Department of Agriculture, Fisheries and Forestry

# Asian honey bee Transition to Management Program

## Detection efficacy of Asian honey bees (*Apis cerana*) in Cairns, Australia

Great state. Great opportunity.

Queensland
Government

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# Contents

# Summary

A project under the Asian honey bee (AHB) Transition to Management Plan was to validate the efficacy of detection and destruction methods and strategies as essential elements of managing Asian honey bees (*Apis cerana* Java genotype) in Australia. Here, detection efficacy was determined in two separate ways: (1) by analysing historical data (previously collected and stored data) for public reporting, standard AHB traps, Rainbow bee-eater roost/pellet surveillance and bee lining; and (2) by conducting experimental field trials to determine the comparative effectiveness of two different floral observation methods (general and timed floral observations). As part of this, detection methods suitable for high-risk areas as well as for the edge of the Known Infested Area (KIA) were reviewed.

Direct comparison of the different detection methods was difficult due to a systemic lack of record-keeping in the historical data, meaning that determining of efficacy (i.e. number of bees found per hour or person-hour) was not possible for most methods.

Efficacy could be estimated for AHB traps and bee-eater surveillance, and directly calculated from field trials for general and timed floral observations. Rainbow bee-eater surveillance was the most efficacious of all methods, followed by timed floral observations, general floral observations, and lastly AHB traps with the worst of all efficacies. Rainbow bee-eater-surveillance was 10 times more efficacious (in terms of bees found per person-hour) than floral observations, and 36 times more efficacious than AHB traps.

For some methods, a success percentage could be calculated or estimated (number of successful detections relative to the total effort). Public calls and Rainbow bee-eater surveillance were by far the most successful detection methods, followed by the AHB detection dog. However, it needs to be noted that additional resources required for these particular methods were not taken into account (e.g. time spent for laboratory analysis of pellets; maintenance and training of the dog). Using AHB traps was the worst method.

Field trials showed that timed floral observations were more efficacious than general floral observations (transect walks), and both methods were greatly influenced by the abundance of bees and floral resources in an area. Floral observations mostly differed from transect walks in that fewer flowering plants were observed per site but each flowering plant was observed for much longer (10 minutes per flowering plant). This methodology appeared to greatly improve the chances of detecting AHB compared to scanning plants while walking.

These results were likely due to the much more scarce distribution, and lower numbers, of AHB across all sites. In comparison, European honey bees (EHB) were detected in much higher numbers across all sites, utilising a much larger number of plant species than AHB. This could potentially be a reflection of EHB having much larger colonies, and/or due to either competitive exclusion of AHB by EHB, and/or a difference in preferred floral sources between the two species.

Further research is recommended to determine the actual AHB and EHB density in different areas (habitats) in order to optimise detection methods. In addition, floral preferences appear to differ greatly between the species, with AHB utilising a very narrow range of the available flowering plants. Thus, research into the floral preferences of AHB in the presence and absence of EHB may also lead to improved detection methods.

Due to the need to find nests in high-risk areas, timed floral observations are recommended as these will detect foragers that can be bee lined to their nest. A detection dog may speed up bee lining in certain circumstances. Recommended detection methods on the edge of the KIA depend on the purpose of finding AHB: to find and destroy nests, the same methods as

in high-risk areas should be employed; to track the spread of AHB, Rainbow bee-eater pellet surveillance is the recommended detection method as it is the most efficacious method but does not result in finding nests.

# Introduction

Since the first detection of the Asian honey bee (AHB), *Apis cerana* Java genotype, in Cairns (Queensland, Australia) in May 2007, there have been over 800 detections of AHB nests and swarms. Until April 2012, all detections were destroyed. After April 2012, some nests and swarms were kept for research, and post November 2012, all destruction within the Known Infested Area (KIA) ceased.

Various methods to detect and destroy nests and swarms were deployed between 2007 and 2012. Surveillance and detection methods varied, including for example floral sweeping, tracing of public reports, and using Rainbow bee-eaters (*Merops ornatus*) for bee-surveillance (Table 1). However, efficacy of any of these methods was never formally validated.

Part of the AHB Transition to Management Plan stipulates to "Validate the efficacy of detection and destruction methods and strategies as essential elements of deploying different control methods", which includes determining rates of effort and validation of all methods (AG2 Bi). "All methods" was taken as those methods currently used as well as some of those that were of no or limited success in the past, but that may, with improvement, show promise of detecting or destroying nests and swarms.

Therefore, the goal of this report was to determine and compare the rates of effort required for each of the identified detection methods, as well as validate their efficacy, where data was available. Efficacy of destruction methods are presented in a separate report: Destruction efficacy of Asian honey bees (*Apis cerana*) in Cairns, Australia (Wittmeier *et al.*, 2013).

Specifically, the report aimed to

- (i) determine efficacy of detection methods in two separate ways:
    - Analysis of historical data based on previously collected and stored data (public reporting, standard AHB traps, Rainbow bee-eater roost/pellet surveillance, bee lining).
    - Experimental field trials to determine the comparative effectiveness of general versus timed floral observations.
- (ii) extend the outcomes from these analyses to provide advice on the most appropriate methods for detection in high-risk (Port) areas and along the edge of the KIA.

**Table 1: Asian honey bee (*Apis cerana*) detection and destruction methods (including type, purpose and status) that are or have been used by Biosecurity Queensland, Department of Agriculture, Fisheries and Forestry, Queensland, Australia (Biosecurity Queensland DAFF).**

| Type | Purpose | Status |
|---|---|---|
| ***Active detection methods*** | | |
| Floral sweeping/observations | Finding foragers on flowers | Used until 2013 |
| Targeted* floral observations | Finding foragers on flowers | Used until 2013 |
| Bee-eater roosts/pellets | Detect presence of *A. cerana* in a general area | Used until 2013 |
| Sugar feeding stations | Finding foragers, bee lining | Used until 2012 |
| Sugar feeding traps | Trapping foragers | Used until 2012 |
| Genetic testing for *A. cerana* in bee-eater pellets and syrup of feeding stations | Detect presence of *A. cerana* in a general area | Trials concluded |
| Genetic testing for *A. cerana* in trap liquor | Detect presence of *A. cerana* in a general area | Trials concluded |
| Sticky traps | Trapping foragers | Deemed ineffective in the past |
| Sticky frames | Trapping foragers | |
| Pheromone log traps | Trapping swarms | |
| Bait hives | Trapping swarms | |
| Various other traps (incl. Lucitraps; using palm tree flowers as attractants) | Trapping foragers | |
| Scenting (melting honeycomb) | Finding foragers | |
| Odour detection dog | Finding nests | |
| Mega Garden (AHB-preferred floral sources in a movable trailer) | Finding foragers | |
| ***Passive surveillance*** | | |
| Public calls & reports | Find nests, swarms & foragers | Used until 2013 |
| ***Nest detection*** | | |
| Bee lining | Find nests from foragers | Used until 2013 |

* Targeting specific, previously mapped flowering plants.

# Historical data

## Methods

### Data sources

Data was sourced from BioSIRT (a computer database used by Biosecurity Queensland for managing routine and emergency incidents for disease, pest or residues, in plants, animals or in the environment), covering over five years of data from May 2007 until 31October 2012. Data included the infested property (IP) number, date of detection, location information (GPS coordinates, suburb), initial detection method and final detection method. Data was downloaded as a Microsoft Excel file, and graphing was performed in Excel. Any statistical analyses performed were done in GenStat (14th Edition; VSN International, 2011).

Data entered into BioSIRT included information about the detection method such as initial detection methods (public report, trap, floral sweep, other) and final detection methods (bee lining by field staff, bee lining by dog, and public report).

More specific methods relating to each detection method will be described in the relevant methods sections, if necessary and appropriate.

### General analysis of AHB detection

All AHB nests and swarms detected between May 2007 and 31 October 2012 were used to summarise overall detection methods. This included overall frequency of different detection methods, of nests versus swarm detection methods, as well as of initial versus final detection methods.

### Public reports

Public reporting utilises "citizen science", i.e. the involvement of the community in finding foragers, nests and/or swarms and reporting these to a free-call number.

Data entered into BioSIRT included information about detection methods, one of which is "public report". Any detections by public report since 2007 were summarised and analysed.

In addition, detections following public reports were analysed for location (suburb) and whether there was a relationship between the suburb's human population size and density as well as suburb area. Suburb population sizes were sourced from 2006 census data[1,2]. Suburb areas were based on census-specific state suburbs (SSCs; Australian Bureau of Statistics) in ArcGIS.

Data was also sourced from the Biosecurity Queensland Call Centre (BQCC; "public calls"). Such data was available from January 2009. In particular, the number of bee-related public calls made to BQCC was determined. BQCC classifies bee-related calls into three categories: (1) AHB General, (2) AHB Suspect Bee, (3) AHB Swarm, and (4) AHB bird roost (Rainbow bee-eater roost). Public calls between January 2009 and October 2012 were summarised overall as well as analysed by time (month/year/season).

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[1] Demographic, Social and Economic Profile of Cairns Regional Council – report; http://www.cairns.qld.gov.au/__data/assets/pdf_file/0018/5733/DemographicProfile.pdf (accessed 11/12/2012)

[2] Qpzm LocalStats Australia: www.localstats.qpzm.com.au (accessed 11/12/2012)

In order to determine a public call success rate, the number of detections by public report was divided by the total number of AHB related public calls to BQCC, overall as well as according to time (month/year/season).

The relationship between the number of AHB calls (swarm & suspect bee combined, swarm only, and suspect bee only) and the number of detections during the same month as well as during the following month was determined using a simple linear regression analysis.

## Standard AHB traps

Standard AHB traps were used for surveillance of AHB between 2010 and 2012.

Standard AHB traps consisted of a round, plastic self-filling water dish of various colours (17.5cm internal diameter) with a connection fitting most soft plastic soda bottles. The dish contained washed, fine grade sand shaped into a sloping beach and well. The dish was then filled with a lavender-scented sugar syrup solution as a reward (2kg of refined sugar, 1 teaspoon of powdered gelatine and approximately 5mL of lavender essential oil dissolved in 1.5 litres of tap water). Gelatine was added to the syrup in order to make the solution 'gluggy' and trap foraging bees. The sugar syrup solution was poured into the well to a height such that the beach was one third the size of the well in area providing a small platform from which bees could land and feed (please see Commerford *et al.*, 2013 for more detail).

AHB bee traps were placed on timber platform stations consisting of a hardwood stake with marine grade plywood top-plates attached. All stations were approximately 1.3m high.

Initially, an inverted soft drink bottle with excess sugar solution was then attached to the container. However, due to issues associated with fermentation of the sugar solution in the exposed bottles over time, bottles were no longer used after the end of 2010. Instead, traps were manually re-filled every two weeks.

To reduce loss of sugar solution from the dish over the two-week period due to evaporation in the sun and dilution of the syrup by rain, a small plastic garbage bin (hood) was modified by excising multiple rectangular entrances 4-8cm above the rim of the upturned bin. This allowed bees to enter the trap while providing some protection against evaporation or dilution. The lid was secured in place using plastic flagging tape of various colours. A thick layer of adhesive Tangle-Trap Insect Trap Coating Paste Formula (herein referred to as sticky paste) was applied around the wooden stake section of the platform 5-10cm below the top-plate to prevent insects (especially ants) from carrying off trapped specimens.

Seventy traps were placed around the edges of the KIA and within 'high-risk' areas (namely the port area of Cairns and around major transport nodes) in January 2011. In September 2011, trap numbers were increased to 101 traps, which stayed in place until July 2012 (Table 2; Figure 1).

Approximately one-third of the traps were placed each in urban environment (including industrial areas), agricultural environment and relatively natural environment (defined as very low or no housing surrounded by large tracts of uninterrupted forest; Table 3). Field personnel inspected the traps and remaining sugar syrup levels fortnightly.

