Asian honey bee (*Apis cerana*) remote nest treatment

Asian honey bee Transition to Management Program

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Summary

A project under the Asian honey bee Transition to Management Plan was to investigate alternative control techniques and attractants and to finalise development of remote poisoning by validating techniques and refining protocols to reduce risk of non-target poisoning and minimising adverse effects on environment and native fauna. This project was to be delivered by 30 June 2012.

Biosecurity Queensland consulted the Scientific Advisory Group to develop a research proposal with operational protocols and it was agreed that the aim of the research was to:

i. determine the effectiveness of remotely killing individual, feral *Apis cerana* nests using fipronil,

ii. investigate the potential of this method as a useful management tool for *A. cerana*, and

iii. determine the potential effects of this treatment method on non-target species.

Between February and June 2012, 19 remote treatment trials with fipronil-laced sugar syrup were conducted on 15 *A. cerana* nests.

The treatments showed that fipronil was very effective at suppressing and killing individual *A. cerana* colonies if more than 20% of bees relative to nest entrance activity took fipronil back to the nest. The percentage of bees taking back fipronil relative to the nest entrance activity was the best predictor of treatment success.

However, the usefulness of remote treatment as a method to manage *A. cerana* in Australia is doubtful due to several reasons:

1. Not all targeted nests died as a result of remote treatment, even when more than 1000 bees took fipronil back to the nest.

2. Some colonies increased in activity as early as five days after treatment and needed a second treatment. However, treating a second time was not always possible due to difficulties in re-training bees back onto a feeding station.

3. There is a risk to non-target species from fipronil residue in dead and dying bees (bees contained up to 0.130 µg fipronil/bee) and in the comb (0.096 µg fipronil/g of comb). Particularly at risk are native invertebrates (e.g. *Tetragonula* sp.) and birds (e.g. Rainbow bee-eater), as well as feral and managed *Apis mellifera*.

4. A vast amount of time and effort is required to conduct trials in accordance with the required permit and WH&S regulations. In total, 1767.5 hours were spent on the treatments, which equal an average of 117.8 hours per trial, or 93 hours per treatment. The most time was spent bee-lining, training and maintaining bees on a feeding station, as well as monitoring nests after treatment.

5. Knowing the number of bees taking back fipronil is not sufficient to confidently predict success. It is necessary to know the nest entrance activity to determine a target number of bees, and to confidently predict success. To know the nest entrance activity, a nest needs to be found. And once a nest is found, then manually killing the nest is vastly more time and cost-effective than remote treatment.

6. Based on research to date, it is considered that sufficient data has been collected to evaluate the effectiveness and usefulness of remote treatments (i.e. agreed research

aims (i) and (ii)), but further research should be conducted on research aim (iii) by determining the effect that dead bees and comb containing fipronil has on non-target species, and ideally the toxicity of fipronil for *A. cerana* and any non-target species that may come into contact with fipronil.

Introduction

Honey bee colonies (both feral and managed) may need to be destroyed for various reasons. In particular, effectively destroying unwanted honey bee pests such as *Apis cerana* in Australia is highly desirable. Because feral colonies are generally difficult to find, baited sugar feeding stations are often used, where bees collect sugar syrup (laced with bait), which is then taken back to the nest, killing or suppressing the entire nest (Taylor, Goodwin et al., 2007). To achieve this, sufficient amounts of bait need to be taken back to the nest, which means that a delayed response to the bait is required so that foragers can make several trips between the feeding station and the nest. In addition, the bait station needs to attract sufficient numbers of bees (Taylor, Goodwin et al., 2007). Finally, the bait used needs to be safe for humans to use, and it needs to have low environmental impact, particularly on non-target species (Taylor, Goodwin et al., 2007).

A number of different bait chemicals have been trialled, with varying success, for their effectiveness in destroying or suppressing feral colonies, including, for example, Gramoxone, Avermectin and Ivermectin, Orthene 75S (acephate) and fipronil (reviewed in Taylor, Goodwin et al., 2007). Taylor et al (Taylor, Goodwin et al., 2007) trialled seven different chemicals in New Zealand and found that fipronil-containing insecticide was the most effective to destroy feral *Apis mellifera* colonies, i.e. of the seven chemicals, fipronil was the most toxic at low concentrations with a 3-hour response delay, while being relatively safe for humans.

Insecticides that contain fipronil as the key active constituent have also been trialled for controlling *A. mellifera* bees in Queensland, New South Wales and Western Australia (Keshlaf, Spooner-Hart et al.; Warhurst, 2001; Clark, T. et al., 2006) and for *A. cerana* in the Solomon Islands (Anderson, 2010). Two preliminary trials using fipronil on *A. cerana* were carried out in Cairns by Biosecurity Queensland in early 2011 (De Jong, 2011). These trials determined a high effectiveness of fipronil as a means of eliminating or suppressing bee colonies.

The goal of this study was to gain a better understanding of how varying forager levels of *A. cerana*, carrying fipronil back to the nest from a remote treatment station, would suppress or kill an *A. cerana* nest of a certain size.

The specific aims of our study were (1) to determine the effectiveness of remotely killing individual, feral *A. cerana* nests using an insecticide containing fipronil as the only active constituent, (2) to investigate the potential of this method as a useful management tool for *A. cerana*, and (3) to determine the potential effects of this treatment method on non-target species.

With this project due to be finalised by 30 June 2012, the purpose of this report is to update the Scientific Advisory Group (SAG) and Management Group with research details and results for the 15 trials (19 treatments) conducted by Biosecurity Queensland and to seek advice on any next steps.

Methods

Throughout the report, a “trial” is any treatment(s) conducted on a particular nest, i.e. we conducted 15 trials on 15 nests. “Treatment” is the actual treatment using a fipronil-baited feeding station. One nest (or trial) may involve several treatments. We conducted 19 treatments on 15 nests (=15 trials).

