues were detected in any of the honey or wax samples from the APITHOR™-treated hives or from the untreated control hives.

Discussion

The criteria for inclusion in the trial were that hives should be healthy, beetle-infested, and contain similar numbers of adult bees. As such, the 26 trial colonies were estimated to be of similar strength at the pre-treatment inspection. However, similarity in terms of adult bee numbers did not guarantee that mean brood area or mean beetle numbers would also be similar in the hives allocated to the two groups and, for mean brood area, this was shown to be the case (Table 1). We do not believe this initial difference impacted on the results in any way. The hives were typical of those used in Australian commercial beekeeping and were adequately uniform for our purpose.

The conditions under which the study was conducted were also suitable for the purpose of measuring the key parameters of colony health over a six-months interval. In keeping with normal commercial beekeeping practice in Australia, the hives were relocated as necessary to where major flowering events were occurring. Seasonal conditions on the southwest slopes of New South Wales from early spring until early summer 2012 were difficult and both the control and APITHOR™-treated hives struggled during the early phase of the trial. However, after the hives were moved to the south coast of NSW, prolific eucalypt flowering from early to mid-summer provided excellent conditions for the bees and both treatment groups thrived. At the beginning of the trial, the hives were infested with moderate numbers of small hive beetles (Table 1) and live beetles were recorded in all untreated hives throughout the entire trial period (Tables 2–4). That there were fewer live beetles in the control hives at the six-month assessment (Table 4) than were present at three-months assessment (Table 2) is thought to reflect seasonal conditions, reinfestation from outside the apiary and the effectiveness of APITHOR™ in eliminating small hive

Table 2. Comparison of key indicators of bee health in control and APITHOR™-treated hives[a] after three-months continual exposure to APITHOR™.

Treatment[a]	Mean net gain in hive weight (kg) (S.E.)	Mean brood area (No. of 5 × 5 cm squares) (S.E.)	Mean proportion of available hive frames occupied by bees (S.E.)	Mean no. live beetles (S.E.)
Control (n = 10)	31.15 (3.30)a	208.3 (11.0)a	.99 (.01)a	45.60 (5.18)b
APITHOR™ (n = 15)	29.18 (2.69)a	204.9 (9.0)a	.99 (.01)a	3.93 (1.24)a

Notes: Data in individual columns followed by the same letter were not significantly different (p > .05). S.E. = standard error of mean.
[a]Data for the queenless hive were excluded from the analyses.

Table 3. Comparison of key indicators of bee health in control and APITHOR™-treated hives prior to the second three-month-long APITHOR™ treatment.

Treatment	Mean hive weight (kg) (S.E.)	Mean brood area (no. of 5 × 5 cm squares) (S.E.)	Mean no. live beetles (S.E.)
Control (n = 10)	36.74 (.90)a	208.3 (10.7)a	45.60 (5.49)b
APITHOR™ (n = 15)	36.88 (.71)a	205.1 (8.5)a	4.81 (1.41)a

Notes: Data in individual columns followed by the same letter were not significantly different (p > .05). S.E. = standard error of mean.

Table 4. Comparison of key indicators of bee health in control and APITHOR™-treated hives after six-months continual exposure to APITHOR™.

Treatment	Mean net gain in hive weight (kg) (S.E.)	Mean brood area (no. of 5 × 5 cm squares) (S.E.)	Mean proportion of available hive frames occupied by bees (S.E.)	Mean no. live beetles (S.E.)
Control (n = 10)	31.5 (1.6)a	109.8 (8.0)a	.94 (.3)a	13.70 (1.94)b
APITHOR™ (n = 15)	30.5 (1.6)a	107.5 (7.9)a	.90 (.3)a	1.73 (.6)a

Notes: Data in individual columns followed by the same letter were not significantly different (p > .05). S.E. = standard error of mean.

beetles from the hives. Over the course of the trial, far more dead beetles (1746) were retrieved from within the traps deployed in the APITHOR™-treated hives or from the bottom boards of these hives (380) than were present in all 26 hives at the beginning of the trial. Clearly, reinfestation from outside the apiary was occurring but pressure probably varied between locations and with the season. It is also possible that there was some movement of beetles from control to treated hives. If so, the presence of APITHOR™ in more than half of the hives may have contributed to the overall reduction in the number of live beetles in the apiary, including the controls.