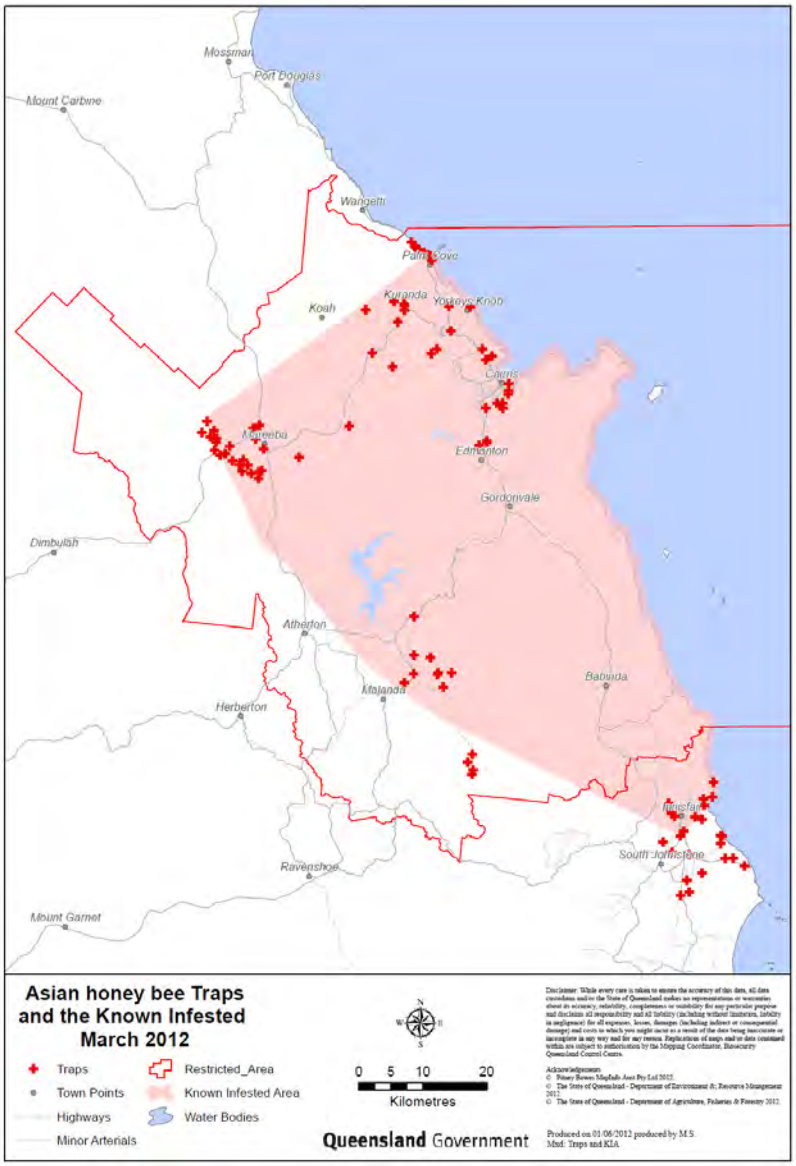


**Figure 1:** Placement of 101 AHB traps (shown as red crosses) across the Cairns/Tablelands region. Also shown are the KIA (red shading) current as at March 2012 and the Asian honey bee restricted area (RA; red line).

**Table 2:** General locations (suburbs) of 101 Asian honey bee traps in the greater Cairns area

| **Area** | **No of traps** |
|---|---|
| Cairns industrial area | 9 |
| Portsmith, Cairns | 8 |
| Northern beaches, Cairns | 13 |
| Innisfail | 25 |
| Malanda | 13 |
| Mareeba | 33 |
| **Total** | **101** |

**Table 3: Environment types that 101 Asian honey bee traps were placed in**

| Habitat type | No of traps | % of traps |
|---|---|---|
| Urban (incl. industrial) | 33 | 32.7 |
| Agricultural | 40 | 39.6 |
| Natural environment | 28 | 27.7 |
| **Total** | **101** | |

The efficacy of AHB traps was calculated in several ways:

1. For the period between January 2011 and August 2011 (previous 70 trap locations) the following was calculated:
    - The percentage of positive AHB detections based on the total number of traps checked;
2. For the period between January 2011 and May 2012 (current 101 trap locations) the following was calculated:
    - The percentage of positive AHB detections based on the total number of traps checked;
    - The trap run effort (estimated person hours and days);
    - The effort needed to detect one AHB;
    - The percentage of total operations time taken up by trap runs.
3. The time periods in (1) and (2) were also combined to determine a combined trap success rate between January 2011 and May 2012.
    - Trap efficacy for traps in different environments (using the current 101 traps) was also analysed.

## Rainbow bee-eater pellets

The Rainbow bee-eater (*Merops ornatus*) is one of many predators of honey bees in Australia. It is particularly useful for honey bee surveillance as it preys heavily on Hymenopterans, including bees, it regurgitates non-digestible portions of their prey (e.g. bee wings that can be identified to species level) in form of a pellet, and it congregates in large flocks in roost trees at night. These attributes make the Rainbow bee-eater ideal as a large-scale surveillance tool for the presence/absence of AHB, and may be particularly useful in detecting when AHB first arrive in an area, or in determining area of freedom.

### *Sample sites & collection*

A number of Rainbow bee-eater roosts were checked regularly across the Cairns and Atherton Tablelands region between 2007 and 2012 (Table 4, Figure 2). Nine roosts in six Cairns suburbs were included in the roost surveillance until April 2010, with occasional opportunistic pellet collection outside these nine roosts. From May 2010, roost surveillance encompassed three additional roosts, bringing the surveyed roosts to twelve. From September 2011, further roosts were opportunistically sampled, specifically from near the edge of the KIA. In 2012, surveillance of roosts within the KIA slowed as roost surveillance shifted further focus onto the edge of the KIA.

Pellet collection methods followed closely the methods trialled and established by Bellis and Profke (2003). After collection, pellet samples were sent to a parasitologist (Bill Doherty, Townsville) to be checked for the presence/absence of *A. mellifera* or *A. cerana* fore- or hind wings.

**Table 4: Rainbow bee-eater pellet collection runs and roost locations (suburbs) in the Cairns and Tablelands areas. The number of roosts per location is given in brackets.**

| **Roost runs** | **Cairns area until April 2009** | **Cairns area from May 2010** | **Cairns North** | **Innisfail** | **Tablelands** |
|---|---|---|---|---|---|
| Roost locations | Cairns City (2)<br>Earlville (1)<br>Edmonton (1)<br>Green Hill (2)<br>Portsmith (2)<br>Smithfield (1) | Cairns City (2)<br>Earlville (1)<br>Edmonton (1)<br>Goldsborough Valley (1)<br>Green Hill (2)<br>Machans Beach (1)<br>Portsmith (2)<br>Smithfield (1)<br>Yarrabah (1) | Clifton Beach (1)<br>Kewarra Beach (1) | Etty Bay (1) | Atherton (1)<br>Kuranda (1)<br>Mareeba (1)<br>Mutchilba (1) |

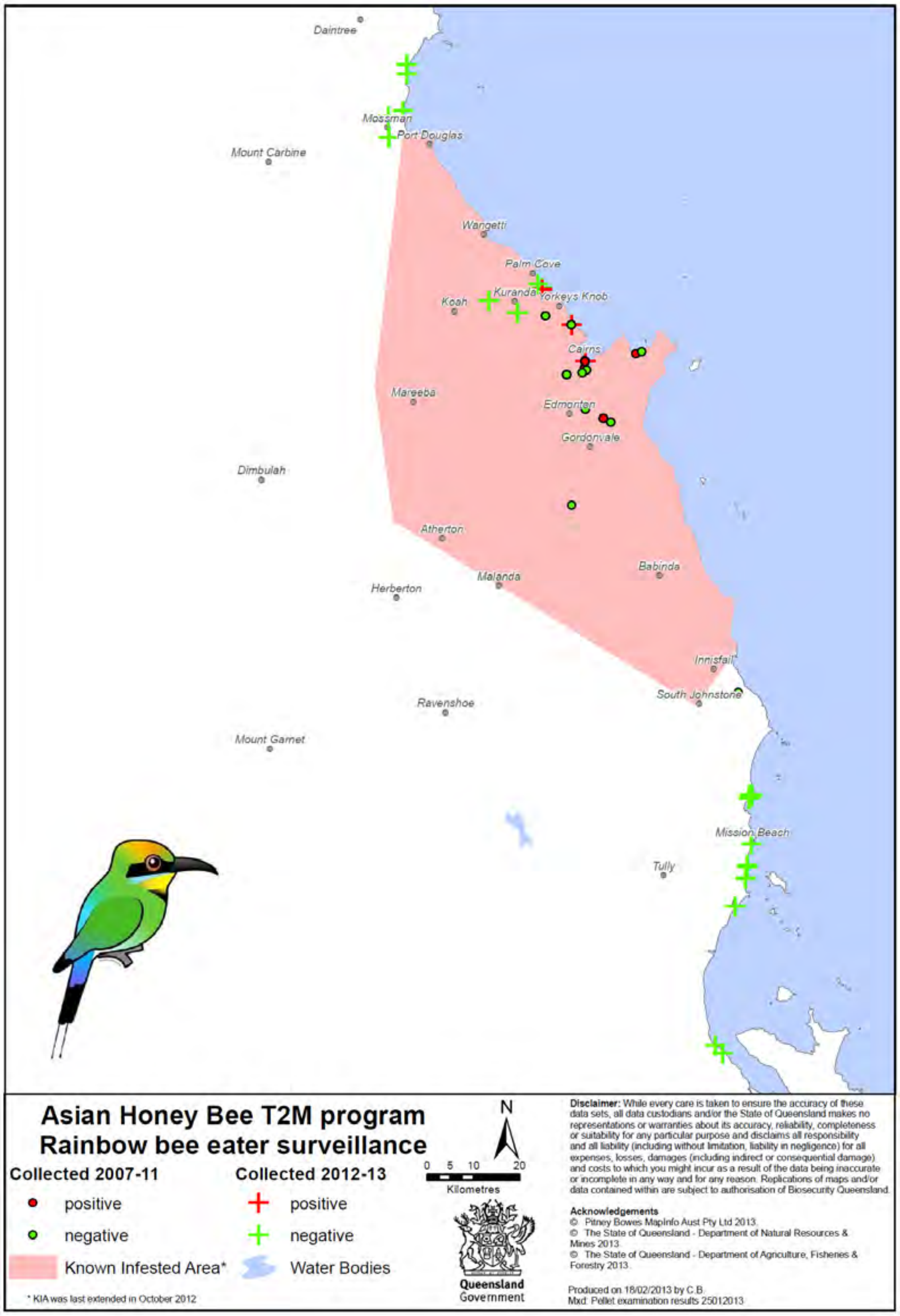


**Figure 2:** **Rainbow bee-eater roost surveillance for presence/absence of AHB in the Cairns and Tablelands area. Red and green circles depict roosts checked for pellets between 2007 and 2011; red and green crosses depict roosts checked for pellets in 2012/2013. Red circles/crosses indicate pellets positive for AHB, green circles/crosses indicate pellets that did were negative for AHB. The KIA (2013) is shaded in pink.**

### *Data analysis – efficacy*

Records were kept on sample collection date and the date when results were received from the parasitologist by facsimile. This data was used to determine the turn-around time for laboratory testing. In addition, all data available on the pathology reports as well as additional information on roost locations were entered into an Excel database. These included the location of the bird roosts (including GPS location), date pellets were collected, presence or absence of *A. mellifera* and/or *A. cerana* wings, and the number of wings present where available.

Unfortunately, no records were taken on the length of time it took to find bee-eater roosts, to drive to and from roosts, or to find and collect pellets at each roost. Therefore, efficacy could not be confidently established. Nevertheless, efficacy was estimated using similar methods to those determining AHB trap efficacy by estimating the drive time for different "roost runs" (Table 5) as well as adding an estimated 15 minutes of searching for pellets at each stop on the "roost run". Drive times for round trips were estimated in Google Maps.

Using the pellet collection data available, an average catch per unit effort was then calculated for different roost runs, for both AHB and EHB. The underlying assumption was that all roosts were checked on each of these roost runs. Roost runs were not conducted as such, rather, roosts were checked opportunistically when an AHB team was in the area for AHB trap runs, public responses, or edge surveillance. Nevertheless, if structured roost runs were to be conducted, then the calculated efficacy will give an indication of the return per effort required.

**Table 5: Rainbow bee-eater pellet collection runs in the Cairns and Tablelands areas to determine presence/absence of AHB. Drive times were estimated using Google Maps. Search time was estimated at 15 minutes per roost.**

| Roost Run | Roost locations | # roosts | Estimated drive time (hrs) | Estimated search time (hrs) | Total time/run (hrs) |
|---|---|---|---|---|---|
| Cairns area until 25/09/2009 | Portsmith<br>Cairns city<br>Smithfield<br>Earlville<br>Edmonton<br>Green Hill | 2<br>2<br>1<br>1<br>1<br>2 | 1.75 | 2.5 | 4.25 |
| Cairns area from 18/05/2010 | Portsmith<br>Cairns city<br>Smithfield<br>Earlville<br>Edmonton<br>Green Hill<br>Machans Beach<br>Yarrabah<br>Goldsborough Valley | 2<br>2<br>1<br>1<br>1<br>2<br>1<br>1<br>1 | 3.5 | 3.0 | 6.5 |
| Cairns North | Kewarra Beach<br>Clifton Beach | 1<br>1 | 1.0 | 0.5 | 1.5 |
| Innisfail | Etty Bay | 1 | 2.75 | 0.25 | 3.0 |
| Tablelands | Kuranda<br>Mareeba<br>Mutchilba<br>Atherton | 1<br>1<br>1<br>1 | 4.0 | 1.0 | 5.0 |

### *Data analysis – spatial analysis*

The locations of positive and negative pellet samples in the two time periods 2007-2011 and 2012/13 were mapped and overlayed over the KIA, using ESRI ArcGIS software (Version 10.5; Figure 2).