Prerequisites for treatments

Treatments commenced when:

i. a suitable *A. cerana* nest was located,

ii. regular movement of bees from the sugar feeding station to the nest was established,

iii. more than 20 bees were on the feeding station at any one time,

iv. the syrup station could be moved to a distance of approximately 80 metres from the nest,

v. the weather was fine, or there was a break in the weather,

vi. a licensed pest controller was available to perform the treatment, and

vii. the nest was able to be checked 24 hours, 48hr and 72 hrs after the treatment.

Feeding station

Bees were trained onto a feeding station containing sugar syrup (2kg of sugar to 1.5L of water plus one drop of lavender oil) by placing the feeding station near a floral source that bees were observed on. Once bees foraged on the feeding station, it was slowly (sometimes over several days or weeks) moved to approximately 80 meters from the nest. The final distance from the feeding station to the nest was measured and recorded. Weather observations including temperature, humidity, and cloud cover were also recorded each time observations of the nest or feeding station were made.

Nest Entrance & Foraging activity

Immediately prior to the remote nest treatment taking place, the level of nest entrance activity was counted at the nest targeted for treatment. This was conducted for a one-hour period (or for a shorter period that was then extrapolated to one hour), using a hand clicker, by clicking every time a bee flew into the nest. The foraging activity at the feeding station was also counted for 10 minutes immediately before the fipronil bait station replaced the feeding station. Counting was not carried out at 72 hours prior to the commencement of the treatment as requested by SAG due to the difficulty and unpredictability of training and maintaining bees on the sugar feeding station, the unpredictability of the weather, as well as staff shortages.

Fipronil treatment

When sufficient numbers of bees were feeding on the station (>20) and all other conditions were in place for a treatment to proceed (see ‘pre-requisites’ above), the regular feeding station dish containing sugar syrup was replaced with the bait station containing Regent 200SC (until 17/04/2012) or Termidor Residual Termiticide (from 17/04/2012) Insecticide and sugar syrup formulation (0.01g fipronil/L).

The baited feeding station was monitored until the target number of foragers feeding on the baited syrup was reached, or after one hour had elapsed (whichever occurred sooner). At this time the treatment was stopped by removing the baited station from the field and immediately replacing it with the original feeding station containing pure sugar syrup (no chemicals). If the targeted level of foraging activity was not achieved within the one-hour time limit, the experiment was stopped and the number of bees that had actually fed on the baited syrup was recorded. At five-minute intervals during the trial, behavioural observations were recorded, as was the number of bees feeding on the station. If non-target species were seen to be entering the station, they were actively discouraged from entering the station or destroyed, and a record of the occurrence made.

Once the baited station was replaced with the feeding station (no chemical), feeding station activity as well as nest entrance activity were monitored for up to 30 minutes, to assess the activity remaining at both.

Weather and time permitting, Biosecurity Queensland staff returned to the nest site every 24 hours after treatment to monitor the nest and feeding station foraging activity over a one-hour period by using the hand clicker. This was conducted for up to one week following a treatment, and every two to three days thereafter.

If nest entrance counts remained at zero for several days, the nest was checked using an endoscope, or, if too high, it was checked by a tree lopper contractor. Nests that were confirmed dead were extracted where possible. When the nest was considered dead (i.e. no bees were seen on the comb or nest activity remained at zero), the nest was extracted by Biosecurity Queensland staff or by a contractor. If the nest was not extractable the endoscope was used to capture photos/video of the dead nest components inside the nesting cavity. Nest entrances were plugged with paper towelling following successful destruction of the nest by remote treatment to reduce the possibility of residual effects of fipronil in the environment.

For extractable nests that were successfully destroyed, nest components were examined in the laboratory. Data recorded included a count of any dead bees found, number, size, area and weight of combs, the number of capped and uncapped worker, drone and queen cells present, and the number of cells containing nectar or pollen.

Second treatments

If the nest was not destroyed and showed signs of increasing nest entrance activity, a second treatment was conducted once nest entrance activity was at similar levels seen prior to the first treatment. The second treatment was done following the same procedures as for the first treatment, but with a higher target number of bees taking fipronil back to the nest if possible.

Target number of bees

One of the main objectives of this study was to determine the number of bees required to take fipronil back to the nest given a nest of a certain size. As the nest size could not be determined until after the nest was destroyed, and then only if a nest was extractable, an alternative, objective measure was needed to determine an *a priori* target number of bees.

Due to the difficulties of extracting most nests and lacking any other measure of nest size, the number of bees entering the nest was used as an alternative to actual nest size. A range of target numbers (expressed as percentage relative to nest entrance activity) were then used in order to determine the minimum number (percentage) of bees feeding on the bait station to effectively kill a nest of a certain size.

Data analysis

Nest size versus nest entrance activity

In order to determine how well nest entrance activity predicted nest size, nest entrance activity was plotted against different measures of nest size (including size, area and weight of the combs, and the number of cells of the combs). However, due to the low number of extractable nests (N = 7) no statistical analyses could be conducted.

Treatment success

The level of suppression of a treated nest was measured as the nest entrance activity after the treatment, relative to the nest entrance activity prior to the treatment, expressed as a percentage, i.e. the nest entrance activity prior to treatment was set at 100%. This allowed comparisons to be made between nests of differing size/activities.

To determine the best predictor of treatment success (treatment success being measured as the number of days until a nest was dead), treatment success was plotted against the following measures as possible predictors:

- The number of bees feeding on the baited station
- The percentage of bees feeding on the baited station relative to the nest entrance activity prior to the treatment
- The percentage of bees feeding on the baited station relative to the feeding station activity prior to the treatment

Nests that did not die after treatment needed to be included in the analysis, and so their time until “death” was set at 39 days – two days longer than the nest that took the longest to die.

