

Asian Honey Bee Remote Nest Treatment

Results from 5 nests (and 2 attempted trials) current at 02/04/2012

IP556 IP557 IP567 IP562 IP566 IP563 IP569

Progress report prepared by Shirin Hyatt
Asian Honey Bee Transition to Management Program

Biosecurity Queensland

02/04/12

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Summary

Preliminary trials of remote nest treatment were carried out on five Asian honey bee nests between 5th March 2012 and 2nd April 2012 in the Cairns region. Further trials on two nests were also attempted during this period; however trials on these nests ceased due to interferences with the nest/trial. For four nests one treatment occurred, and for one nest two treatments occurred. Wet weather was a problem during the duration of trials, and made it difficult to ensure that consistent environmental conditions were maintained between days for bee counts/treatment. 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. One nest was killed within 24 hours following one single treatment (this nest had 973 bees exiting the bait station carrying fipronil). Another nest was killed within 144 hours following a single treatment (42 bees carried fipronil back to a very small nest). The three remaining nests remain active as at 02/04/12 (these nests had a relatively low number of bees exiting the bait station carrying fipronil; 150-400 bees), however nest entrance activity has slowed considerably since the 24 hours after treatment. Nest activity for these four nests remains considerably low as time goes by. Only one of the nests that have been treated in this preliminary experiment was able to be completely extracted (IP 566) to allow for an examination of comb and accurate assessment of nest size.

Introduction

This preliminary experiment was conducted on five Asian honey bee (AHB) nests identified by Biosecurity Queensland. It was designed to gain a broad understanding of how varying forager levels of AHB, carrying fipronil back to the nest from a remote treatment station, would suppress or kill an AHB nest of a certain size.

Aim

To remotely kill individual nests using an insecticide containing fipronil as the only active constituent and to investigate the potential of this method as a useful management tool of Asian honey bee. The purpose of this report is to provide SAG with comprehensive details and results for each of the first five trials conducted by Biosecurity Queensland so that a clearly defined procedure can be developed and implemented for the remainder of the remote treatment trials.

Materials

- For each bait station:
 - Multi-flow plastic feeding tray x 2 (one to be used with regular syrup and the other one to be used with Regent 200SC for half hour poisoning trials)
 - Washed river sand (enough to cover the bottom of the feeding trays 2-3cm deep). Sand must be completely dry when used for trials (to avoid dilution of chemical).
 - All weather hoods for feeding trays
 - Collapsible card table or wooden garden stake with flat wooden square board (30cm x 30cm) affixed to top.
 - Personal Protective Equipment (PPE) as per MSDS for handling Regent 200SC Insecticide
 - Sugar solution – 2 litres of warm water to one kilo of white sugar, when dissolved, add 2 drops of lavender oil as a scent attractant.
 - Regent 200SC Insecticide (containing 200g/L fipronil as its only active constituent)
 - Glass pipette
 - Container for mixing chemical and syrup
 - Warning signage for placement around treatment area
- Lockable storage boxes for contaminated materials (PPE, used pipette, used feeding tray containing syrup and sand, used mixing container)
- Vehicle with appropriate warning signage and lockable chemical storage facilities.
- Chemical Spill Kit

- Stop watch
- Mobile phone (in case of emergency)
- GPS
- Trundle wheel (to measure distance from nest to feeding station)
- Thermometer (to measure air temperature)
- Endoscope (to examine cavities inside small openings)

Methodology

Trials

Trials commenced after:

- 1) a suitable AHB nest was located,
- 2) regular movement of bees from the sugar feeding station to the nest was established,
- 3) the syrup station could be moved to a distance of approximately **80 metres*** from the nest and
- 4) the weather was fine, or there was a break in the weather.

**note: The syrup station for IP 566 was unable to be moved to a distance of 80m. A distance of 25m for this trial was used instead. This was because after several attempts to move the station to the preferred distance over a number of days, the feeding bees would not cooperate.*

Prior to the remote nest treatment taking place, the level of foraging activity was counted at each of the nests identified. This was conducted for a 1-hour period, with a hand clicker, clicking every time a bee flew into the nest. The foraging activity at the nest entrance was conducted for the 1 hour immediately* before the fipronil bait station was placed into the field.

**note: counting was not carried out at 72 hrs prior to the commencement of the trial owing to time constraints and also because trials had to be opportunistic due to the unpredictability of the weather and bee numbers on sugar stations from day to day.*

The exact distance from the feeding station to the nest was measured and recorded. Weather observations including temperature, humidity, and cloud cover were also recorded.

The regular feeding station on which *A. cerana* were currently foraging on was then replaced with the feeding station containing the Regent 200SC Insecticide and sugar syrup formulation (mixed at a rate of 0.025ml per 500ml of sugar syrup)

Each of the 5 nests identified in this experiment required a different level of foraging activity to explore/determine how many foragers might be needed to kill a nest of a certain size. Once the targeted level of foraging activity was reached to the best of Biosecurity Queensland's ability at the bait station, the trial stopped.

Counting was achieved by using a hand clicker to count bees leaving the bait station until the specific level* of foraging activity was achieved.

**note: the target foraging activity to be achieved was determined on a nest to nest basis by Biosecurity Queensland. Target forager numbers were based on the activity observed at the nest entrance as well as the regular feeding station, and a range of targets were trialled (between 40 and 1000 bees).*

The baited feeding station was monitored until the target level of forager activity feeding on the syrup containing fipronil was reached for that specific nest and then treatment was stopped. If the targeted level of foraging activity was not achieved within the 1 hour time limit as stated on the permit, the experiment was stopped and the number of bees that had actually fed on the fipronil syrup was recorded. Behavioural observations during the trials were also recorded. If other non-target species

were seen to be entering the station, they were actively discouraged from entering the station or destroyed.

When the 1 hour time limit was reached, or the target number of foragers was reached, the baited feeding station was removed from the field and immediately replaced with the original syrup station containing pure sugar syrup (no chemical).

The original feeding station was then monitored for periods of up to 30 minutes to assess the frequency of foraging remaining at the station.

Weather and time permitting, BQ staff returned to the nest site every 24 hours after treatment to monitor the nest and sugar station foraging activity. Nest activity was assessed over a 1 hour period, with a hand clicker, clicking every time a bee flew into the nest and the numbers were recorded.

If the nest was dead as a result of the fipronil at 48 hours after treatment, an extraction of the nest was conducted where achievable. If the nest was not able to be extracted, then an endoscope was used to capture photo and video of the dead nest components inside the nesting cavity. If the nest was not completely destroyed after 2 days or at 48 hours, it was left for another two days (and monitored at 72 hours where possible). If at 96 hrs, the nest still had not died then the nest was exposed to another* dose of Fipronil, using exactly the same methodology and conditions as described above.

**note: it was not always possible to do a second treatment at 96 hours after the initial treatment. This was because no foraging bees were evident on the regular sugar feeding station at 96 hours even though the nest was still active at this time. BQ had to try and 'train' the bees back onto the station and return the station to the appropriate distance from the nest so that a 2nd treatment could occur – this proved difficult and time consuming to achieve – especially in wet weather.*

Where a second treatment was not able to occur at 96 hours due to absence of bees on the station, then the nest activity was still monitored every 24 hours (where possible) until foragers again returned to feed on the sugar station. When foragers returned, then a second treatment took place using the methodology and conditions described above.

Advice received towards the end of the trials on these five nests (from Plant Health Australia on 16th March) recommended that Biosecurity Queensland only conduct one treatment on each of the nests and monitor them daily to assess the suppression in nest activity over time (rather than do a second treatment at 96 hours as the methodology first suggested). One nest received two treatments before this advice was received.

When the nest was considered dead it was extracted by Biosecurity Queensland staff or by contractor. Otherwise an endoscope was used to capture photos/video of the dead nest components inside the nesting cavity. The only nest that was extractable for these trials was IP 566. An endoscope was used where possible to take photos of the other nests following treatment. Nest entrances were plugged immediately with paper towelling following successful destruction of the nest by remote treatment avoiding the possibility of residual effects of fipronil in the environment.

For extractable nests that were successfully destroyed, nest components were examined in the laboratory. A count of dead bees, estimate age of colony, number of combs, size of combs, queen cells present, amount of nectar or pollen present and any other relevant information were recorded.

After the 5 trials were commenced and adequate data from the trials obtained, a brief experimental report (including introduction, methodology, results, discussion etc.) was written (this document) by the technical advisor of the AHB program and provided to the SAG.

Results

Treatment on 5 nests:

Results suggest that this method of remote nest treatment will severely suppress bee numbers within individual nests within 24 hours regardless of foraging numbers on the baited sugar station.

IP 556 was the only nest to show a 100% reduction in nest entrance activity at 24 hours (this nest was confirmed dead with an endoscope at 48 hours) (Figure 1) whilst IP 557 showed a 81% reduction in activity (Figure 2), IP 567 showed a 94.5% reduction in activity (Figure 3), IP 562 showed a 96.3% reduction in activity (Figure 4) and IP 566 showed a 98.7% reduction in activity at 24 hours (Figure 5).