Location and date of pellet samples positive for AHB were also compared with the location and date of nearest actual AHB nests or swarm detection. This was done to determine instances where Rainbow bee-eater pellets were found prior to actual nests or swarms being found.

## Bee lining

Once foragers were found using any of the above methods, “bee lining” was used to follow foraging bees back to their nest. Therefore, this is not a detection method as such but relies on initial detection of forager.

No detailed records were available on the length of time field staff took to bee line individual nests. Therefore, to determine how long it took to detect a nest by bee lining, for any nest detected by bee lining the date of initial detection and the date of confirmation or destruction (whichever was sooner) were sourced from BioSIRT. From this initial detection the number of days until confirmation/destruction was calculated and taken as an estimate of bee lining effort.

# Results

## General analysis of AHB detection

Overall, 799 nests and swarms were detected between May 2007 and 31 October 2012. Most of these (71.1%) were initially detected following a public report. The remainder was detected by floral observations (11.4%) and a few by trap (2.6%). 14.6% of detections did not report method of initial detection (Table 6).

Most nests were initially detected by public reports (58.6%) and floral observations (16.2%), whereas the vast majority of swarms (97.3%) was initially detected by public reports and very few by floral observations (1.5%; Table 6).

Final detection methods included public report (where no further search action was required; 68.3%), bee lining (29.9%), bee lining by detection dog (0.3%) and other (1%). In 0.5% of cases, final detection method was not recorded (Table 6).

Final detection of nests was most often done through public reports (54.7%) or bee lining (43.2%). Two nests were found by detection dog. Final detection of swarms was through public reports in the majority of cases (96.5%). Six swarms were found by bee lining (Table 6).

Initial detection by public report was most often followed by no further search (96.3% of cases) but a small number required bee lining (3.7%). Initial detection by floral sweeping was most often followed by bee lining (92.3%) with a few unknown or other final detection methods (7.7%). All detections by trap were followed by bee lining. Of the 119 detection that had no initial method of detection recorded, most were followed up by bee lining (96.6%) in the remaining cases the final detection was unknown or other (Table 6).

**Table 6: Number of AHB nests and swarms found through different initial and final detection methods for 799 AHB detections between May 2007 and 31 October 2012.**

| Initial Detection | Final detection | Nest | Swarm | Total |
|---|---|---|---|---|
| Public report | Public report | 296 | 251 | 547 |
| | Bee lining | 20 | 1 | 21 |
| Floral observations | Bee lining | 81 | 2 | 83 |
| | Dog bee lining | 1 | | 1 |
| | Unknown or other | 4 | 1 | 5 |
| Trap | Bee lining | 20 | | 20 |
| | Dog bee lining | 1 | | 1 |
| Unknown or other | Bee lining | 112 | 3 | 115 |
| | Unknown or other | 4 | 1 | 5 |
| Total | | 539 | 260 | 799 |

## Public reports

Between May 2007 and October 2012, 566 detections (71.2% of all detections) were made following a public report. During this period, slightly more nests (311, 55.5%) than swarms (252, 44.5%) were detected by public reports.

The number of detections following public reports was very low in 2007 and 2008. Detections by public reporting increased slightly in 2009 and then dramatically from June 2010. Following June 2010, levels of detections by public reports fluctuate greatly (Figure 3). Peaks of detections by public reports do not seem to coincide with specific seasons or months (Figure 3).

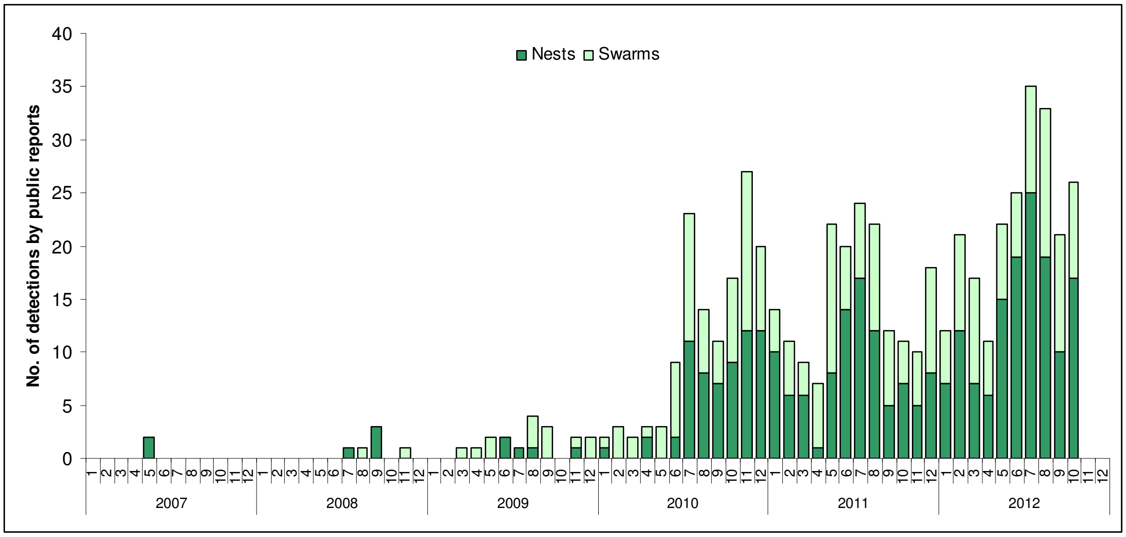


**Figure 3: The monthly number of AHB swarm and nest detections following a public report between May 2007 and October 2012.**

### *Public reports by suburb*

Between May 2007 and October 2012, detections by public reports were made in 75 different suburbs in the Cairns and Tablelands region. Gordonvale and Portsmith had the most detections by public reports (>50 detections), followed by Edmonton and Cairns City (>30 detections), and Aeroglen and Bentley Park (>20 detections; Figures 4 & 5). The remaining four suburbs in the top ten include Mount Sheridan, Mareeba, Aloomba and Parramatta Park (Figure 4). These ten suburbs account for more than half (53%) of all detections by public reports.

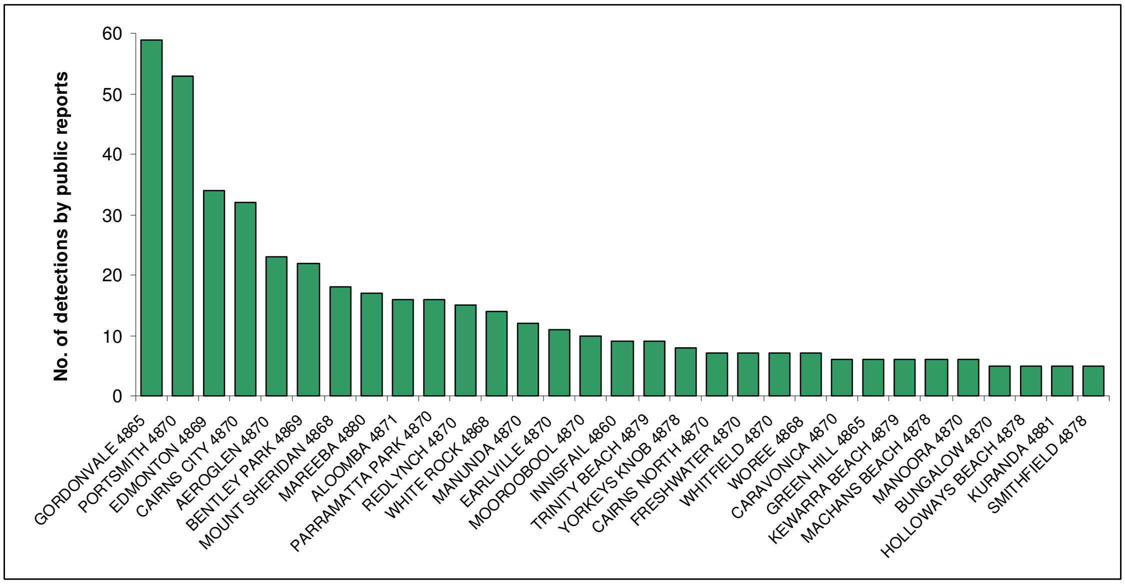
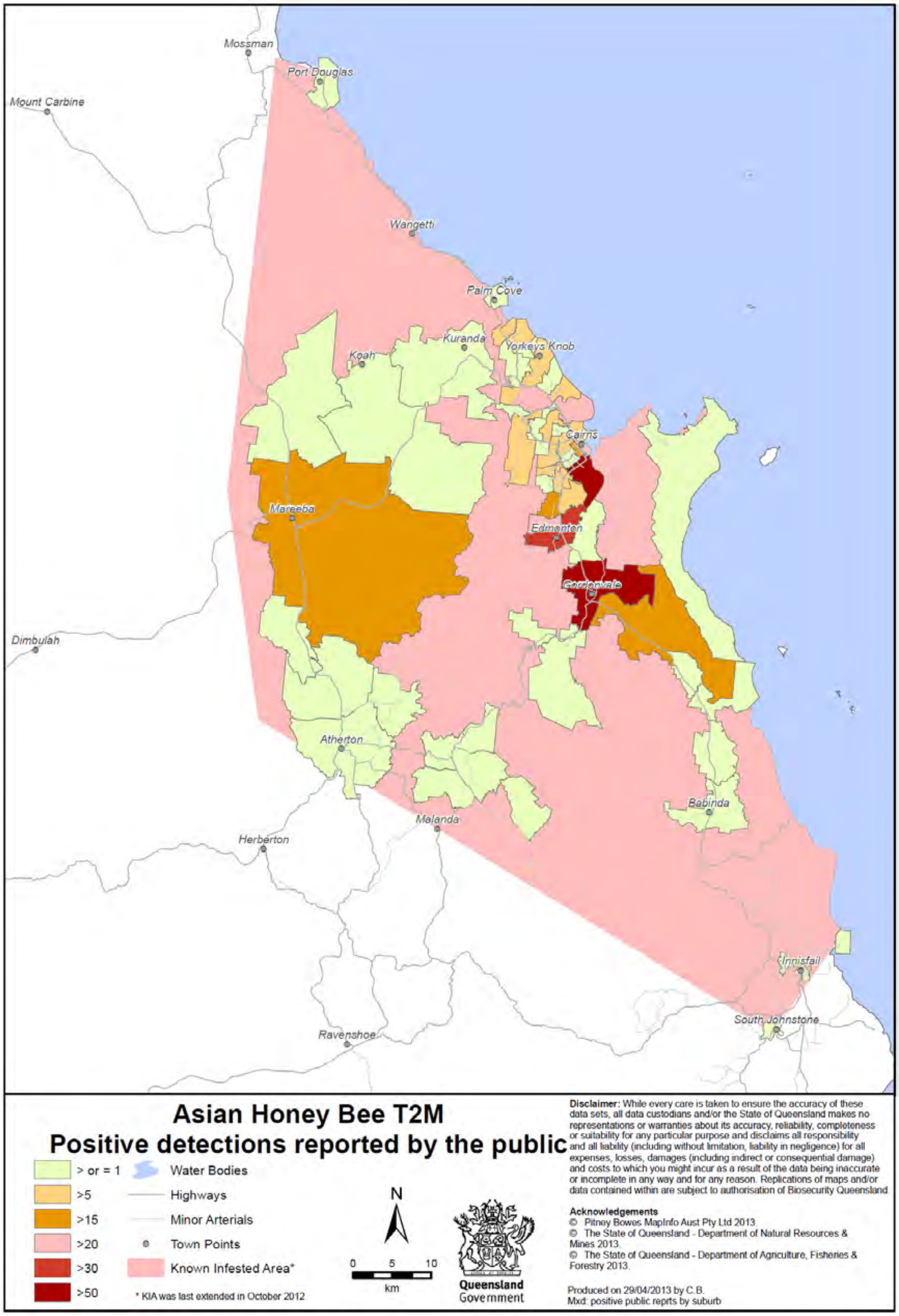


Figure 4: **The number of AHB detections by public report in different suburbs in the Cairns and Tablelands area between May 2007 and October 2012 (suburbs with less than five detections were excluded).**



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Produced on 29/04/2013 by C.B.
Mxd: positive public reprts by suburb

**Figure 5: The number of AHB detections by public report in different suburbs in the Cairns and Tablelands area between May 2007 and October 2012. Darker colours depict greater numbers of detections by public reports.**

A linear regression showed no relationship between the number of detections and the suburbs' population densities ($F_{1,67}$=0.15, p>0.05). Similarly, there was no relationship between the number of detections and the suburbs' area ($F_{1,70}$=0.02, p>0.05). This means that public reports did not depend on a suburb's density or area.