A regression analysis is still to be performed to determine statistical significance and validate any trends.

Treatment effectiveness/efficacy

The number of total person-hours required to conduct the remote treatment trials was recorded for each treatment in order to determine the efficacy of remote treatment.

Effects on off-target species

All efforts were made to exclude off-target species from the bait station. However, any non-target species that came close to landing on the baited station, or that landed on the baited station had to be destroyed. Any species observed foraging on dead or dying bees, or robbing the weakened nest of nectar or pollen, were recorded.

In addition, *A. cerana* that had been feeding from the baited station were collected from several trials and sent to the Biosecurity Queensland Residues Testing Laboratory, Brisbane, to be tested for fipronil residues. Bees collected included those flying off the station, as well as those fitting/seizing on the ground. Residue testing was also carried out on bees collected from the nest entrance 48 hours following treatment. Comb from one treated nest was also sent to be tested for fipronil residue.

Where possible, non-target invertebrates were also collected for fipronil residue testing.

Results

Between February and June 2012, 19 treatments were conducted on 15 nests. Eight of these nests were located in an urban/residential area, four in sclerophyll woodland, two in rainforest and one in a rural/agricultural setting (Appendix 1). Seven nests were extractable, eight could not be extracted.

Seven nests (46.6%) were successfully destroyed after one treatment. Four nests (26.6%) were successfully destroyed after a second treatment. In total, 11 nests (73.2% of nests) were destroyed by remote treatment. Of the four remaining nests (26.6%), two were not destroyed after the first treatment but a second treatment was not possible as bees could not be re-trained back onto the feeding station. Another nest was not destroyed after the first treatment but a second treatment could not be done as by the time a second treatment could proceed, the target nest was occupied by *A. mellifera*. The fourth nest was treated and nest activity highly suppressed after 24 hours. However, *A. mellifera* were found robbing the nest and so the remaining colony (including any *A. mellifera*) was manually destroyed and the trial aborted.

Treatments were also attempted but could not proceed at two nests. One nest was prepared and ready for treatment but on the day of treatment it was found that the colony had absconded and the nest was overrun by green ants. Bees from the second nest could not be trained onto a feeding station despite weeks of field effort. These two nests are not included in the 15 treated nests or in any of the results.

At two nests, the feeding station could not be moved to a distance of 80m due to the fact that even after several attempts to move the station to the preferred distance over a number of days, the feeding bees would not cooperate. Instead, a distance of 15m and 25m was used.

Nest size

Seven of the 15 nests were extractable. The remaining eight nests could not be extracted as they were found within house wall cavities that could not be dismantled. Although the area and number of cells of the combs are yet to be determined, combs

have been weighed in order to categorise the nests into different sizes. Nests varied from 10g to 1803g and were categorised into three size classes (Table 1).

Nest entrance activity showed a slight increase with nest seize (weight; Figure 1). A statistical test still needs to be conducted to confirm the significance of this trend. However, nearly 30% of the variation in the data is explained by comb size, an indication that there is some merit to this relationship.

Table 1: Weight of combs, and activity at nest and feeding station (actual and percent) prior to treatment as well as time until nest died for seven remotely treated *A. cerana* nests that were extractable (sorted by weight).

IP	Weight of combs (g)	Activity at nest (1 hr)	Activity at station (10 mins)	No. of bees taking fipronil	% (rel. to nest activity)	% (rel. to station activity)	Time until nest dead (days)
566	10	606	60	42	7%	70.0%	6
606	95	1060	356	60	6%	16.9%	Did not die
591	176	3096	282	484	16%	171.6%	Did not die
558	392	5916	255	1022	17%	400.8%	5
609	511	1294	134	222	17%	165.7%	Did not die
609*	511	3576	864	1250	35%	144.7%	2
578	610	unknown	303	578	na	190.8%	Did not die
578*	610	3992	207	921	23%	444.9%	7
589	1803	unknown	377	1110	na	294.4%	Did not die

*A second treatment was conducted on these nests.