All nests showed considerable decline in nest entrance activity in the days following initial treatment. Activity for IP 567 began to decline as time lapsed after treatment; however this nest has recently shown increased activity since 504 hours. IP 562 has shown fluctuations in nest entrance activity as time goes by, but still remains low (less than 250 bees entering nest in 60 minutes). IP 557 was the only nest where nest entrance activity began to increase within merely 48 hours following treatment. A second treatment was conducted on this particular nest (after considerable time was spent to re-train bees back onto the syrup station) at 240 hours after which nest activity again plummeted in the 24 hours following the second treatment and has since remained fairly steady (Figure 2). IP 566 was dead at 144 hours following one treatment (Figure 5)

Figure 1: NEST IP 556

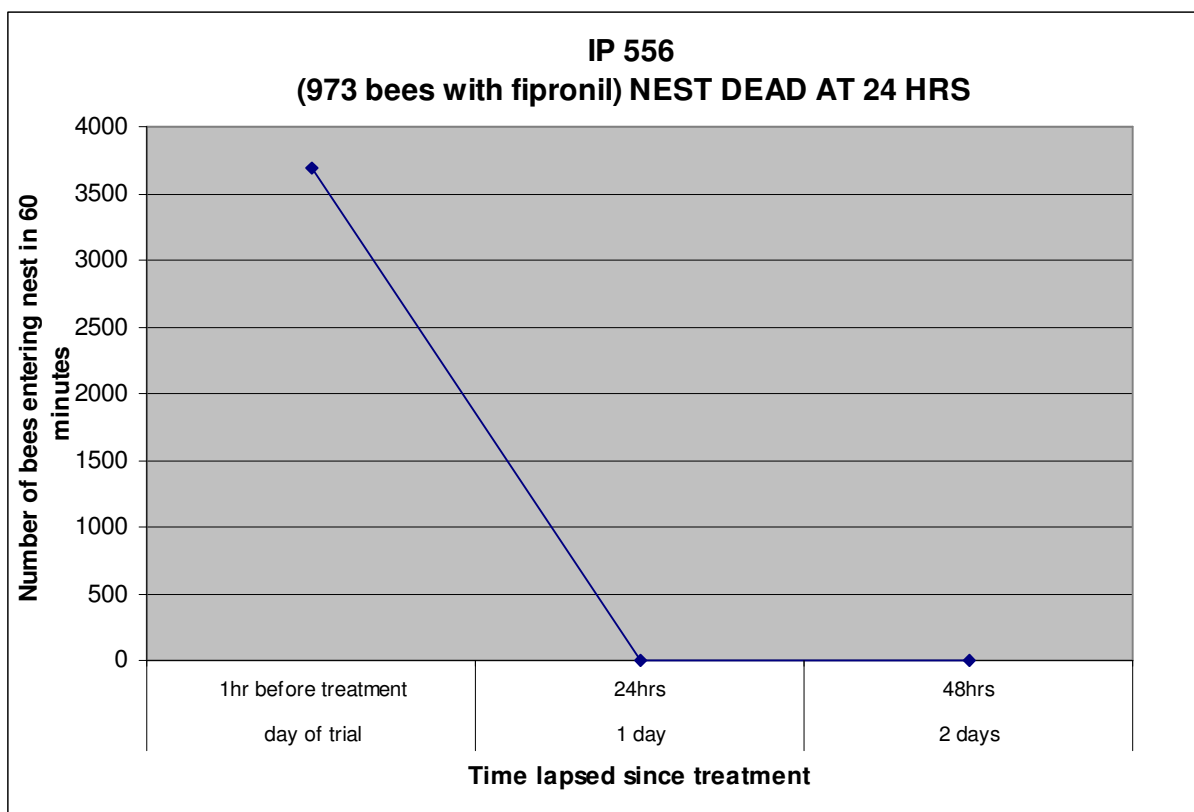


Figure 2: NEST IP 557

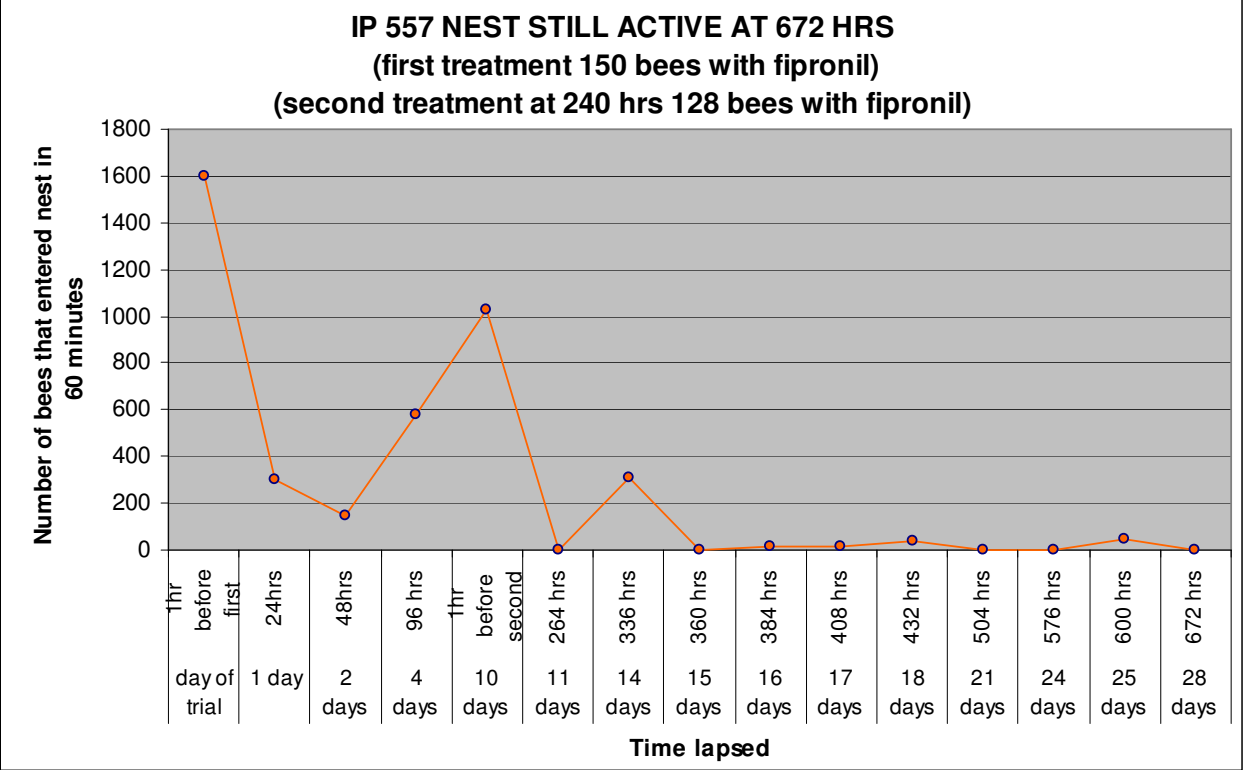


Figure 3: NEST IP 567

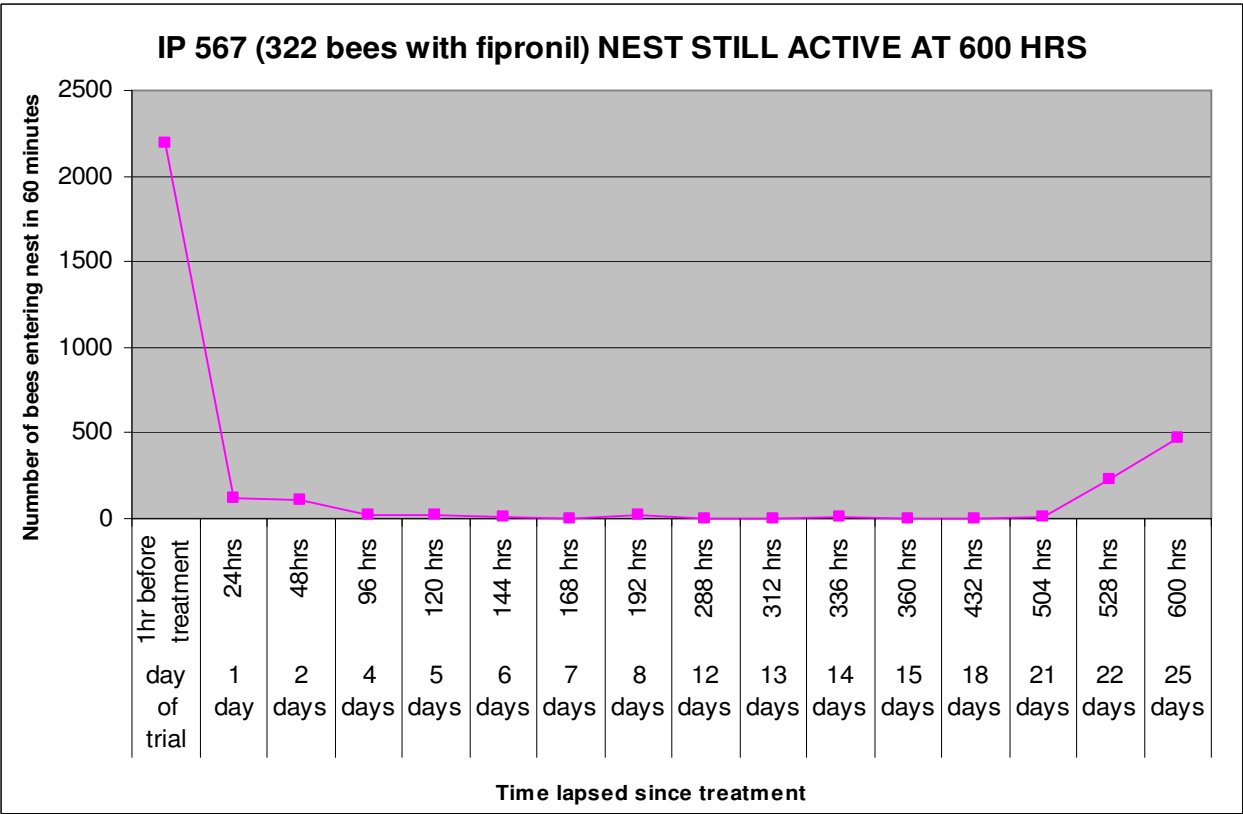


Figure 4: NEST IP 562

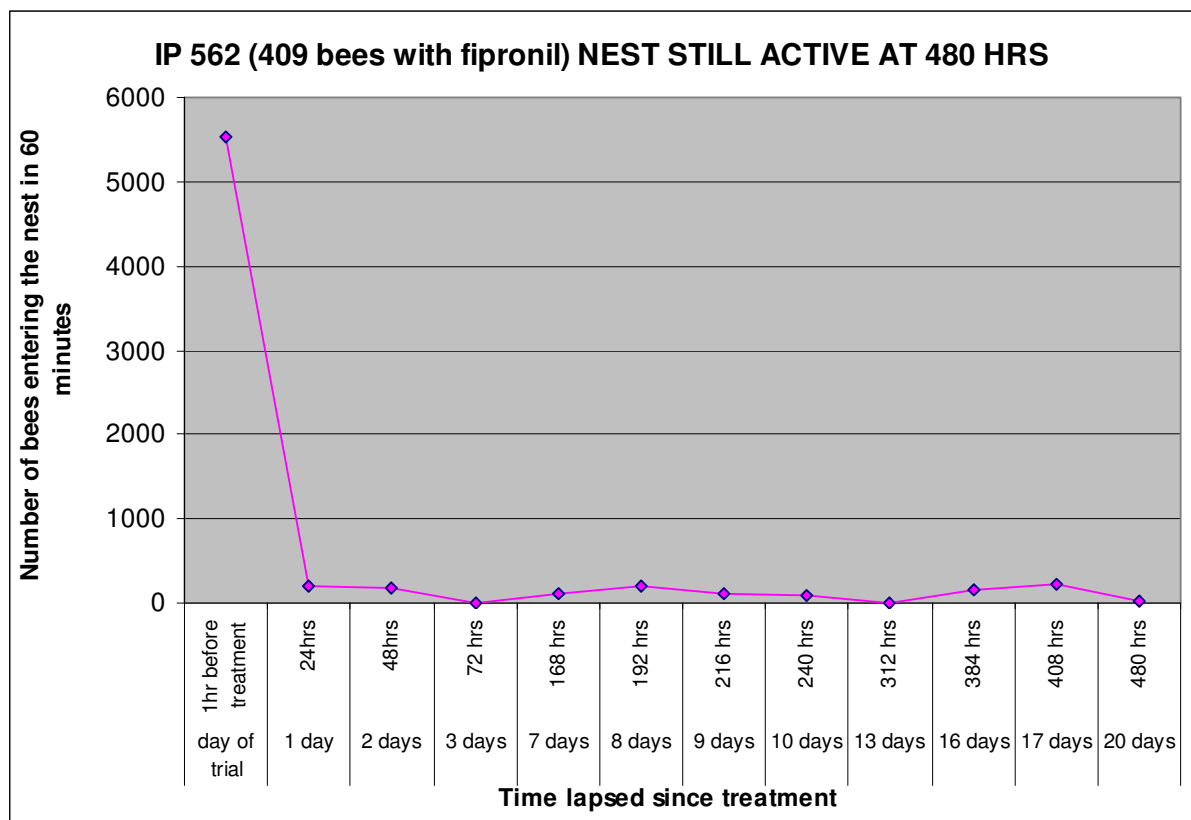
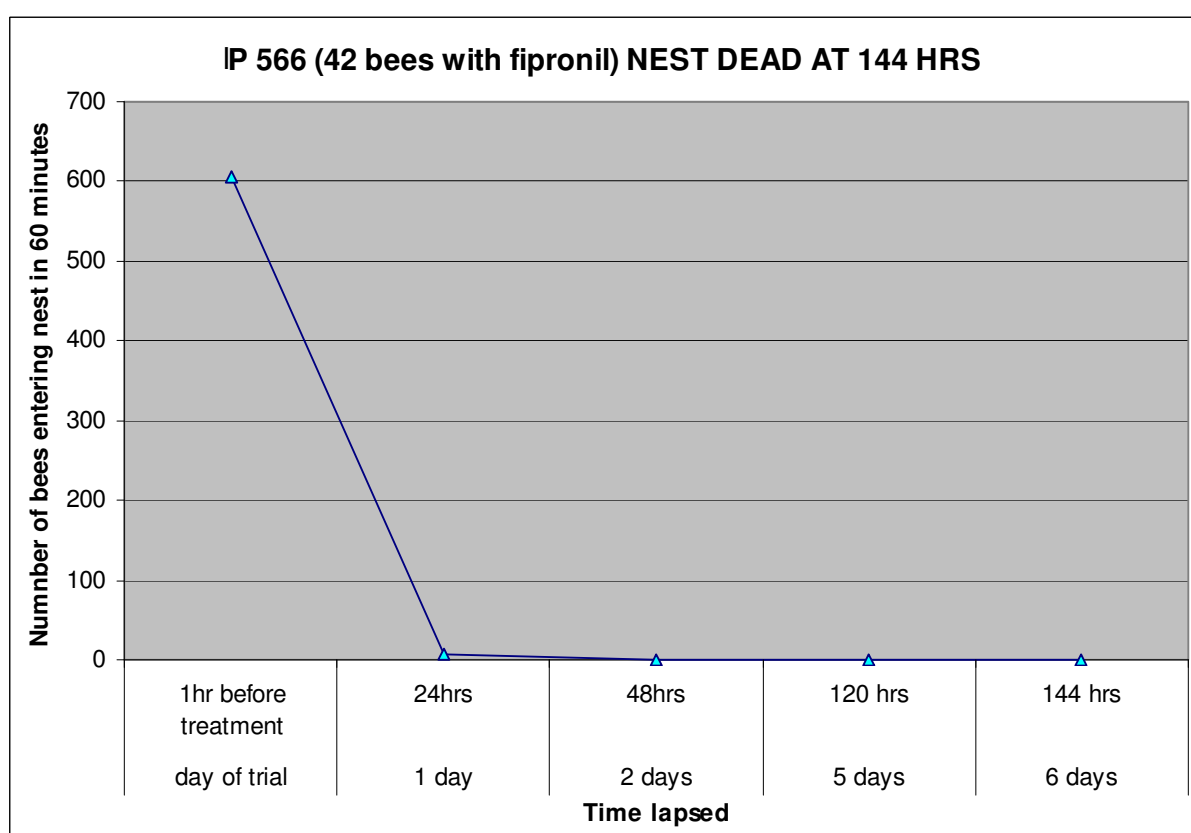


Figure 5: NEST IP 566



Discussion

Insecticides that contain fipronil as the key active constituent have been previously trialled for controlling *Apis mellifera* bees in Queensland, Western Australia, New Zealand (Warhurst 2001, Clark et. al., 2006, and Taylor et. al. 2007) as well as for *Apis cerana* in the Solomon Islands (Anderson 2010). Two preliminary trials using fipronil on Asian honey bees were carried out in Cairns by Biosecurity Queensland in early 2011 (De Jong 2011). All of the authors above have noted the effectiveness of fipronil as a means of eliminating or suppressing bee colonies. It is difficult to compare these studies to the trials that Biosecurity Queensland are currently conducting due to the unknown number of foragers carrying fipronil back to individual nests in these studies. Also, many of the trials were aimed at eliminating or suppressing more than one nest from a single bait station in environments likely containing higher densities of bees than in Cairns.