However, there was a weak but significant correlation between the number of detections and the suburbs' population size ($F_{1,67}$=9.56, p=0.003, $r^2$ = 0.113), i.e. the larger the suburb's population size, the more detections by public reports were made (Figure 6). However, only 11.3% of the variation in the data can be attributed to population size, meaning that other factors are also influencing the number of public calls.

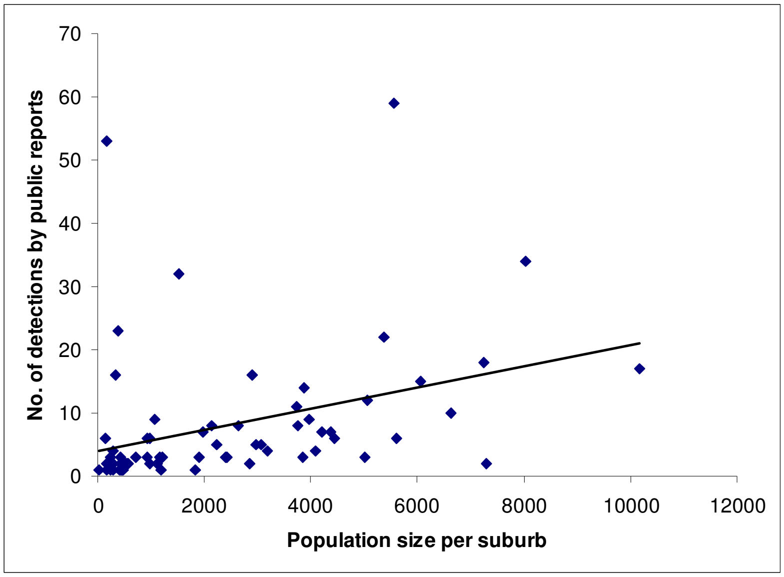


**Figure 6: Relationship between the number of AHB detection by public report and the population size of the suburb the detection was made. Line of best fit shown ($r^2$ = 0.113).**

When correcting the number of detections for population size (i.e. when dividing the number of public detections by the suburb's population size), the suburbs with the highest relative detections were centred slightly south of Portsmith. Portsmith had the highest number of detections relative to population size (0.32), followed by Fitzroy Island (0.08), Aeroglen (0.06), Aloomba (0.05), Green Hill (0.04), Cairns City (0.02), Wright's Creek (0.01), Little Mulgrave (0.01), Lake Barrine (0.01) and Gordonvale (0.01).

## *Public calls*

3113 bee-related calls were made to BQCC between January 2009 and October 2012 with an average of 67.7 calls per month. 2823 (90.7%) of those calls were related to suspect bees or swarms (average of 61 calls per month), the remainder (290 calls or 9.3%) was classed as general or relating to bee-eater roosts.

In the same time period (January 2009 and October 2012), 546 detections (70.3% of all the detections in this time period) were made by public reports. This equates to 17.5% of all calls and 19.3% of all "suspect bee/swarm" calls to BQCC resulting in a positive detection of AHB.

The following analyses will be based on calls related to suspect bees or swarms only.

Over time, both the calls to BQCC and the detections by public reports fluctuated (Figure 7). However, these fluctuations were not correlated, i.e. the number of total calls (swarms and suspect bees) to BQCC each month was not correlated with the total number of detections by public reports in the same month ($F_{43}$=2.88, p>0.05). That means that more calls made to BQCC did not result in more successful detections by public reports.

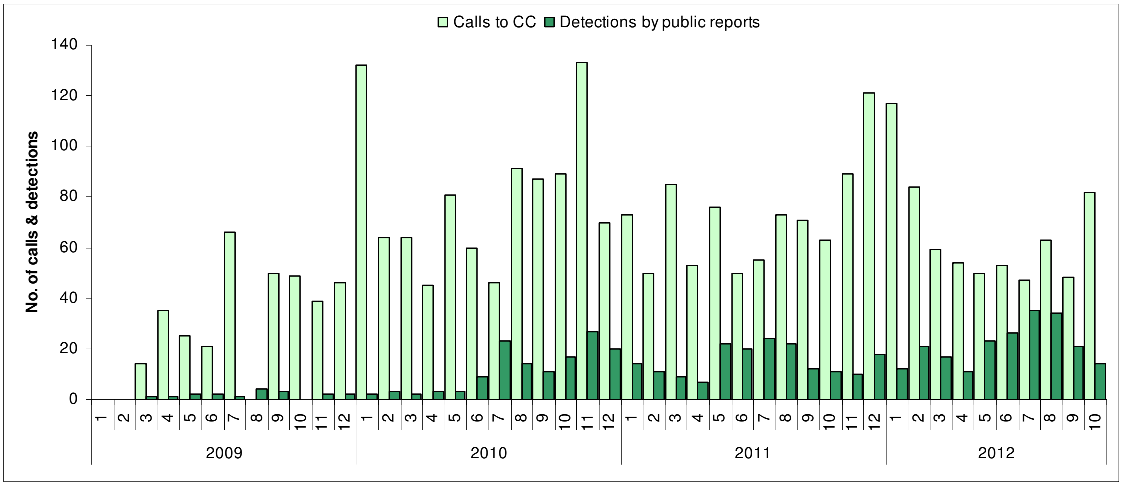
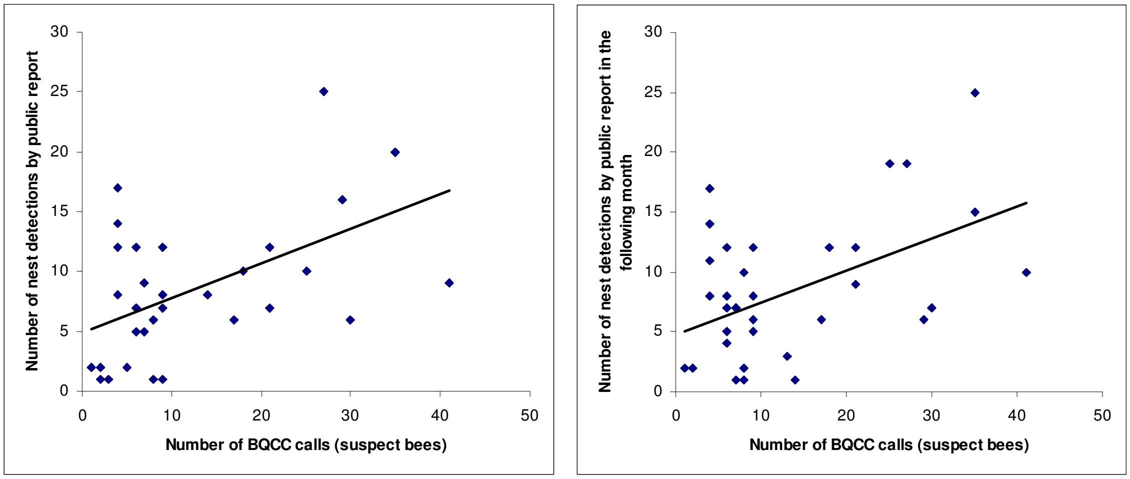


**Figure 7: The number of bee-related calls (suspect bees or swarms only) to BQCC and the number of AHB detections by public reports between January 2009 and October 2012.**

However, there was a positive relationship between the number of "suspect-bee" calls and the total number of public detections, the number of nests detected as well as the number of swarms detected, in the same month (total: F=15.32, $r^2$=0.264, p<0.001; nests: F=13.05, $r^2$=0.274, p=0.001; swarms: F=5.77, $r^2$=0.114, p=0.022; Figure 8a). This means that the more suspect-bee-related calls are made in one month, the more AHB nests and swarms are found through public reports in that same month (Figure 8a).

In addition, there was also a positive relationship between the number of "suspect-bee" calls and the total number of public detections in the following month, the number of nests as well as the number of swarms detected in the following month (total: F=14.33, $r^2$=0.255, p<0.001; nests: F=11.04, $r^2$=0.223, p=0.002; swarms: F=5.97, $r^2$=0.118, p=0.020; Figure 8b). This means that the more suspect-bee-related calls are made in one month, the more AHB nests and swarms are found through public reports in the following month (Figure 8b).

There was no relationship between swarm-related calls and the number of swarms detected in the same or the following month (F 0.68, p>0.05 and F 0.01, p>0.05, respectively). This indicates that detections of nests rather than swarms drive these relationships.



a) b)

**Figure 8: The relationship between the number of BQCC calls (suspect bees) and the number of nest detections by public report in the same month (a) and the following month (b). Line of best fit shown.**

The monthly success rate of public calls (i.e. the number of public calls to BQCC that result in a detection) fluctuated over time. However, the success rate of public calls was significantly higher during the dry months (May to October) than during the wet months (November to April; $t_{df=31.1}$=-2.84, p=0.008; Figure 9).

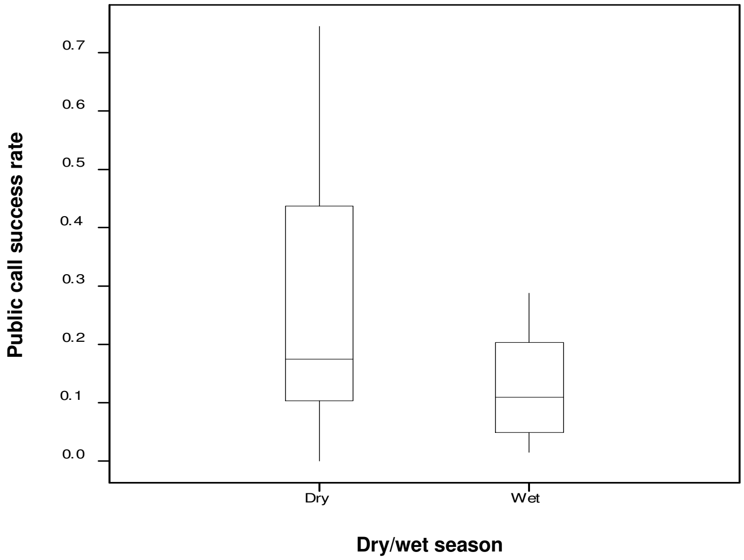


**Figure 9: Boxplot showing the success rate of public calls (number of public calls reporting a suspect bee or swarm resulting in detection) for dry season months (May to October) and wet season months (November to April) between 2009 and 2012.**

## Standard AHB traps

Trap success was found to be exceedingly low (Table 7). Only four AHB detections were made in traps between January 2011 and May 2012 (74 weeks = 37 trap runs = 1609.5 person hours), leading to a trap success rate of 0.14% (Table 7).

In 2012, the operations team had 48 person-days available per week (= 4 field staff x10 days/fortnight + 1 field staff x8 days/fortnight). It took an estimated six person-days/fortnight (or 43.5 hours) to do a trap run. Therefore, the field team spent 12.5% of their time (each fortnight) checking traps. Catch per effort was 0.0025 bees/person hour, or 402.4 person hours to find one bee (given 37 trap runs = 1609.5 person hours).

Three of the four AHB detected in traps were found in industrial areas, whereas one was found in natural environment (Eucalypt woodland; Table 8). Therefore, trap success was lowest for agricultural environments (0%). Trap success in natural and urban environments was 0.2% and 0.6%, respectively (Table 9).