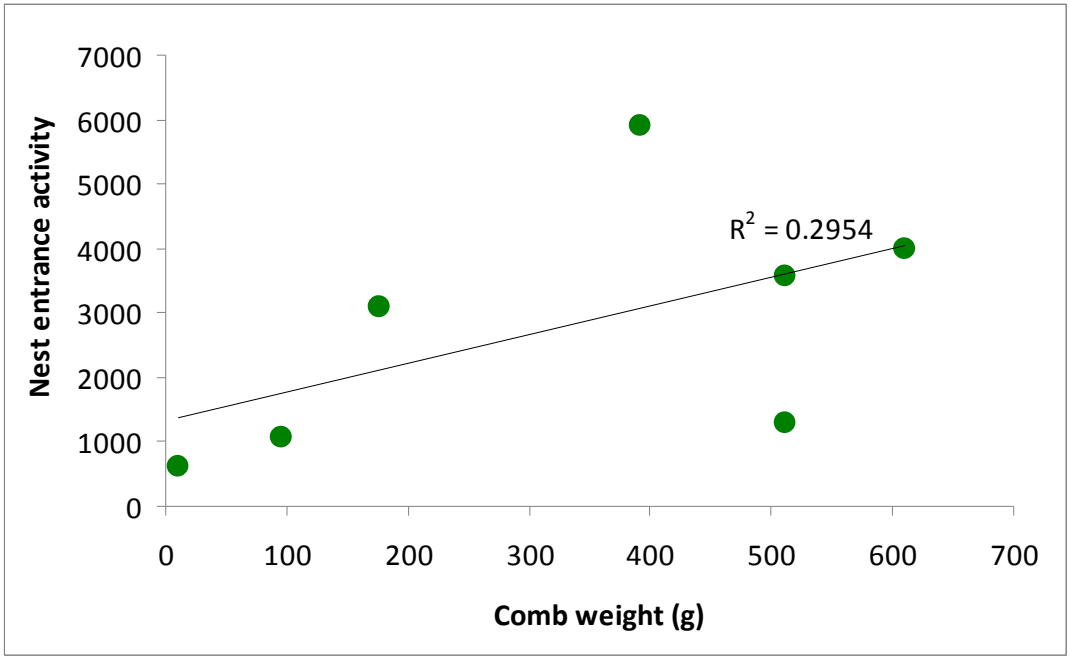

Figure 1: Nest entrance activity versus nest size (comb weight, g) for six remotely treated *A. cerana* nests. One nest was treated twice – it is represented twice in this graph. The second nest that was treated twice (see Table 1) had an unknown nest activity for its first treatment and is only represented once in this graph.

Nest & feeding station suppression

Shaky, fitting bees were observed on the bait station and the flight patterns of feeding bees exiting the bait station appeared disorientated and sluggish within 35 minutes of the treatment (N = 10). Dead and twitching bees were observed at the nest entrances for several days following treatment. On average, immediately after treatment, feeding station activity was reduced by 75% (N = 7) and nest entrance activity was reduced by 81% (N = 6).

In most of the 19 treatments, nest entrance activity was suppressed to at or below five percent (i.e. ≥95% reduction; N = 17) within 24 hours. One nest had a nest entrance activity of 19% (= 81% reduction) at 24 hours, and one nest had an increased nest activity 24 hours after treatment. On average, nest entrance activity 24 hours after treatment was 12.1% (Std. Dev. = 37.1%), i.e. a reduction of 87.9%.

Nest entrance activity generally stayed very low, particularly in those nests that eventually died (Figure 2). Nests that did not die after first treatment either showed no reduction in activity after treatment (IP609) or showed increasing activity from day four (IP557), day 12 (IP567) or day 13 (IP578).

When only considering successful treatments (i.e. nests that died after either the first or the second treatment; N = 12), average nest activity 24 hours after treatment was 1.3% (Std. Dev. = 1.8%), i.e. a reduction of 98.7%.

Nests that died after treatment did so, on average, within 8.1 days (min = 1 day, max = 37 days; N = 11).

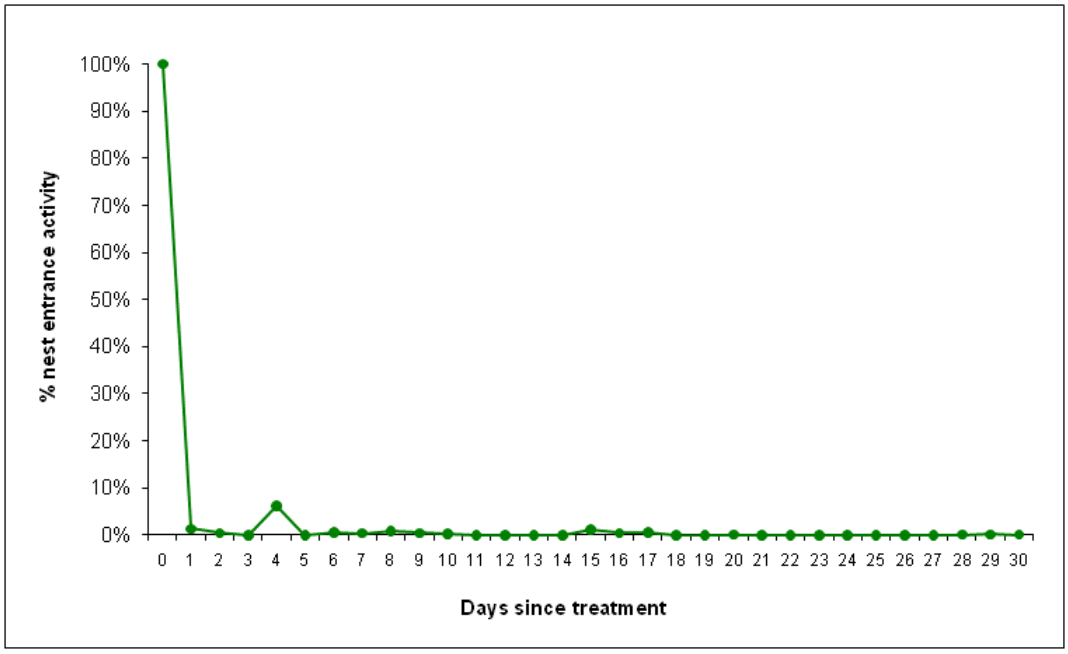

Figure 2: Average *Apis cerana* nest entrance activity of successfully treated nests (N = 11) in the 30 days following remote treatment using fipronil

Predicting treatment success

There was no relationship between the number of bees feeding on the baited station and the days until the nest was dead (Figure 3). In some trials, many bees took fipronil back to the nest but the nest did not die, in other trials very few bees took fipronil back to the nest and the nest did die (Figure 3).

There was also no relationship between the percentage of foraging bees relative to the feeding station activity prior to the trial and the days until the nest was dead (Figure 4). However, there seems to be a very weak trend – higher percentages of bees (>300%) foraging on the baited station result in shorter time until death. Nevertheless, variation is very high.

There seemed to be a weak relationship between the percentage of foraging bees relative to the nest entrance activity prior to the trial and the days until the nest was dead (Figure 5). Higher percentages of bees (>20%) relative to nest entrance activity foraging on the baited station resulted in shorter time until death. Although variation is still high at low percentages of bees (i.e. nests may or may not die when low percentages of bees take back fipronil), variation is much lower when high percentages of bees take back fipronil (i.e. nests die quickly when high percentages are involved; Figure 5).