In this experiment, the effectiveness of remotely treating Asian honey bees nests with fipronil was demonstrated almost immediately and severe suppression of the bee colony was certainly observed within 24 hours of treatment on each nest.

As expected, nest entrance activity following treatment appeared to be related to the number of bees that had carried fipronil back to the nest. Gupta et. al (1984), cited in Hepburn and Radloff (2011), reports that the average nectar load of *A. cerana* foraging on *Plectranthus rugosus* was 18 μ l. The concentration of Regent 200SC Insecticide (active constituent 200g/L fipronil) used for these preliminary trials was 0.05mls of Regent per litre of sugar syrup. From this it can be calculated that an individual AHB took approximately 18×10^{-6} ml of actual fipronil back to the nest after foraging on our bait station – a minute amount. This calculation could be used to determine the relative amounts of actual fipronil contained within an insecticide solution that is needed to kill individual nests of a certain size.

It is largely unknown exactly how long these low amounts of fipronil will last in a natural setting and what the effects on non target organisms may be. It has been reported that fipronil degrades much faster under tropical conditions than temperate (Tingle et al 2000) however literature mainly relates to studies carried out in temperate environments. Half lives of fipronil have been calculated in soil (in various soils maintained at 22-25°C under anaerobic conditions, half life was 18-308 days dependant on soil type, pH and organic matter). Fipronil's half life on treated surfaces has been determined at 3-7 months depending on the substrate and the habitat where it is applied (Tingle et al 2000). Given the extremely low amounts of actual fipronil that is carried back to the nest by individual bees (when using Regent at the concentration specified in Biosecurity Queensland's permit), it would be expected that is unlikely that residual levels will cause detrimental effects for non target species within the natural environment, however a more accurate assessment may need to be made.