**Table 7: AHB trap success presented as percent of traps containing AHB for two different time periods (January - August 2011 and September 2011 - May 2012) as well as the combined period (January 2011 - May 2012).**

| Time period | No of traps checked | No of AHB found | Trap success |
|---|---|---|---|
| January - August 2011 | 1120 | 4 | 0.36% |
| September 2011 - May 2012 | 1818 | 0 | 0% |
| January 2011 - May 2012 (combined) | 2938 | 4 | 0.14% |

**Table 8: Date, location and vegetation type of the four Asian honey bees found in AHB traps between January 2011 and May 2012.**

| Date | Trap number & location | Vegetation |
|---|---|---|
| 5/08/2011 | AHB13403, Portsmith, Cairns | Industrial |
| 17/08/2011 | AHB13388, Kuranda | Eucalypt woodland |
| 11/07/2011 | AHB13403, Portsmith, Cairns | Industrial |
| 11/07/2011 | Hargreaves Rd, Edmonton | Industrial/agricultural |

**Table 9: AHB trap success for different environment types between September 2011 and May 2012.**

| Environment type | No. of traps | No of traps checked | No of AHB found | Trap success |
|---|---|---|---|---|
| Urban (incl. industrial) | 33 | 528 | 3 | 0.6% |
| Agricultural | 40 | 640 | 0 | 0% |
| Natural environment | 28 | 448 | 1 | 0.2% |

## Rainbow bee-eater pellets

Between May 2007 and 31 October 2012, 174 pellet samples were sent away for testing, 125 (71.8%) of which were positive for the presence of *Apis* wings (both AHB and/or EHB). Half of all samples were positive for *A. mellifera*, and one-fifth were positive for *A. cerana* (38 samples, 21.8%).

37 of the 38 positive AHB samples had an associated wing count, with an average of 12.2 wings found per pellet (range: 1-190; median = 3) and the majority of pellets contained one to ten AHB wings. Only nine of the 87 EHB samples had an associated wing count, which were only recorded as "numerous".

Records for 33 "roost runs" in the Cairns area were available, 19 of which included nine roosts (until late 2009) and the remainder included 12 roosts (from early 2010). In total, an estimated 339 roosts were checked between 2007 and 2012, and an estimated 171.75 hours was spent doing so.

Of the 339 roosts checked, 31 roosts (9.1%) were positive for AHB and 65 (19.1%) were positive for EHB. 71.7% were negative for both AHB and EHB. Surprisingly, pellets never contained both AHB and EHB wings. Catch per unit effort was 0.18 per hour for AHB and 0.38 per hour for EHB, or 5.5 hours spent to find one AHB and 2.6 hours spent to find one EHB.

Some limited data for other roost runs was available. Records for 10 roost runs to two roosts in the northern beaches (Kewarra Beach and Clifton Beach) were available. In total, these roosts were checked 20 times, and an estimated 20 hours was spent doing so. Five roosts (25%) were positive for AHB and six roosts (30%) were positive for EHB. Catch per unit effort was 0.25 per hour for AHB and 0.30 per hour for EHB, or 4 hours spent to find one AHB and 3.3 hours spent to find one EHB.

Neither the Atherton/Mareeba run nor the Etty Bay run yielded any positive AHB samples despite detecting the presence of EHB. EHB was detected at similar catch per unit effort rates as the previous runs, i.e. 0.25 per hour for Atherton/Mareeba run and 0.33 per hour for the Etty Bay run.

Whether or not Rainbow bee-eater pellets return a positive or negative result depends in part on whether the roosts are located within or outside the KIA at the time of pellet collection.

In most instances, roosts were located within the then KIA, and pellets positive for AHB merely confirmed AHB presence in an area. In three instances, AHB negative pellets were found within the KIA, indicating that false negatives are possible. However, there were four instances where pellets positive for AHB were found outside the then KIA.

The Rainbow bee-eater roost in Smithfield yielded AHB positive pellets both in May and June 2010. In addition, Machans Beach roost also yielded AHB positive pellets in May 2010. At that time, the KIA only extended as far north as Aeroglen/Stratford, which is approximately 2km south of the Machans Beach roost, and approximately 8km south of the Smithfield roost (Figure 2). The first nest/swarm detection in Machans beach occurred in November 2010, 162 days after the AHB positive pellet was found. Similarly, the first nest/swarm detection in Smithfield occurred in September 2010, 111 days after an AHB positive pellet was found.

Finally, the first nest/swarm in Bayview Heights was detected 139 days after the Rainbow bee-eater roost yielded a positive AHB pellet, even though Bayview Heights was technically inside the then KIA.

Apart from these four instances, no AHB positive pellets have been found outside the KIA (Figure 2).

## Bee lining

There were 239 detections recorded in BioSIRT that involved bee lining. Most of these were initially detected through floral sweeping (34%). Low numbers were initially detected through public calls (9%) or AHB trap (8%); 48% had no initial detection method recorded.

On average, it took 3.1 days to bee line a nest following initial detection, with a minimum of zero days (nest found on the same day as initially detected) and a maximum of 82 days. Given that the data are highly left-skewed (many small numbers, very few large numbers; Figure 10) the median number is more informative. The median time it took to detect a nest by bee lining was zero days.

Most nests (60.7%) were found by bee lining on the same day they were initially detected, or within the first two days thereafter (84.1%). The majority of nests were found by bee lining within four days of initial detection (90.4%).

However, some nests took longer to find (>20 days). However, this may not be due to bee lining taking that long, but rather due to the fact that some nests, once detected, were left for scientific research and not checked for some time. Unfortunately, no written records were kept on which nests were detected but then kept for later research.

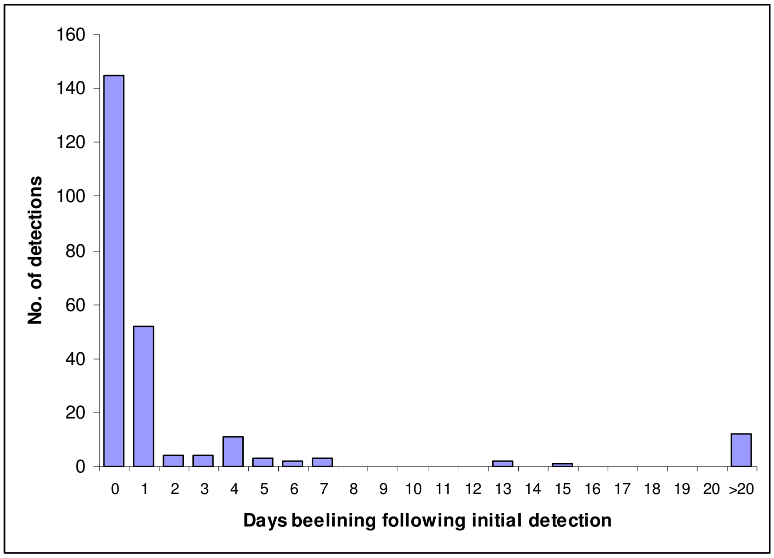


**Figure 10:** Length of time (days) it took to find nests by bee lining

# Field trials

Floral observations were utilised for the entire AHB Program between 2007 and 2013. They involve scanning flowering plants for the presence of AHB, and, when necessary, catching bee samples using a net. This use of a net resulted in these methods being referred to as sweep netting. However, these methods should be more correctly referred to as floral observations.

Targeted floral observation required extensive floral mapping of an area, and then targeting flowering plants according to season. In this report, targeted floral observations were not tested. Instead, “timed” floral observations were tested, which did not require floral mapping, but involved timed observations of specific flowering plants across a field site rather than generally scanning for flowers while walking a transect (= general floral observations).

Thus, the aim of the efficacy field trial experiment was to determine whether walking along a transect (general floral observations) or stopping only at specific flowering plants for a set amount of time (timed floral observations) resulted in better detection rates of *A. cerana*. Based on the outcomes of this trial, recommendations will be made on the most effective way of conducting surveillance for *A. cerana*.

## Methods

### Study sites

Four locations were chosen across the greater Cairns area (Figure 11) to represent different levels of assumed *A. cerana* densities, as well as different habitats (Table 10). Within these locations, two replicate sites were placed in suitable areas, i.e. where transects could be placed along roads in such a way that they came to approximately 2-2.5km within a square of 500x500m (Figure 12). As roads within rainforest were found to be straight, rainforest transects were linear.

Each location was surveyed once a month starting in September 2012 and finishing in March 2013. Each monthly surveillance trial took four consecutive days (Table 11). On each surveillance day, both sites within a location were visited twice – once in the morning (8.00-10.00) and once in the early afternoon (12.30-14.30) (Table 11). The order in which locations were visited each month was randomised such that each location was visited on a different day for the first four months. This same order was then repeated for the remaining three months (Table 11).

Standardised transect walks and timed floral observations were trialled concurrently at the two study sites within a location, in order to determine the differential effectiveness of each method. This is a standard experimental design used in biodiversity and abundance studies (e.g. Roulston *et al.*, 2007; Westphal *et al.*, 2008), albeit modified to target AHB rather than determining general bee diversity within an area.

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**Acknowledgements**
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Figure 11: **Eight study sites within four locations for surveillance trials in or near Cairns, Queensland, Australia. Yellow – Kuranda sites; green – rainforest sites; orange – Cairns City sites; blue – Gordonvale sites.**

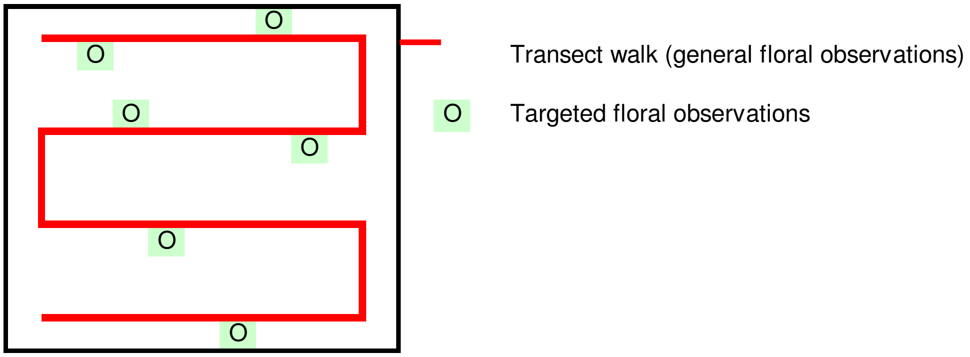


**Figure 12:** Diagram of a study site showing the transect placement and example locations of timed floral observation (observation 'plots').

**Table 10:** Locations of study sites showing habitat type, assumed Asian honey bee density and the number of study sites within each location.

| Location | Habitat | Assumed density | # trial sites |
|---|---|---|---|
| Cairns City | Urban | High | 2 |
| Gordonvale | Urban/rural | High | 2 |
| Kuranda | Rural/Rainforest | Unknown/low | 2 |
| Rainforest | Rainforest | Low | 2 |

**Table 11:** The order in which locations were visited each month between September 2012 and March 2013. Each location was visited on a different day between September 2012 and December 2012. This order was then repeated from January 2013.

| | Day 1 | Day 2 | Day 3 | Day 4 |
|---|---|---|---|---|
| September 2012 | City | Gordonvale | Kuranda | Rainforest |
| October 2012 | Gordonvale | Rainforest | City | Kuranda |
| November 2012 | Rainforest | Kuranda | Gordonvale | City |
| December 2012 | Kuranda | City | Rainforest | Gordonvale |
| January 2013 | City | Gordonvale | Kuranda | Rainforest |
| February 2013 | Gordonvale | Rainforest | City | Kuranda |
| March 2013 | Rainforest | Kuranda | Gordonvale | City |

## Surveillance/detection methods

Two different detection methods were trialled: general floral observations and timed floral observations.

Within the AHB Program, floral surveillance has been termed 'floral sweeping' or 'floral sweep netting'. However, in line with the scientific literature, it should be called 'floral observation' along a variable transect walk where bees are identified 'on the wing' (i.e. without catching in a net). *Not* catching targeted insects is routinely done in scientific studies where the species is easily identifiable without catching (e.g. Roulston *et al.*, 2007; Westphal *et al.*, 2008). Thus, in this document, we will refer to general and timed 'floral observations' rather than 'floral sweeping'.

### *Timed floral observations*

Timed floral surveillance commenced at the same time as transect surveillance. An observer would start walking along the transect until a suitable flowering plant was encountered. Suitable flowering plants were any flowering plants with focus on those preferred by *A. cerana* according to the "Field Guide to Hosts of *Apis cerana*" (Durkan, 2010). Once such a plant or group of plants (floral source) was encountered, the observer scanned the flowers of the plant(s) for 10 minutes, noting down the start time, GPS location, plant name(s), as well as how many AHB and European honey bees (EHB; *Apis mellifera*) were seen on the flowers over the 10-minute period.

Between four and six such spot observations were made within a two-hour period, depending on the availability of suitable flowering plants. Locations of spot observations were spread across the study site, and generally changed between months due to changing flowering seasonality.

The same plants that were chosen for timed surveillance during the morning session were then used again in the afternoon session.