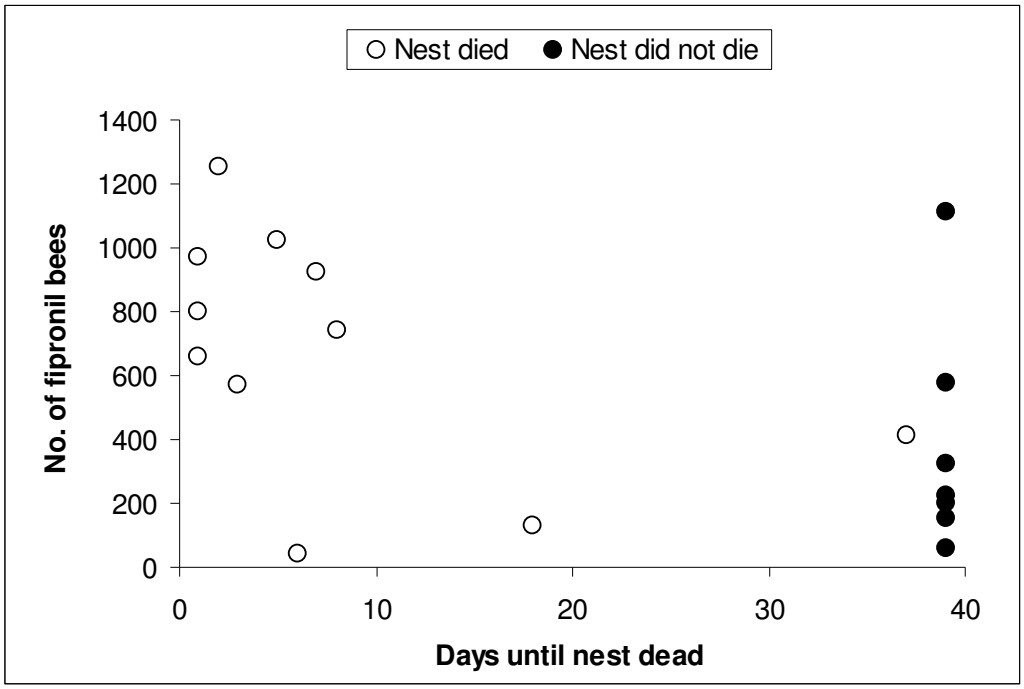

Figure 3: **Number of days until *A. cerana* nests died (or did not die) after a certain number of bees foraged on a fipronil-bated station. Nests that died are depicted as clear circles, whereas nests that did not die after treatment are depicted as black circles.**

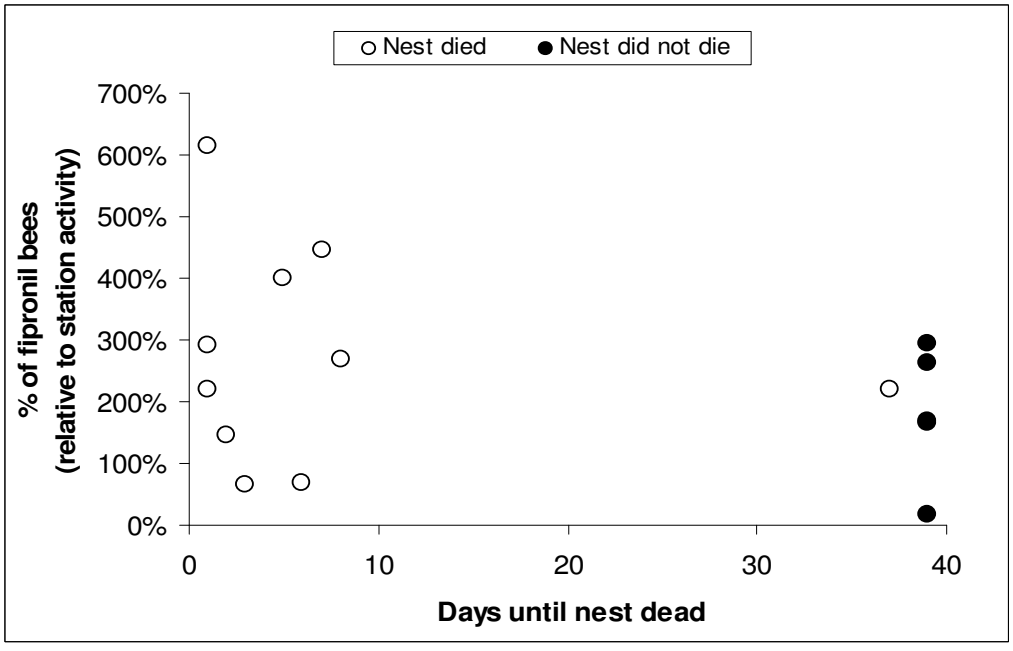

Figure 4: **Number of days until *A. cerana* nests were dead after a certain percentage of bees (relative to feeding station activity prior to treatment) forage on a fipronil-bated station. Nests that died are depicted as clear circles, whereas nests that did not die after treatment are depicted as black circles.**