Whilst the relative sizes of individual nests could not be accurately compared for these particular 5 trials (due to only one of the nests being suitably extractable from the nesting cavity), it would be expected that the size of the nest would also have some impact on the effects of the insecticide. It was difficult to gauge nest size based on the activity at the nest entrance before trials commenced. Past experience (as part of the AHB eradication program) has indicated that although a feral bee nest may appear 'busy', it is still possible for the nest to be quite small as foragers may be collecting nectar or pollen from floral resources close by to the nest and the same bees are entering and exiting the nest frequently (also, activity may depend on the time of the day and the type of floral source that the bees are targeting). An attempt to locate literature that suggests a reliable 'method' for estimating the size of feral bee nest colonies resulted in little information being obtained regarding this issue.

An endoscope was used where possible (at least 48 hours after treatment and when minimal disturbance to the nest could be ensured) to try and get an idea of the nest comb size/live bees left within the cavity. Nest IP 557 was unable to be looked at with the endoscope as the nesting cavity entrance was too small to insert the endoscope and the nest appeared to be behind a flat wooden pole within the wall. IP 562 was also unable to be looked at by field staff with an endoscope due to the height of the nest. For most of these trials, dead bees were not able to be accurately counted as they mainly died within the nesting cavity (inside building walls) and were impossible to collect. Ants carrying away dead bees at nest entrances were also a problem.

IP 556 was completely extinguished within 24 hours following treatment; this nest had a very high number (almost one thousand bees) carry fipronil back to the nest during the trial, and the number of combs evident (seen with the endoscope) inside the nesting cavity indicates that the nest was probably of a fairly large size (perhaps 10 000 bees or more).

Two nests had close to 400 bees carry fipronil back to the parental colony (IP 567 and IP 562). Both these nests are currently showing symptoms of severe poisoning and also showed behavioural indications of poisoning during treatment. Nest entrance activity for these nests has dropped considerably following treatment. Shaky, fitting bees were observed on the bait station during trials and flight patterns of feeding bees exiting the bait station were disorientated and sluggish during these two trials. Dead and twitching bees have been observed at the nest entrances at virtually each daily monitoring following treatment. This behaviour was also observed in the trials conducted by Taylor et. al (2007) and Anderson (2010). Nest entrance activity for IP 567 was extremely low between 288 hours and 504 hours, however endoscope photos at 312 hours showed that at least 30 bees were still present on comb inside the nesting cavity. Activity of this nest has increased considerably since 504 hours. At this stage nest entrance activity for IP 562 appears slowed, with activity being variable day to day. We continue to monitor the activity of these nests to determine whether they are only damaged and will recover over time, or whether they will eventually die out.

Treatment on IP 557 provided variable results. This nest was attacked by green ants in the 48 hours following initial treatment, which may have played a part in reducing the nest entrance activity recorded by field staff when the nest was monitored at 48 hours and at 72 hours. At 48 hours green ants were present at the nest entrance in large numbers, by 72 hours most of the green ants had disappeared. It is possible that the green ants were affected by the insecticide contained within dead bees that they had consumed and this is why they weren't so evident in the 24 hours following first observations of the ants by field staff. No green ant nest was noticed or located in the vicinity of the AHB nest, so the off target effects on these ants was not assessed. Ants consuming dead bees may prove a problem for these trials in the future, especially if ant species are also noticeably suppressed in the immediate vicinity of treated AHB nests.

It was clear that by 96 hours nest entrance activity for IP 557 was certainly increasing, and efforts were made to conduct a second treatment. Following the second treatment on this nest, activity has been severely slowed, but green ants have again recently been observed at the nest entrance. It would be worthy to observe the size of this particular nest within the nesting cavity (although this will not be possible – unless the wall of the building is removed) as it seems that 150 bees carrying fipronil back to this nest (as well as a green ant attack) was not enough to drastically suppress nest entrance activity with one treatment. We continue to monitor this nest to see if the secondary treatment successfully kills the nest.

IP 566 is the only nest used in these first 5 trials that could be totally extracted from the nesting cavity after being destroyed by treatment. Initial examination (before commencing the trial) showed a very small and young colony containing approximately 500-1000 bees. 42 bees carried fipronil back to the nest from the bait station during treatment. This nest died out relatively quickly due to the small size of the colony. Monitoring at 120 hours after treatment showed that less than 20 bees remained on the comb and observations at 144 hours confirmed that the colony had died. This nest contained two small pieces of comb (pictured in the photo gallery on page 36) and was approximately 1-2 months of age. Dead bees were scooped up from inside the bird box and counted (along with a lot of debris). Mostly only parts of exoskeletons of the bees remained and whole bees were rare (ants and other insects were evident both on the comb and within the debris on the bottom of the bird box and most of the dead bees were probably consumed by these animals). Approximately 40-50 dead bees/parts could be counted. Comb pieces were dry and void of honey and pollen (presumably consumed by ants). However it was evident that the cells around the edge of the comb (perhaps 10% had previously contained pollen). Approximately 15% of the total area of comb contained capped worker brood although it looked like up to 30% had earlier contained brood before being eaten by ants. Results showed that 42 bees was a sufficient number of bees carrying fipronil to kill this small nest within 144 hours following initial treatment.

It was difficult for field staff to ensure that consistent environmental conditions were maintained between days for bee counts/treatment due to erratic weather conditions typical to North Queensland at this time of 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.

Trials for this preliminary experiment 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 suitably applied in the field could be made. The process proved to be quite 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 high (some individual nests have taken up to 55 contact hours for a team of two field officers to maintain, treat and monitor, with time taken likely to be much longer for these nests as monitoring continues).