### *General floral observations*

General floral surveillance was conducted along a standardised transect walk across the 500x500m study site. Two observers walked slowly along the transect, one person on each side of the road, scanning for *A. cerana* and *A. mellifera* on any flowering plants. In residential areas, any flowering plants in front yards were included as long as entering private property was not necessary. The entire transect was to be covered in exactly two hours. When *A. cerana* or *A. mellifera* were encountered on a flowering plant (floral source), this was noted down, including information such as the time, GPS coordinates, plant species and number of bees present.

The two observer teams (one team at each site) would swap sites between the morning and afternoon sessions. The only exception was the rainforest sites. Due to the distance and driving time between the sites it was impractical to swap teams.

## Analysis

Any single flowering plant or group of plants in close proximity that *A. mellifera* and/or *A. cerana* were found on will be referred as 'floral source'. As such, a floral source is a flowering plant at a specific location on the transect that what positive for either AHB or EHB. As the current report focuses on detection, 'floral source' rather than 'number of bees' was used for analysis.

Analysis will be done in three parts: (1) Catch-per-unit-effort (based on the number of AHB/EHB positive floral sources per transect) between detection methods, specifically focussing on *A. cerana*; (2) catch-per-unit-effort (based on the number of AHB/EHB positive floral sources per transect) between *A. cerana* and *A. mellifera*; (3) a comparison of numbers of individual *A. cerana* and *A. mellifera* found in different areas and on different plants. A full analysis of plant preferences of *A. cerana,* as well as comparisons between *A. mellifera* versus *A. cerana* floral preferences, can be found in the report "Ecology and Behaviour of Asian honey bees (*Apis cerana)* in north-eastern Australia" (Commerford and Koetz, 2013).

For each surveillance method, a catch-per-unit-effort (henceforth called catch-per-effort, CPE) was calculated, for both AHB and EHB. CPE was calculated by dividing the number of individual locations (floral sources) within a study site where AHB or EHB were found (the catch) by the time it took to conduct the surveillance (the effort).

As the transect walks were done by two people and the timed floral observations by one person, for transect walks the number of floral sources found positive for AHB, as well as the total number of AHB/EHB, was divided by two in order to make the data (catch) comparable between methods. Hence, CPE is presented per person unless otherwise stated.

Effort was measured in three ways: (1) the actual surveillance time (per person), i.e. time spent actively looking for bees (i.e. the entire time for transect walks, but for timed floral observations only the time actually looking at flowers), (2) the total time it took for the surveillance (per person; from the beginning of the transect to the end of the transect, including walking time between observation plots for timed floral observations).

Catch-per-effort was then compared between surveillance methods while taking into account effects of site, month and floral diversity. A General Linear Mixed Model (GLMM, using restricted maximum likelihood, REML) was used to statistically distinguish between the catch-per-effort of different detection methods (using “detection method” and “site” as fixed effects and “month” and “floral diversity” as random effects). A value of 1 was added to each CPE value before analysis to prevent values of zero being disregarded as missing values.

# Results

## General

Eight different sites were visited once a month between September 2012 and March 2013. Each month, each site was surveyed twice on one day (am and pm) using two different detection methods concurrently. Thus, over the seven months, 224 surveys were done (28 per site), taking a total of 389.5 hours (599.3 person hours).

Of the 224 individual surveys, 83 detected AHB (37.1%) and 191 detected EHB (85.3%). Of 112 transects surveys, 33.0% found AHB, whereas 90.2% found EHB. Of the 112 floral observation surveys, 41.1% found AHB and 77.7% found EHB.

Sites that one was most likely to encounter AHB on a survey were City 2 (residential Cairns City – 92.9%), and Gordonvale 1, City 1 (Cairns CBD) and Gordonvale 2 (57.1%, 50% and 50%, respectively). Kuranda sites and Rainforest sites had a probability of <10%. In contrast, most sites had a probability of encountering EHB of >90%, with the exception of Cairns City sites (City1 – 14.3%, City 2 – 64.3%) and Rainforest 2 (50%).

Time of day (am/pm) did not matter in terms of detecting AHB on transects surveys. Slightly more floral observation surveys were successful in the morning (37.5% morning versus 28.6% in the afternoon).

A total of 1733 AHB were counted on 212 floral sources, 7283 EHB were counted on 1521 floral sources, and only 79 instances (floral sources) were found that had both AHB and EHB present. Thus, 7.2 times more EHB-positive floral sources were found than AHB-positive floral sources, and the number of individual EHB was 4.2 times higher than that of AHB.

Therefore, overall efficacy was 0.53 AHB-positive floral sources per hour (0.35 per person hour) and 3.91 EHB-positive floral sources per hour (2.54 per person hour). Thus, using the exact same methods and locations, efficacy of finding EHB was 7.8 times greater.

## Time spent on surveys

On average, transect walks took 22.6 minutes longer (total time) than timed floral observations of the same area (Table 12). Actual surveillance time was twice as long for transect walks than timed floral observations (Table 12). When accounting for the fact that two people conducted each transect walk and one person conducted timed floral observations, person-hours doubled for transect walks but remained the same for timed floral observations (Table 12).

**Table 12: Average surveillance time (mins ± StDev) for each detection method (timed floral observations and transect walks), showing total time (total time taken for surveying a transect and for timed floral observations including walking between observation plots), actual time (actual time spent actively looking for bees, which does not include walking from one observation plot to another) and time in person-hours.**

| Detection method | Total time (mins ± StDev) | Actual time (mins ± StDev) | Person-hours (mins ± StDev) |
|---|---|---|---|
| Timed floral observations | 94.0 (±20.4) | 56.3 (± 5.7) | 94 (± 20.4) |
| Transect walk | 116.6 (±8.2) | 116.4 (± 8.2) | 230.0 (± 8.2) |

## Catch-per-unit-effort between detection methods

### *Total time*

When considering the total time of surveillance, there was no significant difference in CPE between the detection methods (Table 13; Figure 13 & 14a).

However, $CPE_{total}$ was significantly different between sites (Table 13). Generally, $CPE_{total}$ was highest in Cairns City (particularly site “City 2”), followed by Gordonvale sites, and lowest in Kuranda and the rainforest (Figure 14b).

Although detection methods did not differ for most sites, transect walks at “City 1” yielded particularly low $CPE_{total}$, similar to Kuranda and rainforest sites (Figure 14b).

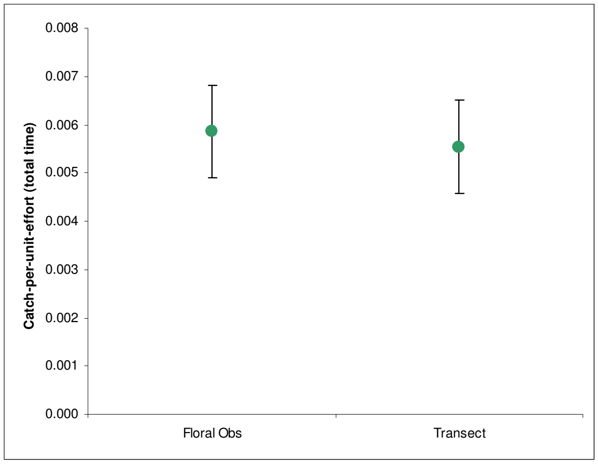


**Figure 13:** Catch-per-unit-effort (total surveillance time) ± 1 S.E. for two different detection methods (timed floral observations and transect walks). Catch per unit effort (total time) was calculated by dividing the number of individual locations (floral sources) within a study site where AHB were found (the catch) by the total time it took to conduct the surveillance (the effort).

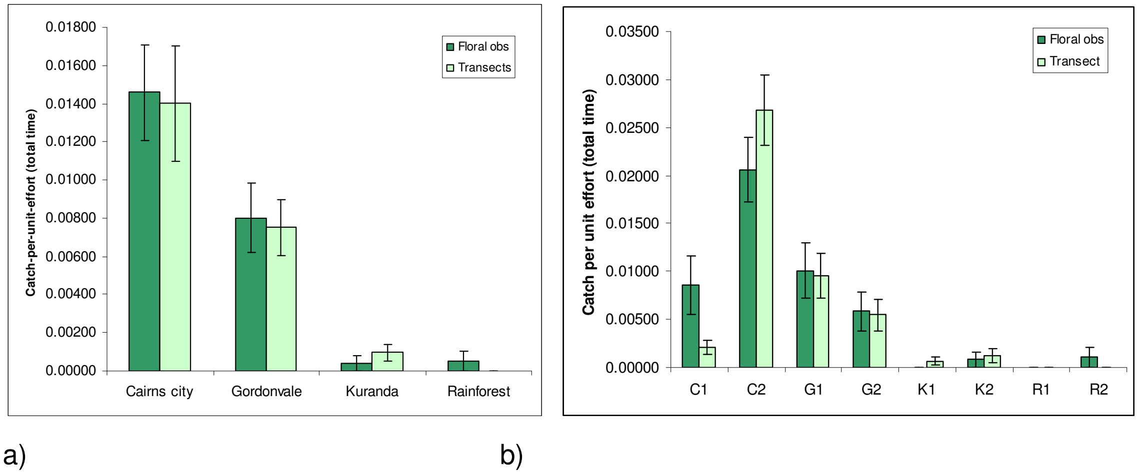


**Figure 14:** Catch-per-unit-effort (total surveillance time) ± 1 S.E. for two different detection methods (dark green: timed floral observations; light green: transect walks) at four different locations (a) and eight different sites (b). Catch per unit effort (total time) was calculated by dividing the number of individual locations (floral sources) within a study site where AHB were found (the catch) by the total time it took to conduct the surveillance (the effort).

### *Actual time*

When considering the actual time of surveillance, there was a significant difference in CPE between detection methods as well as between sites, without an interaction effect (Table 13). Overall, $CPE_{actual}$ was significantly greater for timed floral observations (Figure 15), with the only exception being “City 2” where transects had much higher $CPE_{actual}$.

$CPE_{actual}$ was greatest for Cairns City (particularly “City 2”), followed by Gordonvale sites, and lowest for Kuranda and Rainforest sites (Figures 16 a & b).

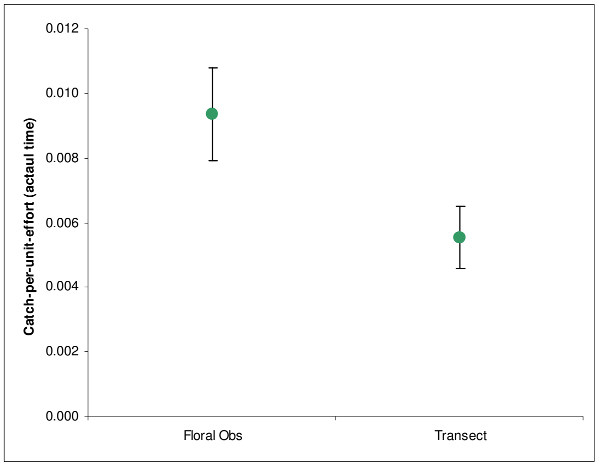
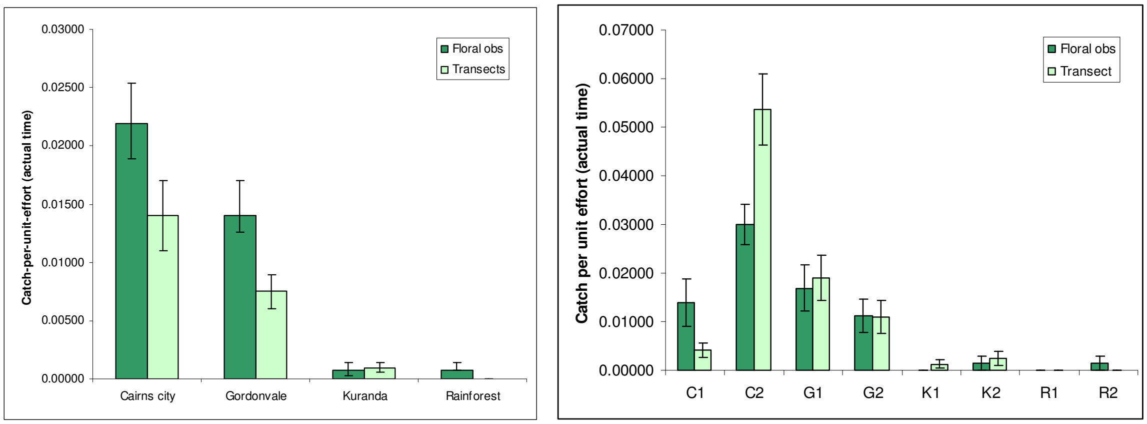


**Figure 15:** **Catch-per-unit-effort (actual surveillance time) ± 1 S.E. for two different detection methods (timed floral observations and transect walks). Catch per unit effort (actual time) was calculated by dividing the number of individual locations (floral sources) within a study site where AHB were found (the catch) by the actual time looking for bees (the effort).**



a) b)

**Figure 16:** **Catch-per-unit-effort (actual surveillance time) ± 1 S.E. for two different detection methods (dark green: timed floral observations; light green: transect walks) at four different locations (a) and eight different sites (b). Catch per unit effort (actual time) was calculated by dividing the number of individual locations (floral sources) within a study site where AHB were found (the catch) by the actual time looking for bees (the effort).**

**Table 13: Statistical results from Linear General Mixed Modeling analysis for AHB catch-per-unit-effort (CPE) total survey time and actual survey time per person (fixed effects: detection method & site, random effect: month). Significant effects are indicated in bold.**

| **Fixed term** | **Wald statistic** | **n.d.f.** | ***F*** | **d.d.f.** | ***P*** |
|---|---|---|---|---|---|
| **CPE (total time)** | | | | | |
| Detection method | 0.02 | 1 | 0.02 | 207.0 | 0.887 |
| Site | 264.42 | 7 | 37.77 | 207.3 | **<0.001** |
| Interaction | 11.96 | 7 | 1.71 | 207.0 | 0.109 |
| | | | | | |
| **CPE (actual time)** | | | | | |
| Detection method | 8.30 | 1 | 8.30 | 207.0 | **0.004** |
| Site | 212.21 | 7 | 30.32 | 207.3 | **<0.001** |
| Interaction | 10.52 | 7 | 1.50 | 207.0 | 0.168 |

## Floral sources & sites

In total, AHB and EHB were detected on 74 different plant species across all sites and months. AHB were found on 27 (36.5%) and EHB on 73 (98.7%) of the 74 recorded plants species.