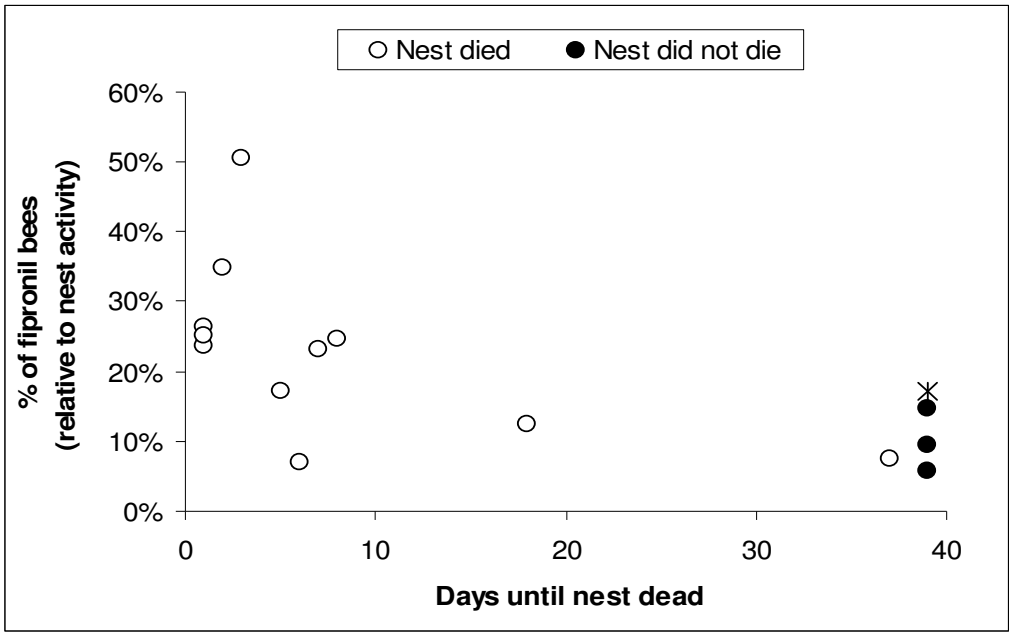

Figure 5: Number of days until *A. cerana* nests were dead after a certain percentage of bees (relative to nest entrance activity prior to treatment) forage on a fipronil-bated station. Nests that died are depicted as clear circles, whereas nests that did not die after treatment are depicted as black circles.

Treatment Efficacy

For all treatments combined, 1767.5 hours were required to conduct the 19 remote treatments on 15 nests, which equals an average of 117.8 hours per trial, or 93 hours per treatment (combined hours for a field team of two people, plus one scientist and one pest controller for the actual treatments).

The minimum amount of time needed was 33.5 hours (IP556) due to its proximity to the Biosecurity Queensland offices as well as the ability of field officers to conduct the treatment themselves. Once the safety measures were reviewed by Biosecurity Queensland WH&S officers, a trained pest controller was the only person allowed to conduct the treatment (i.e. handle the chemical). The maximum amount of time taken for a trial was 320 hours (IP578).

Hours include driving to and from the site, bee-lining nests, setting up feeding stations, training bees onto a station, maintaining bees on the station, nest and feeding activity counts prior to and following treatment, preparing for, conducting and cleaning up after the treatment, as well as a small amount of time for data entry and report writing. However, the estimate does not include any time spent by the scientist and senior scientist, operations coordinator, data entry clerk or program manager (including, for example, meetings, operations planning, revising and re-writing experimental procedures etc.).

Non-target species

Non-target species that were observed coming close to the bait station, or that did land on the bait station and had to be destroyed, included native bees (mostly *Tetragonula* sp. as well as bees of the family Halictidae), wasps, flies, and *A. mellifera*.

Non-target species that were observed to rob honey or pollen from the treated nest or to eat dead or dying bees include *A. mellifera*, green ants (*Oecophylla smaragdina*), sugar ants (*Camponotus sp.*), cockroaches (common house cockroach variety), lizards and cane toads (*Bufo marinus*).

Residue testing on dead and fitting bees and comb showed presence of fipronil and its metabolites, i.e. fipronil desulfinyl, fipronil sulphide, and fipronil sulfone. Highest levels of total fipronil (0.130 µg/bee) were found in dead or fitting bees immediately after the end of treatment, i.e. after one hour. Fipronil levels then decreased over time but were present at detectable levels for 48 hours (Table 2). Comb also showed relatively high levels of total fipronil after 24 hours.

A. mellifera were also collected for residue testing. However, the number collected was too low to be able to detect the presence or absence of fipronil. No other non-target species were collected or tested.

Figure 6: Levels of fipronil detected in bees and comb.Bees/comb samples for residue testing were collected from a range of trials.

Bees/comb tested	Sample	Total fipronil reported
After 2-3 feeds on bait station	Bees	0.020 µg/bee
Immediately after end of treatment (multiple feeds over 1 hr)	Bees	0.130 µg/bee
24 hours following treatment	Bees	0.038 µg/bee
Comb (24 hours following treatment)	Comb	0.096 µg/g
48 hours following treatment	Bees	0.004 µg/bee

Discussion

In this study, the effectiveness of remotely treating individual, feral *A. cerana* nests with fipronil was demonstrated, as an almost immediate and severe suppression of the bee colony was observed within 24 hours of treatment for most nests. Indeed, bees foraging on the baited station showed adverse effects within 30 minutes. Similar immediate responses were found previously (Keshlaf, Spooner-Hart et al.; Warhurst, 2001; Taylor, Goodwin et al., 2007; Anderson, 2010; De Jong, 2011).

The number of bees as well as the percentage of bees relative to the feeding station activity, prior to the treatment that took back fipronil, did not seem to be good a predictor of success (Figures 3 & 4). However, the percentage of bees taking back fipronil relative to the nest entrance activity prior to the treatment did seem to predict whether or not a nest would be dead within a few days (Figure 5). All nests that had more than 20% of bees taking back fipronil died within seven days (Figure 5). Nests that had a lower percentage of bees taking back fipronil mostly died much later or not at all (Figure 5).

Plotting the number of bees taking back fipronil against treatment success did not determine a minimum number needed in order to kill a nest (Figure 3). However, if we take the nest with the largest nest entrance activity that was successfully destroyed within one week (IP558 – 5918 bees/hour), the number of bees required in this instance was 1022. So if an inference of a minimum number of bees that needs to take back fipronil is to be made, one could say that at least 1000 bees are needed to take fipronil back to the nest. This is a rather large number that was only achieved in three of the 19 treatments – two of these were destroyed successfully within one week, one nest still did not die.