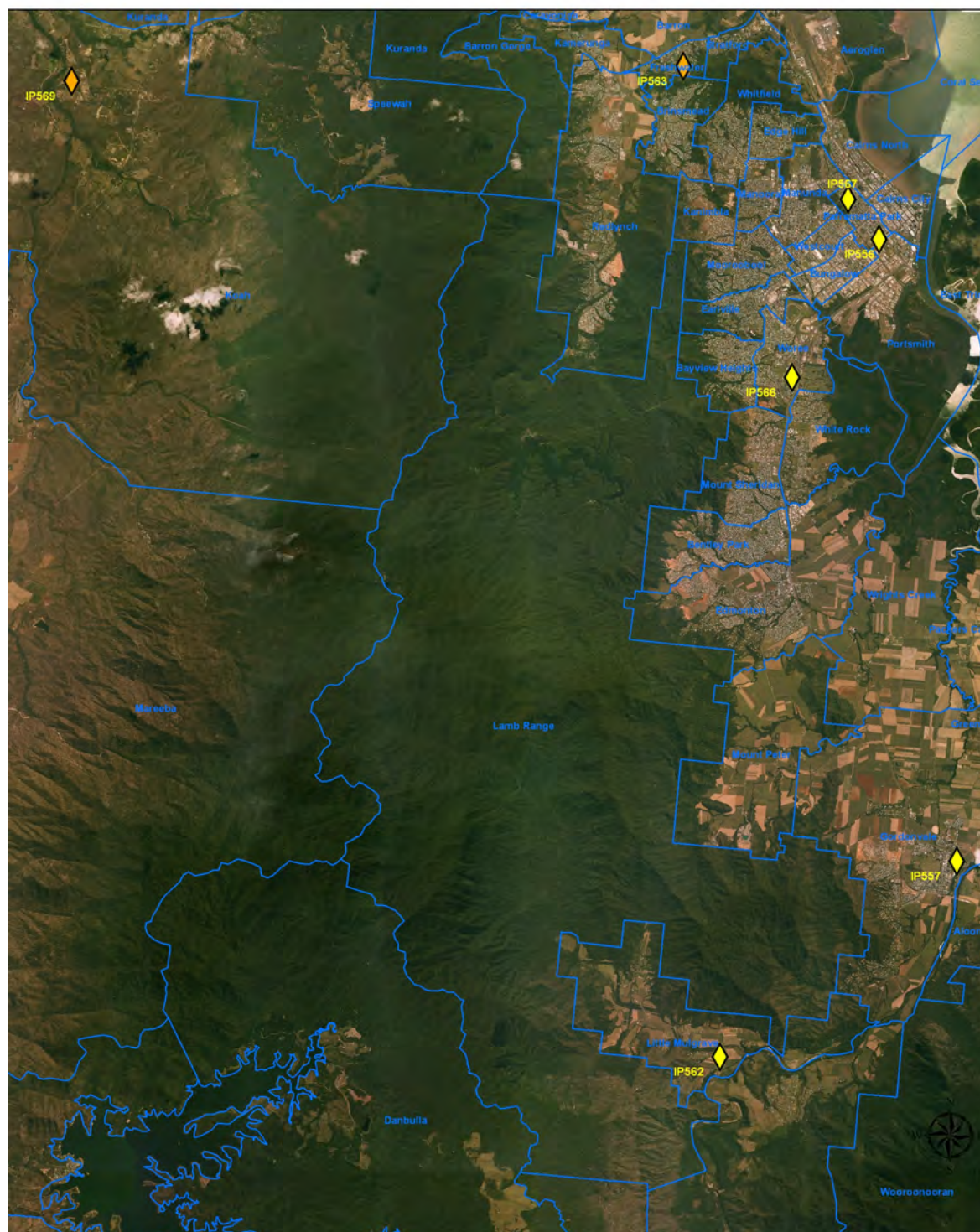
Conclusion

The outcomes of these preliminary trials demonstrates the potential of using Regent 200SC Insecticide at a concentration of 0.05ml per litre of sugar syrup to remotely destroy or suppress single Asian honey bee colonies. Early results show that this treatment method is very successful in suppressing bee entrance activity in the 24 hours following treatment regardless of the number of foragers that have fed on the bait station, and in most cases the bee numbers will remain suppressed for at least 14 days (further monitoring will most likely show that this will continue being the case for even longer periods of time). 973 bees carrying fipronil was adequate to destroy a nest of suspected large size, while 42 bees carrying fipronil was adequate enough to destroy a small colony of 500-1000 bees. Future trials will need to determine the minimum number of foragers required to feed on a bait station in order to successfully eliminate a nest of any size over time. Confidence levels also need to be tested in various environments and at varying distances of the bait station from the nest. It will also need to be determined that if a 'kill' is the aim of this treatment, then in what time frame does this need to be achieved (i.e do we want the nest eliminated in 24 hours for all treatments, or is it reasonable that the nest is slowly killed over weeks following treatment). Ideally, nests that are located within cavities that can be wholly extracted following successful remote treatment (and where dead bees can be easily collected from cavities daily) will provide the most reliable results in terms of determining the number of foragers required relative to the size of nest in trial. In these preliminary trials, due to time constraints, treatment and daily monitoring was carried out in bad weather. As we move into our dry season, trial results should become more reliable and consistent.

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Map showing locations of nests used for trials



REMOTE TREATMENT TRIALS

Legend



Attempted Trials



Nests in Trial



Suburbs



Queensland Government

A scale bar labeled "Kilometers" with markings at 0, 1, 2, 4, 6, and 8.

Disclaimer: While care is taken to ensure the accuracy of this data, all data contained herein and the State of Queensland makes no representations or warranties about its accuracy, reliability, completeness or suitability for any particular purpose and disclaims all responsibility and all liability (including without limitation, liability in negligence) for any loss or damage (including consequential or compensatory damages) that may be suffered by any person in reliance on the data. The data is provided "as is" and without warranty. Replications of maps and/or data contained herein are subject to authorization by the Mapping Coordinator, Bioscience Queensland Control Centre.

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APPENDIX B:

Detailed results/observations and time log for each nest (current at 02/04/2012)

NEST IP 556

TRIAL DETAILS	IP 556
Nest Number/ID	IP 556
Address	Palm Ave
Suburb	Paramatta Park
Environment	Residential/Urban, various floral sources
Nest Details	Nest within a wooden wall of a Queenslander residence. Nest entrance approximately 2.2m from ground. This nest will NOT able to be extracted.
FIRST TREATMENT	28/02/2012
Weather	70% cloud cover, muggy, no rain. Temp: 35.0% Humidity 61%
Distance between station and nest	82m
Foraging activity at regular station before treatment	8.55am – 9:05 am (over 10 min) 335 bees exited the regular station (approximately 50 -100 bees on station at any one time)
Foraging activity at nest entrance before treatment	9:12am – 10:12 am (over 1 hour) 3692 bees entered nest (very busy)
TARGET FORAGING NUMBER TO ACHIEVE	1000 bees to exit bait station
Treatment Commenced	10:20am
Treatment Completed	11:20am
Total time bait station in field	60 minutes
Actual foraging activity achieved on bait station	973 bees exited the bait station with fipronil
OBSERVATIONS DURING TREATMENT	0-10 minutes: 50 – 100 bees on station at any one time. Normal behaviour 10-20 minutes – 50 -100 bees on station at any one time. Normal behaviour 20-30 minutes: at 29 minutes only 15-20 bees on station. Bees starting to appear sluggish and flight away from station is slow and disorientated. 30-40 minutes: 2-5 bees on station (very disorientated behaviour and flight) 40-50 minutes: at 45 minutes 0 foragers (2 dead bees on station) 50-60 minutes: 0 foragers
Nest immediately after treatment	Very low activity (11:35am) 10 bees entered nest in one minute (most carrying pollen) 0 bees on regular syrup station
Nest 24 hrs after treatment	8am 29/02/2012 No activity
Regular station 24 hrs after treatment	8am 29/02/2012 0 bees
Nest 48 hrs after treatment	11am 01/03/2012 No activity Video/photos taken of nest with endoscope to get estimate of nest size (6 layers of comb – not sure how 'long' comb layers are as cavity is very narrow).
Regular station 48 hrs after treatment	10:45am 01/03/2012 No activity
	NEST DEAD

**TIME LOG IP556
(Palm Ave, Paramatta Park)**

DATE	JOB	TIME TAKEN (2 x staff)
17/01/2012	Nest detected by public report (attending to report, following up with house owner)	1.0 hrs
06/02/2012	Sweeping for foragers	2.0 hrs
06/02/2012 – 09/02/2012	Training bees on station	6.0 hrs
10/02/2012 – 24/02/2012	Maintaining sugar station and bees on station (making syrup, driving to site and topping up station, changing sand etc)	8 hrs
24/02/2012	Getting owner to sign consent form (phone call and meeting)	0.5 hrs
27/02/2012 (attempted treatment – too wet to continue)	Making syrup, organising equipment and driving to site	0.5 hrs
	Bee count at nest entrance	1.0 hrs
	Bee count at regular syrup station	0.5 hrs
28/02/2012 (1 st treatment)	Bee count at nest entrance	1.0 hrs
	Bee count at regular syrup station	0.5 hrs
	Preparation for trial (measuring distance etc.) and mixing chemical	0.5 hrs
	Trial (1000 bee target)	1.0 hrs
	Packing away	0.25 hrs
29/02/2012	Visiting site and checking nest @ 24 hrs	0.50 hrs
01/03/2012	Visiting site and checking nest @ 48 hrs	0.50 hrs
	Setting up ladder, taking video/photos with endoscope	0.50 hrs
02/03/2012	Downloading photos/video and writing up trial results	2.0 hrs
		26.25 hrs (total)

NEST IP 557

TRIAL DETAILS		IP 557	
Nest Number/ID	IP 557		
Address	56 Gordon St		
Suburb	Gordonvale		
Environment	Residential/Urban, various floral sources		
Nest Details	Nest within wall of old Queenslander. Nest entrance approximately 1m from ground. This nest will NOT be able to be extracted and cavity is too small to insert endoscope		
FIRST TREATMENT	05/03/12		
Weather	50% cloud cover, no rain. Temp. 33.7°C, Humidity 76%		
Distance between station and nest	74m		
Foraging activity at regular station before treatment	12:40am – 12:50am (over 10 min) 150 bees exited the regular station (approximately 40-50 bees on station at any one time)		
Foraging activity at nest entrance before treatment	1:00pm – 2:00pm 1600 bees entered nest		
TARGET FORAGING NUMBER TO ACHIEVE	150 bees to exit bait station		
Treatment Commenced	2:10pm		
Treatment Completed	2:30pm		
Total time bait station in field	20 minutes		
Actual foraging activity achieved on bait station	150 bees exited the bait station with fipronil		
OBSERVATIONS DURING TREATMENT	0-10 minutes: 40 – 50 bees on station at any one time. Normal behaviour 10-20 minutes: 40-50 bees on station at any one time. Normal behaviour 20-30 minutes: treatment completed 30-40 minutes: treatment completed 40-50 minutes: treatment completed 50-60 minutes: treatment completed Foraging activity on regular station (following treatment at 30 minutes) is active (still 10-30 bees on station – some may possibly be carrying some fipronil).		
Nest immediately after treatment	Still active		
DATE/ TIME LAPSED SINCE TREATMENT	Nest Activity (60 min)	Feed Station Activity (30 min)	Comments
06/03/12 (24hrs)	304 bees	3 bees	Pile of dead bees on ground. Bees falling from nest
07/03/12 (48hrs)	150 bees	0 bees	Nest still active, but being attacked by green ants
08/03/12 (72hrs)	NO COUNT	0 bees	Bees displaying 'swarming' behaviour? Not entering and exiting the nest as normal. Large numbers of bees flying in cloud around entrance. Lots of drones. Still a few green ants around (but not as many as day before) Cannot accurately count bees.