Kuranda and Gordonvale had the highest bee-visited plant diversity recorded (48 and 47 plant species, respectively), followed by Cairns City (25 plant species) and the Rainforest (14 plant species).

Within sites, Gordonvale 2 had the highest bee-visited plant diversity with 42 plant species, followed by Kuranda sites and Gordonvale 1 (Table 14). City 2 also had relatively high bee-visited plant diversity (>20 plant species per site), while City 1 and the rainforest sites all had the lowest bee-visited plant diversity recorded (≤11 plant species per site; Table 14).

A detailed analysis of floral preferences for both AHB and EHB was beyond the scope of the current document and can be found in *Ecology and behaviour of Asian honey bees* (Apis cerana) *in Cairns, Australia* (Commerford and Koetz, 2013).

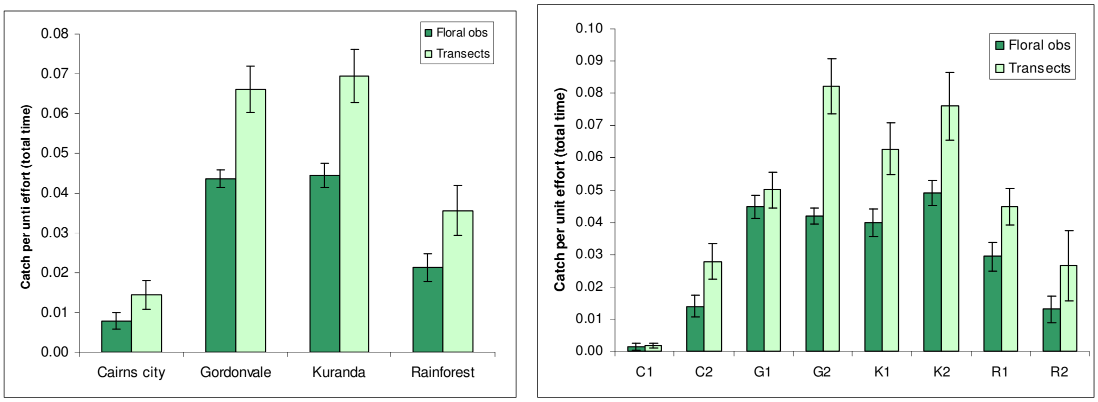
**Table 14: Number of AHB/EHB-visited plant species and number of AHB and EHB found at each of the eight sites during the detection efficacy field trials, September 2012 to March 2013.**

| **Site name & location** | | **# of *Apis*-visited plant species** | **# of *Apis* bees found** | |
|---|---|---|---|---|
| | | | AHB | EHB |
| City 1 | Cairns CBD | 9 | 18 | 8 |
| City 2 | Cairns North | 22 | 114 | 103 |
| Gordonvale 1 | Gordonvale CBD | 27 | 43 | 204 |
| Gordonvale 2 | Suburban Gordonvale | 42 | 28 | 322 |
| Kuranda 1 | Kuranda south | 33 | 2 | 254 |
| Kuranda 2 | Kuranda west | 39 | 4 | 318 |
| Rainforest 1 | Kuranda rainforest | 11 | 0 | 165 |
| Rainforest 2 | Cairns rainforest | 8 | 1 | 103 |

## Catch-per-unit-effort for EHB

$CPE_{total}$ for EHB was significantly different between detection methods and between sites, with an interaction effect (Table 15). Transects tended to yield higher CPE, particularly in Gordonvale and Kuranda (Figure 17a). However, the interaction effect indicates that this relationship changed between different sites (Figure 17b).

$CPE_{actual}$ for EHB was not significantly different between detection methods, and either method yielded similar CPE. However, $CPE_{actual}$ was significantly different between sites (Table 15).



a) b)

**Figure 17: European honey bee catch-per-unit-effort (total surveillance time) ± 1 S.E. for two different detection methods (dark green: timed floral observations; light green: transect walks) at four different locations (a) and eight different sites (b). Catch per unit effort (total time) was calculated by dividing the number of individual locations (floral sources) within a study site where EHB were found (the catch) by the total time it took to conduct the surveillance (the effort).**

**Table 15: Statistical results from Linear General Mixed Modeling analysis for EHB catch-per-unit-effort (CPE) total survey time and actual survey time per person (fixed effects: detection method & site, random effect: month). Significant effects are indicated in bold.**

| **Fixed term** | **Wald statistic** | **n.d.f.** | ***F*** | **d.d.f.** | ***P*** |
|---|---|---|---|---|---|
| **CPE (total time)** | | | | | |
| Detection method | 34.35 | 1 | 34.35 | 208.0 | **0.001** |
| Site | 195.08 | 7 | 27.87 | 208.0 | **0.001** |
| Interaction | 15.98 | 7 | 2.28 | 208.0 | **0.029** |
| | | | | | |
| **CPE (actual time)** | | | | | |
| Detection method | 0.23 | 1 | 0.23 | 207.0 | 0.633 |
| Site | 267.34 | 7 | 38.19 | 207.4 | **0.001** |
| Interaction | 9.56 | 7 | 1.37 | 207.0 | 0.221 |

## Comparison of AHB and EHB

Nearly seven times more EHB-positive floral sources than AHB-positive floral sources were found overall. Similarly, four times more total individual EHB than AHB were counted over the entire experiment. The majority of individual AHB were found in Cairns City and Gordonvale, whereas most EHB were found in Gordonvale and Kuranda (Figure 18).

There was no correlation between the number of AHB and the number of EHB across the sites (Spearman's rank: $r_s(206) = 0.164$, $p = 0.558$), i.e. more EHB in an area did not result in fewer AHB in that area. However, sample size was very small for this analysis.

CPE was generally much higher for EHB than AHB, irrespective of the detection method (Figure 19). CPE for EHB was highest in Gordonvale and Kuranda, whereas AHB CPE was highest in Cairns City and Gordonvale (Figure 20).

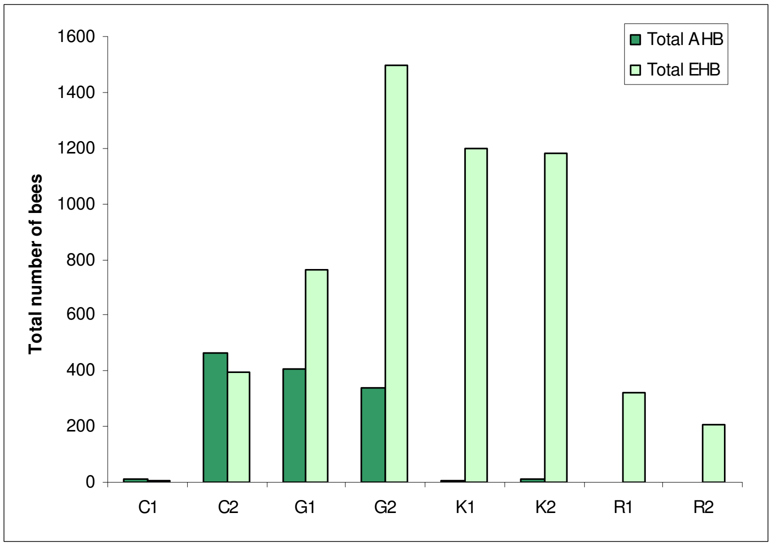


**Figure 18: Total number of individual Asian honey bees (dark green) and European honey bees (light green) found at eight different sites across the Cairns region.**

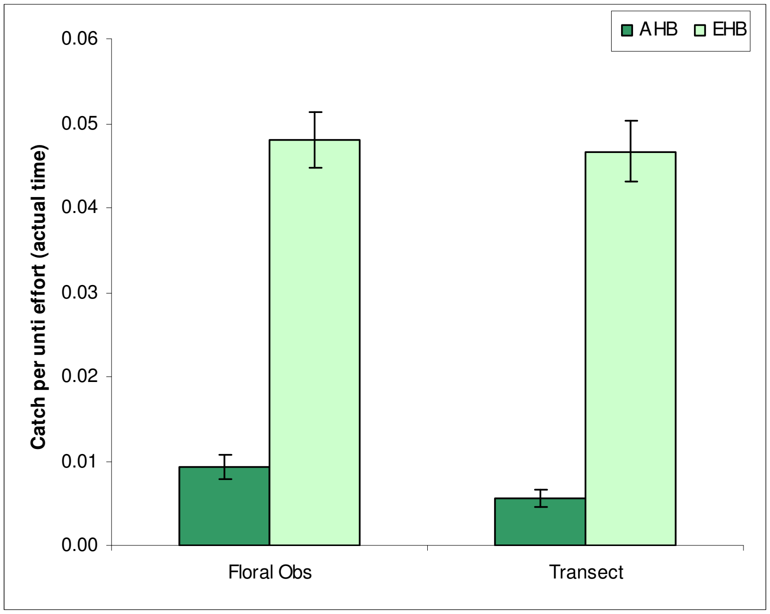


**Figure 19:** **Comparison of catch-per-unit-effort (actual surveillance time) ± 1 S.E. between Asian honey bees (dark green) and European honey bees (light green) across two different detection methods.**

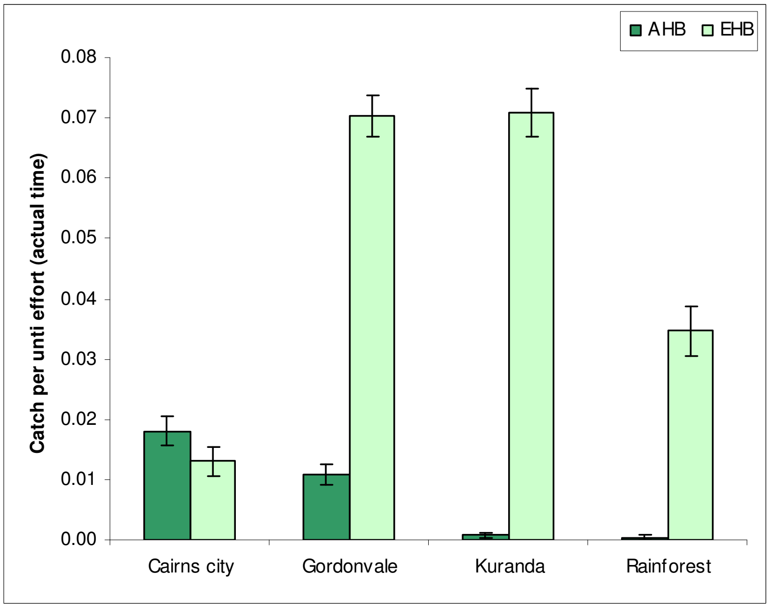


**Figure 20:** **Comparison of catch-per-unit-effort (actual surveillance time) ± 1 S.E. between Asian honey bees (dark green) and European honey bees (light green) at four different locations in Cairns, Australia.**

The average number of AHB and EHB detected across all sites each month remained relatively steady across time, fluctuating around 10-20 AHB and 50-60 EHB between September 2012 and March 2013 (Figure 21). There was no obvious decline in average numbers in the traditional wet season months, although a drop in both AHB and EHB average numbers occurred in February 2013, following a marked increase in rain in January 2013 (Figure 21). When looking at total numbers of AHB and EHB found across all sites each month, the same trends were apparent, with numbers fluctuating around 90 AHB and 800 EHB between September 2012 and March 2013. Interestingly, AHB numbers appear to fluctuate less from month to month than EHB numbers (Figure 21).