This result means that even a minimum number of 1000 bees taking back fipronil cannot guarantee success in remotely treating a feral *A. cerana* nest. Instead, to predict success with some confidence, it is necessary to find the nest and calculate a target number of bees relative to the nests' entrance activity. However, if the nest needs to be found, then it may as well be destroyed using, for example, an aerosol spray insecticide, which would kill the nest quickly and immediately, rather than conducting a very time consuming remote treatment.

Although the relative sizes of all individual nests could not be compared (as only seven of the nests were extractable), nest entrance activity of those that could be extracted did seem to increase with increasing nest size (measured as comb weight). Together with the finding that a target percentage relative to nest entrance activity did predict treatment success we can conclude that nest entrance activity can be used as an alternative for nest size for the purpose of remote treatments.

Off-target species

Off-target species may come into contact with fipronil through direct contact on the bait station as well as through robbing nest components (wax, honey, pollen) after a nest has been destroyed, or through eating dead and dying bees. All efforts were made to exclude off-target species from the bait station. However, off-target species that were observed close to or on the bait station, robbing honey or pollen or eating dead bees include native bees, *A. mellifera*, green ants, sugar ants, wasps, flies, cockroaches, lizards and cane toads. Other species that could potentially be affected but have not been directly observed include birds or mammals preying on flying or

dead bees (especially the Rainbow bee-eater, *Merops ornatus*) or robbing honey from dead nests.

Toxicity of fipronil to some organisms has been tested (reviewed in Gunasekara, Truong et al., 2007; DEWHA, 2010). Fipronil is highly toxic to *A. mellifera* at a LD_{50} of 0.004 µg/bee (Gunasekara, Truong et al., 2007). Although toxicity is unknown for *A. cerana* it can be assumed to be similar if not higher due to *A. cerana*'s smaller body size. In fact, fipronil was found to be seven times more toxic to the stingless bee *Scaptotrigona postica* in Brazil (LD50 = 0.00054 µg/bee; Jacob, Soares et al., 2013) compared to its toxicity to *A. mellifera*. Stingless native Australian bees such as *Tetragonula* and *Austroplebeia* species were commonly observed on and around bait stations during the trials and so unless they can be excluded from bait stations it is very likely that small native bees will be affected by off-target impacts of fipronil.

Suggestions have been made to increase the concentration of fipronil in the sugar syrup. However, these are unfounded, and increasing the fipronil concentration may even have adverse effects on the remote treatment. Bees were affected within 20-30 minutes from the start of the treatment – a higher concentration may shorten the time until bees are affected, meaning bees may not find their way back to the nest – crucial for successful remote treatments. Furthermore, bees were found to have fipronil levels thirty times higher than the LD_{50} for *A. mellifera*, and higher concentrations of fipronil would also result in even higher residues found in the bees and nest components, increasing the risk to non-target species.

Fipronil is also highly toxic to cockroaches, which have been observed at dead nests. A German cockroach only needs to consume the equivalent of one-tenth of a bee for a lethal dose (LD50: 0.0046-0.0054 µg/cockroach; Gunasekara, Truong et al., 2007). Many native cockroaches are smaller than German cockroaches, and so are likely to be affected by fipronil residue.

Lizards were also observed at dying and dead nests. Scientists studying the toxicity of fipronil in West Africa reported that fipronil were highly toxic to the Fringe-toed lizard *Acanthodactylus dumerili* (Peveling and Demba, 2003). An LD_{50} in the order of 30 µg fipronil/g bodyweight was calculated for this species. If the toxicity of fipronil to native lizards here in Australia is similar to Peveling and Demba (2003)'s findings, it would seem that the concentrations used in this experiment are unlikely to affect lizards of the same size or larger – more than 1000 fipronil-affected bees would need to be consumed. However, because fipronil toxicity for native lizards is unknown, precaution needs to be taken.

Birds such as Rainbow bee-eaters prey on bees and could potentially be affected by fipronil if they catch bees that have just taken fipronil. Similar to many other free-living bird species, the toxicity of fipronil to Rainbow bee-eaters is unknown. However, several studies have shown that accidental consumption of fipronil by some birds has the potential to adversely affect their reproduction, development and behaviour (Kitulagodage, Buttemer et al., 2011; Kitulagodage, Isanhart et al., 2011). Fipronil is deemed to be highly toxic to the Bobwhite quail (LD_{50}: 11.3 µg/g), Red-legged partridge (LD_{50}: 34 µg/g) and Pheasant (LD_{50}: 31 µg/g), while fipronil toxicity is somewhat lower in the House sparrow, Pigeon and Mallard duck (LD_{50}'s: >1000 µg/g) (DEWHA, 2010). Again, a precautionary approach should be applied by assuming that fipronil may be toxic to Rainbow bee-eaters until it is shown otherwise.

While it appears that fipronil breaks down rather quickly in bees (Table 2), the level of residue testing conducted throughout this experiment was limited. It is not known,

for example, how quickly fipronil will degrade in hive comb over time in various Australian environments. More research is essential to investigate the risk of fipronil residue to non-target species.

Treatment Efficacy & Difficulties

It was difficult for field staff to ensure that consistent environmental conditions were maintained between days for bee counts and treatments due to erratic weather conditions earlier in the year. It also proved difficult to ensure that bees were continuously foraging on the sugar feeding station so that a second treatment could be carried out on those nests that were not killed with one treatment. Bees seemed to 'go off' the syrup within 24 hours of treatment. During trials using fipronil on bees in New Zealand, Taylor et al. (2007) also found that any disturbance that caused a break in recruitment such as weather or lack of syrup required the bees to be retrained onto the bait stations. They also noted that when more attractive or plentiful nectar sources were available, foraging at the bait station may not be successful (Taylor, Goodwin et al., 2007).