09/03/12 (96hrs)	576 bees	0 bees	Trying to train bees back onto syrup as they seem to have gone off it. Nest still active, no green ants.
12/03/12 – 14/03/12 (168hrs, 192hrs and 216hrs)	TOO WET	TOO WET	Pouring with rain – no counts conducted
15/03/12 (240 hrs)	2 nd treatment	2 nd treatment	Successfully trained bees back onto syrup. Second treatment able to occur (see table below)
SECOND TREATMENT	15/03/12		
Weather	90% cloud cover, no rain. Temp. 32°C, Humidity 81%		
Distance between station and nest	77m		
Foraging activity at regular station before treatment	11:30am – 11:40am (over 10 min) 153 bees exited the regular station (approximately 50-70 bees on station at any one time)		
Foraging activity at nest entrance before treatment	10:30pm – 11:30pm 1030 bees entered nest		
TARGET FORAGING NUMBER TO ACHIEVE	<u>150</u> bees to exit bait station		
Treatment Commenced	1:08pm		
Treatment Completed	1:28pm		
Total time bait station in field	30 minutes		
Actual foraging activity achieved on bait station	128 bees exited the bait station with fipronil		
OBSERVATIONS DURING TREATMENT	0-10 minutes: 40-50 bees on station at any one time. Normal behaviour 10-20 minutes: 40-50 bees on station at any one time. Normal behaviour 20-30 minutes: treatment completed 30-40 minutes: treatment completed 40-50 minutes: treatment completed 50-60 minutes: treatment completed Foraging activity on regular station (following treatment at 20 minutes) is active (still 30-40 bees on station – some may possibly be carrying some fipronil).		
Nest immediately after treatment	Still active		
DATE/ TIME LAPSED SINCE 2nd TREATMENT	Nest Activity (60 min)	Feed Station Activity (30 min)	Comments
16/03/12 (24hrs)	0 bees	0 bees	Nest is dead? Will confirm Monday (96hrs)
19/03/12 (96hrs)	306 bees	0 bees	Bees entering and exiting nest again!
20/03/12 (120hrs)	0 bees	0 bees	Nest is dead?
21/03/12 (144hrs)	18 bees	0 bees	Low activity entering nest.

22/03/12 (168 hrs)	16 bees	0 bees	Low activity
23/03/12 (192 hrs)	36 bees	0 bees	No dead or dying bees observed at entrance. Bees appear to be stronger in flight than previous days
26/03/12 (264 hrs)	0 bees	0 bees	Very wet day
29/03/12 (336 hrs)	0 bees	0 bees	Green ants noticed around nest entrance
30/03/12 (360 hrs)	84 bees	0 bees	Most number of bees seen since 96 hours following second treatment
02/04/12 (432 hrs)	0 bees	0 bees	Nest is dead?

TIME LOG IP557 (Gordon St, Gordonvale)		
DATE	JOB	TIME TAKEN (2 x staff)
20/02/2012 – 02/03/2012	Sweeping surveillance (after Church St nest absconded) resulted in detection of foragers. Training bees on station and maintaining bees on station.	8.0 hrs
05/03/2012	Beelining by field staff. Nest detection	4.0 hrs
05/03/2012	Getting mill manager to sign consent form (phone call and meeting)	0.5 hrs
05/03/2012 (1 st treatment)	Making syrup, organising equipment and driving to site	1.0 hrs
	Bee count at nest entrance	1.0 hrs
	Bee count at regular syrup station	0.5 hrs
	Preparation for trial (measuring distance etc.) and mixing chemical	0.5 hrs
	Trial (150 bee target)	1.0 hrs
	Packing away	0.25 hrs
06/03/2012	Visiting site and checking nest @ 24 hrs	1.50 hrs
	Bee count at nest entrance	1.0 hrs
	Bee count at regular syrup station	0.5 hrs
07/03/2012 (first attempt at 2 nd Treatment)	Making syrup, organising equipment and driving to site	1.0 hrs
	Bee count at nest entrance	1.0 hrs
	Bee count at regular syrup station	0.5 hrs

08/03/2012 (second attempt at 2 nd Treatment)	Making syrup, organising equipment and driving to site	1.0 hrs
	Check nest @ 72 hrs	0.5 hrs
	Check station	0.5 hrs
09/03/2012 (third attempt at 2 nd treatment)	Making syrup, organising equipment and driving to site	1.0 hrs
	Count bees at nest entrance	1.0 hrs
	Check station	0.5 hrs
	Try to train bees back onto station	2.0 hrs
15/03/2012	Making syrup, organising equipment and driving to site	1.0 hrs
	Try to train bees back onto syrup and move station back to 80m	3.0 hrs
	Bee count at nest entrance	1.0 hrs
	Bee count at regular syrup station	0.5 hrs
(2 nd treatment successful)	Preparation for trial and mixing chemical	0.5 hrs
	Trial (150 bee target)	0.25hrs
	Packing away	0.25 hrs
16/03/2012	Visiting site and checking nest @ 24 hrs after 2 nd treatment	1.50 hrs
19/03/2012	Visiting site and checking nest @ 96 hrs after 2 nd treatment	1.50 hrs
20/03/2012	Visiting site and checking nest @ 120 hrs after 2 nd treatment	1.50 hrs
21/03/2012	Visiting site and checking nest @ 144 hrs after 2 nd treatment	1.50 hrs
22/03/2012	Visiting site and checking nest @ 168 hrs after 2 nd treatment	1.50 hrs
23/03/2012	Visiting site and checking nest @ 192 hrs after 2 nd treatment	1.50 hrs
26/03/2012	Visiting site and checking nest @ 264 hrs after 2 nd treatment	1.50 hrs
29/03/2012	Visiting site and checking nest @ 336 hrs after 2 nd treatment	1.50 hrs
30/03/2012	Visiting site and checking nest @ 360 hrs after 2 nd treatment	1.50 hrs
02/04/2012	Visiting site and checking nest @ 432 hrs after 2 nd treatment	1.50 hrs

26/03/2012 – 02/04/2012	Downloading photos/video and writing up trial results	2.0 hrs
		53.25 hrs (total as at 02/04/2012)

NEST IP 567

TRIAL DETAILS		IP 567	
Nest Number/ID	IP 567		
Address	9 Denbeigh st		
Suburb	Paramatta Park		
Environment	Residential/Urban, various floral sources		
Nest Details	Nest under floor boards of high set Queenslander. Nest entrance approximately 2 m from ground. This nest will NOT be able to be extracted.		
FIRST TREATMENT	08/03/2012		
Weather	90% cloud cover, no rain. Temp: 33.0° C. Humidity 69%		
Distance between station and nest	80m		
Foraging activity at regular station before treatment	12:00pm – 12:10pm (over 10 min) 122 bees exited the regular station (approximately 50-60 bees on station)		
Foraging activity at nest entrance before treatment	11:00am – 12:00 am 2198 bees entered nest		
TARGET FORAGING NUMBER TO ACHIEVE	500 bees to exit bait station		
Treatment Commenced	12:15pm		
Treatment Completed	1:15pm		
Total time bait station in field	60 minutes		
Actual foraging activity achieved on bait station	322 bees exited the bait station with fipronil		
OBSERVATIONS DURING TREATMENT	0-10 minutes: 50 – 60 bees on station at any one time. Normal behaviour 10-20 minutes:50-60 bees on station at any one time. Normal behaviour 20-30 minutes: bee numbers starting to drop off 30-40 minutes: 9 bees on station (behavioural problems similar to that displayed in previous nest treatments) 40-50 minutes: 2 bees on station 50-60 minutes: 0 bees		
Nest immediately after treatment	Activity significantly slowed at nest entrance (15 bees entering nest in one minute) 0 bees on regular syrup station		
DATE/ TIME LAPSED SINCE TREATMENT	Nest Activity (60 min)	Feed Station Activity (30 min)	Comments
09/03/12 (24hrs)	121 bees	0 bees	Nest still active Lots of dead bees on ground
10/03/12 (48hrs)	108 bees	0 bees	Nest still active Lots of dead bees on ground Endoscope photo taken

12/03/12 (96hrs)	26 bees	0 bees	Nest still active (but very low activity) Cannot do second treatment (no bees on station)
13/03/12 (120hrs)	18 bees	0 bees	Nest still active (but very low activity) Need to re-train bees onto syrup if to do second treatment on this nest (but forager levels are extremely low and nest is on its way out anyway?)
14/03/12 (144hrs)	10 bees	0 bees	Nest still active (but very low activity) Endoscope photo taken
15/03 (168hrs)	0 bees	0 bees	Extremely wet weather at time of checking nest.
16/03/12 (192hrs)	18 bees	0 bees	Nest still active (but very low activity)
19/03/12 (264 hrs)	NO COUNT	0 bees	Bees hanging around entrance. Small swarm sitting on outside (re-entered cavity whilst team was monitoring). Not entering and exiting as a normal nest, but rather 'swarming around'.
20/03/12 (288 hrs)	0 bees	0 bees	Nest dead?
21/03/12 (312 hrs)	0 bees	0 bees	Looked inside nest cavity with endoscope. Still a few bees (about 20) visible on comb.
22/03/12 (336 hrs)	12 bees	0 bees	Low activity
23/03/12 (360 hrs)	4 bees	0 bees	All 4 bees carrying pollen
26/03/212 (432 hrs)	1 bee	0 bees	Very wet day
29/03/12 (504 hrs)	16 bees	0 bees	Endoscope pictures show that there are still bees on comb and are perhaps on the increase.
30/03/12 (528 hrs)	232 bees	0 bees	Activity increased significantly!
02/04/12 (600 hrs)	472 bees	0 bees	This is the most activity observed since before treatment commenced

TIME LOG IP 567 (Denbeigh St, Paramatta Park)		
DATE	JOB	TIME TAKEN (2 x staff)
28/02/2012	Nest detected by public report (attending to report, following up with house owner)	1.0 hrs
29/02/2012 – 07/03/2012	Sweeping for foragers	2.0 hrs