Bee colony health is reflected in the number of adult bees in the hive (hive strength), the area of brood present, and honey production. Under favorable conditions, healthy hives will increase in weight as honey is produced, and also in strength by producing more brood and hence worker bees. No treatment-related effects other than a significant reduction in the mean number of live beetles in the APITHOR™-treated hives were noticeable at the hive assessments conducted after APITHOR™ had been deployed for three months (Table 2). The mean net increase in hive weight in both groups was approximately 30 kg and although mean brood areas of both groups were smaller than those recorded in the previous spring (Tables 1 and 2), this reflected the changing seasonal conditions.

Similarly, there were no noticeable treatment-related differences at the six-month assessments apart from the significantly lower mean number of live beetles in the APITHOR™-treated hives. Changes in the key indicators of hive health and productivity (Table 4) were similar in both groups and again, reflected the change of season (Tables 3 and 4).

The effectiveness of APITHOR™ in controlling adult beetles inside the hive reported previously (Levot, 2008b; Levot & Somerville, 2012) was again evident in this trial (Tables 2 and 4). The value of APITHOR™ lies in its ability to prevent colonies from being destroyed by beetles – a dead hive having no productivity. Numerous other trapping devices containing various substances such as oils and vinegar (Hood & Miller, 2003) or diatomaceous earth (Cribb, Rice, & Leemon, 2013), sometimes in association with food lures, are available for use inside hives but these require additional or replacement hive hardware (Hood, 2006) and frequent servicing, especially if hives need to be relocated. In the United States of America, traps comprising coumaphos-impregnated plastic strips (Checkmite+™) attached

beneath pieces of stripped-back corrugated cardboard have been used under a permit issued by the Environmental Protection Agency to kill beetles in managed hives (Elzen et al., 1999; Hood & Miller, 2003). They were tested in Australia as well but, perhaps reflecting the inferior performance of coumaphos relative to fipronil (Levot, 2009), achieved reductions of live beetles of only about 53% (Neumann & Hoffmann, 2008). Coumaphos has a strong affinity for bees wax (Kochansky, Wilzer, & Feldlaufer, 2001) and, although there is only a low risk that sub-lethal concentrations have a deleterious effect on bee learning (Weick & Thorn, 2002), its use in the USA is subject to strict conditions (Hood, 2006) to mitigate the danger of coumaphos contamination of honey and wax. Fipronil is extremely toxic to bees (Mayer & Lunden, 1999) and certainly more so than coumaphos which is reported to pose only a slight danger to bees (Weick & Thorn, 2002). However, the results of our residue trial demonstrated that even when APITHOR™ was used continuously for six months, no fipronil or metabolite residues were detected in honey or bees wax extracted from treated hives and no deleterious effects on bee health were observed. This level of safety is achievable because the fipronil-treated cardboard insert is encapsulated within the patented APITHOR™ plastic housing out of reach of the bees or users of the product.

The use of a harborage containing fipronil inside hives has not been without controversy. *Apis mellifera* is extremely sensitive to fipronil (Mayer & Lunden, 1999), fipronil residues have been implicated as a cause of bee colony losses in France (Chauzat et al., 2006) and sub-lethal doses of fipronil were reported to cause reduced foraging (Colin et al., 2004) and poor olfactory learning behavior in honey bees (Decourtye et al., 2005; El Hassani, Dacher, Gauthier, & Armengaud, 2005). However, the harborage design and prescribed use pattern have both been carefully considered. Here, we have demonstrated in a six-month-long trial that APITHOR™ had no measurable, deleterious effect on bee colony health and left no detectable residues in honey or wax. It is innocuous in the hive apart from its ability to quickly and effectively control adult small hive beetles. In December 2013, the safety and effectiveness of APITHOR™ was formally recognized when the APVMA withdrew the temporary permit and granted full product registration in Australia.

Disclosure statement

No potential conflict of interest was reported by the authors.

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