Trials for this preliminary study in Cairns had to be extremely opportunistic due to the unpredictability of the weather and due to the variability in bee numbers feeding on sugar stations from day to day. Visiting each potential nest site frequently was vital so that assessments of when bait stations should be applied in the field could be made. The process proved to be highly labour intensive. The number of human visits (including the driving time between sites) required to keep the stations filled and bees interested as well as monitoring nest activity for hourly periods following treatment were very high. Some individual nests required >300 hours for a team of two field officers to maintain, treat and monitor.

Conclusion

This experiment showed that fipronil is very effective at suppressing and killing individual Asian honey bee colonies if more than 20% of bees relative to nest entrance activity take back fipronil to the nest. However, the usefulness of remote treatment as a method to manage *A. cerana* in Australia is doubtful due to several reasons: (1) not all targeted nests died as a result of remote treatment; some colonies increased in activity as soon as 5 to 12 days after treatment and needed a second treatment; however, treating a second time was not always possible due to difficulties in training bees back onto a feeding station; (2) there is a real risk to non-target species from fipronil residue in dead and dying bees as well as in the comb. Particularly at risk are native invertebrates and birds, as well as feral and managed *A. mellifera*; (3) the vast amount of effort required to conduct trials makes this method very time and resource consuming; and finally, (4) knowing the number of bees taking back fipronil is not sufficient to predict success; it is necessary to know the nest entrance activity to predict success, for which the nest needs to be found; if the nest is found, then manually killing the nest is vastly more time and cost-effective than remote treatment.

Based on this research, it was considered that sufficient data had been collected to evaluate the effectiveness and usefulness of remote treatments for the purpose of the T2M program. Further research should be conducted on residue testing as well as determining the effect that dead bees and comb containing fipronil has on non-target species.

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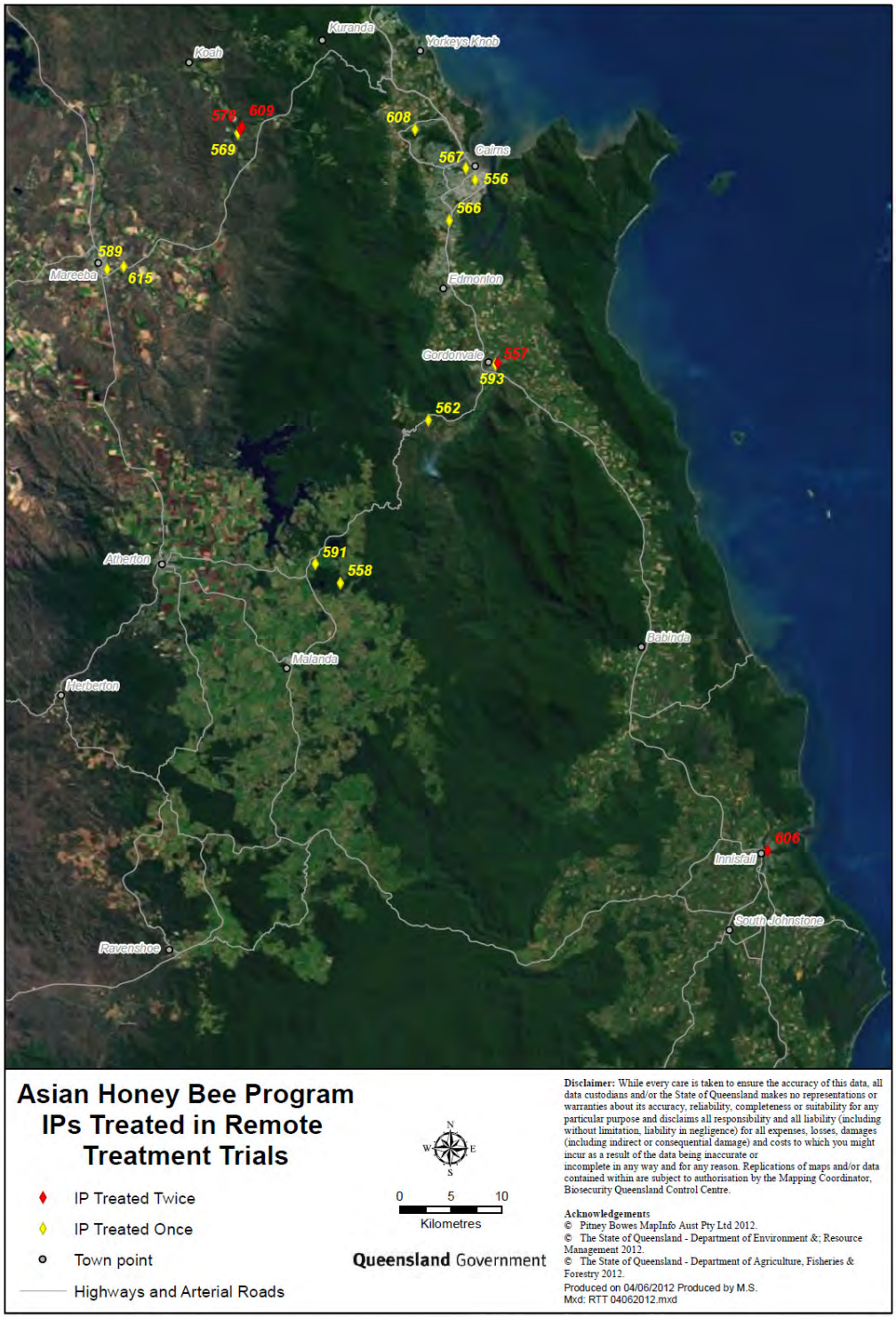
Appendix 1 Map showing locations of nests used for trials



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