	Training bees on station	6.0 hrs
	Maintaining sugar station and bees on station (making syrup, driving to site and topping up station, changing sand etc)	2.0 hrs
06/03/12	Getting owner to sign consent form (phone call and meeting)	0.5 hrs
08/03/2012 (1 st treatment)	Bee count at nest entrance	1.0 hrs
	Bee count at regular syrup station	0.5 hrs
	Preparation for trial (measuring distance etc.) and mixing chemical	0.5 hrs
	Trial (500 bee target)	1.0 hrs
	Packing away	0.25 hrs
09/03/2012	Visiting site and checking nest @ 24 hrs	0.50 hrs
	Bee count at nest entrance	1.0 hrs
	Check station	0.25 hrs
10/03/2012	Visiting site and checking nest @ 48 hrs (on weekend)	0.50 hrs
	Bee count at nest entrance	1.0 hrs
	Check station	0.25 hrs
12/03/2012	Visiting site and checking nest @ 96 hrs	0.50 hrs
	Bee count at nest entrance	1.0 hrs
	Check station	0.25 hrs
13/03/2012	Visiting site and checking nest @ 120 hrs	0.50 hrs
	Bee count at nest entrance	1.0 hrs
	Check station	0.25 hrs
14/03/2012	Visiting site and checking nest @ 144 hrs	0.50 hrs
	Bee count at nest entrance	1.0 hrs
	Check station	0.25 hrs
15/03/2012	Visiting site and checking nest @ 168hrs	0.50 hrs
	Bee count at nest entrance	1.0 hrs
	Check station	0.25 hrs
16/03/2012	Visiting site and checking nest @ 192 hrs	0.50 hrs
	Bee count at nest entrance	1.0 hrs

	Check station	0.25 hrs
19/03/2012	Visiting site and checking nest @ 264 hrs	0.50 hrs
	Bee count at nest entrance	1.0 hrs
	Check station	0.25 hrs
20/03/2012	Visiting site and checking nest @ 288 hrs	0.50 hrs
21/03/2012	Visiting site and checking nest @ 312 hrs	0.50 hrs
22/03/2012	Visiting site and checking nest @ 336 hrs	0.50 hrs
23/03/2012	Visiting site and checking nest @ 360 hrs	0.50 hrs
26/03/2012	Visiting site and checking nest @ 432 hrs	0.50 hrs
29/03/2012	Visiting site and checking nest @ 502 hrs	0.50 hrs
30/03/2012	Visiting site and checking nest @ 528 hrs	0.50 hrs
02/04/2012	Visiting site and checking nest @ 600hrs	0.50 hrs
26/03/2012 – 02/04/2012	Downloading photos/video and writing up trial results	2.0 hrs
		36.25 hrs (total as at 02/04/2012)

NEST IP 562

TRIAL DETAILS	IP 562
Nest Number/ID	IP 562
Address	24 Roos Road
Suburb	Little Mulgrave
Environment	Rural/farmland, various floral sources
Nest Details	Nest within wall of corrugated iron house. Nest entrance approximately 3.5 m from the ground. Nest will NOT be able to be extracted and nest is too high to get endoscope pictures
FIRST TREATMENT	13/03/2012
Weather	100% cloud cover, patchy showers. 27.7 °C, 81% humidity.
Distance between station and nest	80m
Foraging activity at regular station before	12:00pm – 12:10pm (over 10 min)

treatment	185 bees exited the regular station (approximately 50 bees on station at any one time)		
Foraging activity at nest entrance before treatment	11:00am – 12:00pm 5524 bees entered nest		
TARGET FORAGING NUMBER TO ACHIEVE	400 bees to exit bait station		
Treatment Commenced	12:15pm		
Treatment Completed	12:45pm		
Total time bait station in field	30 minutes		
Actual foraging activity achieved on bait station	409 bees exited the bait station with fipronil		
OBSERVATIONS DURING TREATMENT	0-10 minutes: 50 bees on station at any one time. Normal behaviour 10-20 minutes: 70 bees on station at any one time. Normal behaviour 20-30 minutes: bee numbers starting to drop off 30-40 minutes: treatment completed – but 20 bees still on bait station visually dying (fipronil affected) 40-50 minutes: treatment completed 50-60 minutes: treatment completed		
Nest immediately after treatment	Activity slowed at nest entrance 0 bees on regular syrup station		
DATE/ TIME LAPSED SINCE TREATMENT	Nest Activity (60 min)	Feed Station Activity (30 min)	Comments
14/03/12 (24hrs)	208 bees	0 bees	Lots of dead bees on ground and falling out of nest. Nest still active
15/03/12 (48hrs)	184 bees	0 bees	Nest still active.
16/03/12 (72hrs)	0 bees	0 bees	Nest dead? Will confirm on Monday at 144hrs.
19/03/12 (144hrs)	NO COUNT	NO COUNT	Bridge flooded, no access to property
20/03/12 (168hrs)	104 bees	0 bees	Nest active again
21/03/12 (192hrs)	196 bees	0 bees	Nest activity increasing?
22/03/12 (216 hrs)	116 bees	0 bees	fine and sunny day
23/03/212 (240 hrs)	88 bees	0 bees	fine and sunny day
26/03/212 (312 hrs)	1 bees	0 bees	Very wet day
29/03/12 (384 hrs)	156 bees	0 bees	Nest recovering?
30/03/12 (408 hrs)	232 bees	0 bees	Nest recovering? This is the most activity seen since before treatment commenced.

02/04/12 (480 hrs)	20 bees	0 bees	Nest activity lowered again.
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TIME LOG IP 562 (Roos Road ,Little Mulgrave)		
DATE	JOB	TIME TAKEN (2 x staff)
20/02/2012	Nest detected by public report (attending to report, following up with house owner)	1.0 hrs
21/02/2012 – 12/03/2012	Sweeping for foragers	2.0 hrs
	Training bees on station	6.0 hrs
	Maintaining sugar station and bees on station (making syrup, driving to site and topping up station, changing sand etc)	8.0 hrs
	Getting owner to sign consent form (phone call and meeting)	0.5 hrs
13/03/2012 (1 st treatment)	Bee count at nest entrance	1.0 hrs
	Bee count at regular syrup station	0.5 hrs
	Preparation for trial (measuring distance etc.) and mixing chemical	0.5 hrs
	Trial (400 bee target)	1.0 hrs
	Packing away	0.25 hrs
14/03/2012	Visiting site and checking nest @ 24 hrs	1.0 hrs
	Bee count at nest entrance	1.0 hrs
	Check station	0.25 hrs
15/03/2012	Visiting site and checking nest @ 48hrs	1.0 hrs
	Bee count at nest entrance	1.0 hrs
	Check station	0.25 hrs
16/03/2012	Visiting site and checking nest @ 72hrs	1.0 hrs
	Bee count at nest entrance	1.0 hrs
	Check station	0.25 hrs
20/03/2012	Visiting site and checking nest @ 168hrs	1.0 hrs
21/03/2012	Visiting site and checking nest @ 192hrs	1.0 hrs

22/03/2012	Visiting site and checking nest @ 216hrs	1.0 hrs
23/03/2012	Visiting site and checking nest @ 240hrs	1.0 hrs
26/03/2012	Visiting site and checking nest @ 312hrs	1.0 hrs
29/03/2012	Visiting site and checking nest @ 384 hrs	1.0 hrs
30/03/2012	Visiting site and checking nest @ 408 hrs	1.0 hrs
02/04/2012	Visiting site and checking nest @ 480 hrs	1.0 hrs
26/03/2012 – 02/04/2012	Downloading photos/video and writing up trial results	2.0 hrs
		35.25 hrs (total as at 02/04/2012)

NEST IP 566

TRIAL DETAILS	IP566
Nest Number/ID	IP 566
Address	4 Karen Close
Suburb	Woree
Environment	Residential/urban
Nest Details	Nest within bird box in a residential backyard. Nest entrance approximately 1.5 m from the ground. Nest WILL be able to be extracted
FIRST TREATMENT	21/03/12
Weather	20% cloud cover, sunny, light breeze 34 °C, 65% humidity.
Distance between station and nest	25m
Foraging activity at regular station before treatment	1:45pm – 1:55pm (over 10 min) 60 bees exited the regular station (approximately 50 bees on station at any one time)
Foraging activity at nest entrance before treatment	12:45pm – 1:45pm 606 bees entered nest
TARGET FORAGING NUMBER TO ACHIEVE	25-50 bees to exit bait station
Treatment Commenced	14:20pm
Treatment Completed	14:32pm
Total time bait station in field	12 minutes
Actual foraging activity achieved on bait station	42 bees exited the bait station with fipronil

OBSERVATIONS DURING TREATMENT	0-10 minutes: normal behaviour 10-20 minutes: treatment completed 20-30 minutes: treatment completed 30-40 minutes: treatment completed 40-50 minutes: treatment completed 50-60 minutes: treatment completed		
Nest immediately after treatment	10-20 bees still feeding on regular syrup station		
DATE/ TIME LAPSED SINCE TREATMENT	Nest Activity (60 min)	Feed Station Activity (30 min)	Comments
22/03/12 (24hrs)	8 bees	0 bees	Lots of drones hanging around entrance (30 or so drones)
23/03/12 (48hrs)	0 bees	0 bees	Bees still on comb. See photo gallery. Fine and sunny day.
26/03/12 (120 hrs)	0 bees	0 bees	Less than 20 bees on comb
27/03/12 (144hrs)	0 bees	0 bees	NEST DEAD

TIME LOG IP 566		
DATE	JOB	TIME TAKEN (2 x staff)
28/02/2012	Nest detected by public report (attending to report, following up with house owner)	1.0 hrs
01/03/2012- 20/03/2012	Sweeping for foragers	6.0 hrs
	Training bees on station	4.0 hrs
	Maintaining sugar station and bees on station (making syrup, driving to site and topping up station, changing sand etc)	7.0 hrs
	Getting owner to sign consent form (phone call and meeting)	0.5 hrs
21/03/2012 (first trial)	Bee count at nest entrance	1.0 hrs
	Bee count at regular syrup station	0.5 hrs
	Preparation for trial (measuring distance etc.) and mixing chemical	0.5 hrs
	Trial (25-50 bee target)	0.25 hrs
	Packing away	0.25 hrs
22/03/2012	Visiting site and checking nest @ 24 hrs	0.5 hrs
	Bee count at nest entrance	1.0 hrs
	Check station	0.25 hrs
23/03/2012	Visiting site and checking nest @ 48hrs	0.5 hrs

	Bee count at nest entrance	1.0 hrs
	Check station	0.25 hrs
26/03/2012	Visiting site and checking nest @ 120 hrs	0.5 hrs
	Bee count at nest entrance	1.0 hrs
	Check station	0.25 hrs
27/03/2012	Visiting site and checking nest @ 144 hrs	0.5 hrs
28/03/2012	Downloading photos/video, examining comb and writing up trial results	2.0 hrs
		28.75 hrs (total)

APPENDIX C

Attempted treatment on 2 nests

NEST IP 563

TRIAL DETAILS	ATTEMPTED TRIAL IP 563
Nest Number/ID	IP 563
Address	103 Peterson St
Suburb	Freshwater
Environment	Residential/Urban, various floral sources
Nest Details	Nest within a brick wall of a Queenslander residence. Nest entrance approximately 3.5m from ground. This nest will NOT able to be extracted.
ATTEMPTED FIRST TREATMENT	05/03/2012
Weather	80% cloud cover, muggy, no rain. Temp: 34.0% Humidity 61%
Distance between station and nest	79m
Foraging activity at regular station before treatment	05/03/2012 8:30am 0 bees on station
Foraging activity at nest entrance before treatment	05/03/2012 8:30am Nest being attacked by Green Ants!!! (<i>Oecophylla</i> sp) See photos in appendix D Nest is INACTIVE/DEAD CANNOT DO TRIAL

TIME LOG IP563 (Peterson St, Freshwater)		
DATE	JOB	TIME TAKEN (2 x staff)
24/02/2012	Nest Detected by public call out (attending to report, following up with house owner)	1.0 hrs
27/02/2012 – 01/03/2012	Sweeping for foragers (rainy weather)	8.0 hrs
02/03/2012 – 02/03/2012	Training bees on station	4.0 hrs
02/03/2012	Getting owner to sign consent form (phone call and meeting)	0.5 hrs
05/03/2012 (attempted 1 st treatment)	Making syrup, organising equipment and driving to site	0.5 hrs
	Check station	0.25 hrs
	Check nest take photos	0.25 hrs
05/03/2012	Writing up results	0.25 hrs
		14.5 hrs (total)

NEST IP 569

TRIAL DETAILS		ATTEMPTED TRIAL NEST IP 569	
Nest Number/ID	IP 569		
Address	15 Melaleuca Close		
Suburb	Koah		
Environment	Residential/Rural, dry sclerophyll forest		
Nest Details	Nest within a cavity in Eucalypt tree. Nest entrance approximately 8-12 m from ground. This nest WILL be able to be extracted by tree lopper.		
ATTEMPTED FIRST TREATMENT	06/03/12		
Weather	20% cloud cover, no rain. Temp. 36.3°C. Humidity 70%		
Distance between station and nest	76m		
Foraging activity at regular station before treatment	9:55am – 10:05 am (over 10 min) 120 bees exited the regular station (approximately 30 - 40 bees on station at any one time)		
Foraging activity at nest entrance before treatment	nest looks very busy (entrance is too high in tree to count bees – video captured)		
TARGET FORAGING NUMBER TO ACHIEVE	500 bees to exit bait station		
Treatment Commenced	10:15am		
Treatment Completed	11:15am		
Total time bait station in field	60 minutes		
Actual foraging activity achieved on bait station	201 bees exited the bait station with fipronil (ALL flying towards known nest)		
OBSERVATIONS DURING TREATMENT	0-10 minutes: 30 – 40 bees on station at any one time. Normal behaviour 10-20 minutes – 20-30 bees on station at any one time. Normal behaviour 20-30 minutes: Bees starting to drop off and appearing sluggish and flight away from station is slow and disorientated. 30-40 minutes: 10 bees on station (very disorientated behaviour and flight) 40-50 minutes: at 47 minutes 3 foragers on station 50-60 minutes: 0 foragers		
Nest immediately after treatment	Looks similar to activity before trial commenced 0 bees on regular syrup station		
DATE/ TIME LAPSED SINCE TREATMENT	Nest Activity	Feed Station Activity	Comments
14/03/12 (24hrs)	busy	10-20 bees on station at any one time	nest looks very busy (entrance is too high in tree to count bees – video captured)
15/03/12 (48hrs)	busy	5 Bees on station – HOWEVER these 5 bees appear to be from a different nest Beeline is in opposite direction to the nest that we have been	9.30am - nest still looks very busy (entrance is too high in tree to count bees) TRIAL ON HOLD UNTIL SECOND NEST IS BEELINED AND LOCATED!

		watching/treating!	
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TIME LOG IP569
(Melaleuca Close, Koah)

DATE	JOB	TIME TAKEN (2 x staff)
13/02/2012	Routine sweeping surveillance detected foragers	6.0 hrs
14/02/2012 – 28/02/2012	Training bees on station. Beelining by field staff. Nest detection	55.0 hrs
29/02/2012 – 02/03/2012	Maintaining sugar station and bees on station (making syrup, driving to site (40 min drive) and topping up station, changing sand etc)	9 hrs
02/03/2012	Getting owner to sign consent form (phone call and meeting)	0.5 hrs
05/03/2012 (first attempt at 1 st treatment)	Making syrup, organising equipment and driving to site	1.5 hrs
	Check station	0.25 hrs
	Check nest take photos	0.25 hrs
06/03/12 (second attempt at 1 st treatment)	Making syrup, organising equipment and driving to site	1.5 hrs
	Bee count at nest entrance	1.0 hrs
	Bee count at regular syrup station	0.5 hrs
	Preparation for trial (measuring distance etc.) and mixing chemical	0.5 hrs
	Trial (500 bee target)	1.0 hrs
	Packing away	0.25 hrs
07/03/2012	Visiting site and checking nest (@ 24 hrs	2.0 hrs
08/03/2012 (attempt at 2 nd treatment)	Making syrup, organising equipment and driving to site	1.5 hrs
	Check station	0.25 hrs
	Check nest	0.25 hrs
09/03/12	Downloading photos/video and writing up trial results	0.5 hrs
		81.75 hrs (total)

APPENDIX D

Photo Gallery of Trials

IP 556

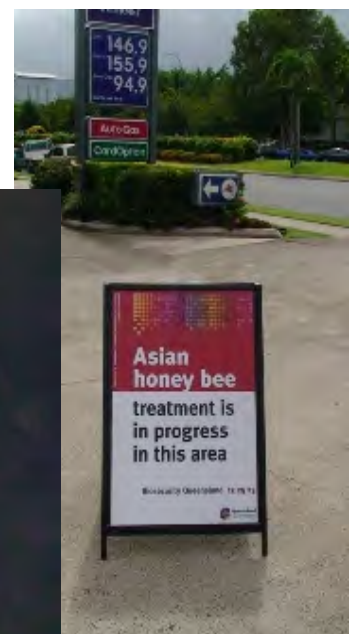


Above: preparing for treatment

Above: counting bees at nest entrance



Above: comb inside nesting cavity 48 hrs after first treatment (nest dead)



Above: treatment sign



Above: nest entrance in the wall of an abandoned worker house belonging to Gordonvale Sugar Mill



Left: nest being attacked by Green Ants. **Top right:** pile of dead bees at base of nest (some being carried away by Green Ants). **Bottom right:** syrup station



Above: Nest Entrance (under floor)



Above: syrup station



Above: nest 48 hrs following first treatment



Right: dead bees (48hrs)



Above: nest 144 hrs following first treatment



Nest Entrances



Above: checking syrup



Above: sweeping up dead bees

Above: nest entrance/s inside corrugated iron wall of residence

IP 566



Above: nest entrance (nest in bird box)



Above: nest 24 hrs following treatment



Above: IP 566 Comb 1 Side A



Above: IP 566 Comb 1 Side B



Above: IP 566 Comb 2 Side B



Above: IP 566 Comb 2 Side B



Above: dead bees and debris collected from bottom of bird box IP 566



Above: nest entrance and Green Ants (*Oecophylla sp*) attacking nest inside wall of residence. Nest activity was nil. Checked and confirmed that nest was dead 24 hrs and 48 hrs after ant discovery.



Above: Green Ants carrying away dead *A. cerana* bees

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Above: nest entrance (8-12m high) in a Eucalypt tree



Above: close up of nest entrance



Above: syrup station



Above: *A. cerana* foraging on syrup station