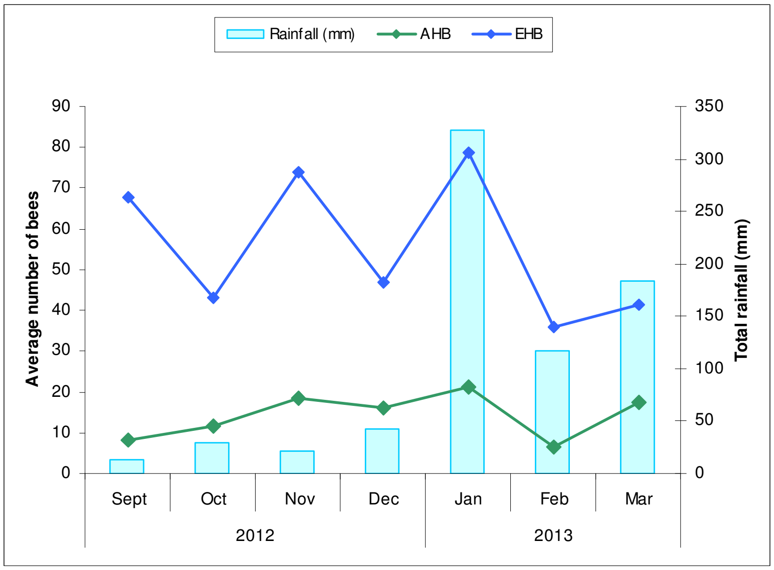


**Figure 21:** **Temporal variation in the average number of Asian honey bees (green) and European honey bees (blue) found at eight different sites in Cairns, Australia. Also shown is the total rainfall (mm) recorded for each month (sourced from www.bom.gov.au, accessed 29/04/2013)**

# Discussion

## Historical data

### Public reports

Between May 2007 and October 2012, nearly three-quarters of all detections were made through public calls, half of those being nests, half swarms. During this time, detections by public reports were made in 75 different suburbs in the Cairns and Tablelands area. Gordonvale, Portsmith, Edmonton and Cairns City had the most detections by public reports (>30 detections). These suburbs are located along Trinity Inlet and south towards the Gordonvale sugar mill, possibly being an indication of the southerly spread from the Cairns Port area in the early years. The top ten suburbs account for more than half (53%) of all detections by public reports.

There was a weak correlation between the number of detections and the suburbs' population sizes, i.e. the larger the population size the more detections by public reports were made. Human density and suburb area did not influence detections. Thus, correcting the number of public detections for population size should give an estimate of AHB population size. Corrected detection numbers showed highest densities somewhat further south than uncorrected detection numbers, with highest numbers in Portsmith and suburbs south and east of Portsmith.

Interestingly, these suburbs are either highly forested (mangrove/rainforest) or adjacent to such habitat (e.g. Trinity Inlet, East Trinity and Green Hill). AHB Program's efforts focussed mostly on populated areas more so than on inaccessible forested areas. Therefore, it is highly likely that these inaccessible, forested areas may be acting as a source for AHB, i.e. it is likely that a relatively undisturbed AHB population resides in those areas, providing a source for swarms that re-populate the populated areas. If so then undisturbed, 'wild' habitat adjacent to populated areas are likely to hamper eradication efforts if neglected.

Some evidence for AHB presence in these areas includes presence of AHB on Admiralty Island since 2008, as well as occasional AHB detections in the forests of East Trinity and Yarrabah.

### Public calls

The majority (90.6%) of bee-related calls made to BQCC were related to suspect bees or swarms, one-quarter of which resulted in a positive detection of AHB. There was a significant, positive relationship between the number of "suspect-bee" calls and the total number of public detections and the number of nests detected. This means that the more suspect-bee-related calls are made in one month, the more AHB nests and swarms are found through public reports in that same month as well as in the following month. The success rate of public calls was significantly higher during the dry months (May to October) than during the wet months.

These results underline the importance of Community Engagement by utilising 'citizen science', i.e. involving the community in finding pest bees. This is also shown in the low public reporting in the early years of the AHB Program when no dedicated Community Engagement staff was available (prior to 2010; Figure 3). Public calls and detection increased rapidly once dedicated Community Engagement began in 2010 (Figures 3 & 7).

## AHB traps

Trap success of AHB traps was exceedingly low (0.14% over 17 months). However, a rather high effort in staff and resources was required to check and maintain the traps.

Over the 17 months that the traps were deployed, the KIA spread and some traps were no longer at the edge of the KIA but within it. Interestingly, despite AHB being present in areas where traps were located, no AHB were trapped towards the end of the trap runs.

The low success rate can most likely be linked to several factors based on field experience by the operations team:

- Sugar syrup stations (traps) were not an attractive food source for honey bees, and bees were unlikely to feed on them unless they were trained to do so. This initial training was crucial in getting bees to feed on a feeding station, but it means that detection of wild AHB is unlikely.

- Even when traps were placed directly underneath an AHB nest or next to floral sources with AHB foragers present, bees preferred flowers to the sugar syrup.

- Despite being covered, traps dried out in dry weather or flooded and diluted in wet weather (especially during the wet season) within the two weeks between trap runs. Dry traps did not attract bees, and diluted trap syrup did not trap bees.

Traps syrup may be used for genetic techniques to detect AHB that have fed on the syrup in the trap. It was confirmed that this could be done when 20 or more AHB have fed on the syrup (Pease, 2012). If so, feeding stations (without being sticky or gluggy) could be left in the field, and syrup liquor could be collected periodically to be genetically checked for AHB DNA. Rainbow bee-eater roosts were used in a similar way to detect presence of AHB. However, unlike Rainbow bee-eater roosts that require no maintenance, feeding stations would need to be checked and maintained regularly to avoid desiccation or dilution.

However, such genetic testing, if done according to a rigorous experimental and sampling design and in conjunction with Rainbow bee-eater surveillance, may be able to confirm an area of freedom of AHB.

## Rainbow bee-eater pellets

Half of all bee eater pellets collected within the greater Cairns area contained EHB, and one-fifth of all samples contained AHB. Catch per unit effort was 0.18 per hour for AHB and 0.38 per hour for EHB, or 5.5 hours spent to find one AHB and 2.6 hours spent to find one EHB.

In three instances, AHB negative pellets were found within the KIA, indicating that false negatives are possible. Therefore, repeated sample collection from the same roost or several roosts within an area is necessary. There were four instances where pellets positive for AHB were found outside the then KIA, up to five months before nests/swarms were found through public reporting.

These results indicate the high effectiveness of using Rainbow bee-eaters for detecting AHB presence in an area. Although it is not a method to find the location of a nest (as both the birds and the bees may have flown some distance before meeting), Rainbow bee-eater surveillance are a highly efficacious method for (i) determining presence or absence of AHB in an area and (ii) tracking the spread of AHB. Thus, this is a good method of determining an area of freedom given the high success rate of finding AHB wings in pellet samples where AHB are present, and the comparatively high efficacy in terms of staff effort, this type of surveillance.

### Bee lining

Most nests (60.7%) were found by bee lining on the same day they were initially detected, or within the first two days thereafter (84.1%). The majority of nests were found by bee lining within four days of initial detection (90.4%).

Thus, once foragers are detected a nest can generally be found quite quickly. Unfortunately, no records were kept on actual human-hours required for bee lining so efficacy cannot be determined.

It must be noted that bee lining can only be conducted once foraging bees have been detected. Thus, it is reliant on any of the above methods for detecting foragers, and cannot be used in isolation from those.

### AHB detection dog

Efficacy of an odour detection dog was determined as part of the AHB T2M Program (R. Gilmour & C. Bell, pers. com.). A brief summary is included here:

During its use between June 2010 and April 2011, the odour detection dog detected all 42 previously-known trial nests and found five additional, previously unknown nests. The dog team performed 316 searches that covered an estimated beeline search area of approximately 180 ha/year. No records of search area, time spent searching, or time spent maintaining and training the dog were kept to assist with calculating rate of effort. Assuming the detection of one nest by the dog required one search, 14.8% of searches resulted in the detection of a nest.

## Floral observation field trials

Nearly 600 person-hours were spent surveying for bees, and 9016 *Apis* bees were counted in total. The majority (80.2%) of detected bees were EHB, whereas 20.2% were AHB. Thus, for each one AHB, four EHB were found. Bees were found on 1708 floral sources (flowering plants), of which AHB utilised very few (12.4%) compared to EHB. Thus, for each one AHB-positive floral source, seven EHB floral sources were found. There were very few occurrences of AHB and EHB foraging on the same floral source.

There could be several reasons for these results: (1) there were seven times as many suitable EHB flowering plants, assuming that EHB and AHB floral preferences vary; and/or (2) EHB and AHB floral preferences are the same but AHB were foraging elsewhere, potentially due to competition with EHB. In terms of individual bee numbers, it may be that the population density of EHB is indeed four times higher than that of AHB, or AHB may have been foraging elsewhere, possibly due to differing floral preferences and/or competition with EHB.

Catch-per-unit-effort (CPE) was nearly eight times higher for EHB than AHB, which is likely a reflection of the greater occurrence of EHB floral sources and a greater number of EHB in general.

AHB CPE was highest in City 2, which was located in a Cairns North residential suburb and also had the highest probability of encountering AHB. Gordonvale sites and City 1 (Cairns CBD) had the next highest AHB CPE. AHB CPE was lowest in Kuranda and the Rainforest, as was the probability of detecting AHB. EHB CPE was highest in Gordonvale and Kuranda, lowest in Cairns City. Interestingly, AHB and EHB CPE were nearly inverse – where CPE was high for one species it was lower for the other (but not statistically so).

EHB CPE matched the general bee-visited floral diversity found in the eight sites – Kuranda and Gordonvale had similarly high levels of bee-visited floral diversity. Interestingly, despite high floral diversity, Kuranda had very low CPE for AHB, whereas CPE for EHB was very similar across those sites. In addition, Cairns City sites had high CPE for AHB (and very low CPE for EHB) despite comparatively low bee-visited floral diversity. Cairns City sites appear to be avoided by EHB, particularly City 1 (Cairns CBD), whereas more AHB are found in City 1 than in Kuranda or Rainforest sites (Table 14, Figure 18).

These results could be explained by competition between the two species, and/or a difference in preferred floral sources and their availability at different sites. Generally, more EHB were found in sites with higher overall observed bee-visited floral diversity. This was not the case for AHB, which could mean that either AHB prefer to forage where EHB numbers are lower, or that AHB has a much narrower range of preferred plants.

### General versus timed floral observations

CPE was not significantly different between methods in terms of total time spent. However, in terms of actual surveillance time, timed floral observation was the preferred method yielding higher CPEs. Therefore, timed floral observation was the more effective surveillance method.

This result may be explained by AHB being much more sparsely distributed than EHB, visiting fewer plants at each site, and visiting each plant at lower numbers. Therefore, during transect surveillance most time would be spent walking without detecting any AHB. However, when observing a single floral source for a set amount of time (in this case 10 minutes), the probability of detecting AHB coming to that floral source is much higher than when glancing at floral sources for only a moment while walking past. On the other hand, EHB are visiting a comparatively large number of plants and each plant at high numbers, so glancing at a plant for a moment is likely to detect EHB.

Thus, it appears that for a sparsely distributed bee such as AHB it may be advisable to spend more time scanning (potentially fewer) individual flowering plants within a site, than scanning a large number of flowering plants with only a glance before moving on.

### Detection methods comparison

Direct comparison of the different methods was difficult due to the systemic lack of record-keeping in the historical data, meaning that determining of efficacy (i.e. number of bees found per hours or person-hour) was not possible for most methods.

Efficacy could be estimated for AHB traps and bee-eater surveillance, and directly calculated from field trials for general and timed floral observations. Rainbow bee-eater surveillance was the most efficacious of all methods, followed by timed floral observations, general floral observations, and, lastly by far, AHB traps (Table 16). Rainbow bee-eater-surveillance was 10 times more efficacious (in terms of bees found per person-hour) than floral observations, and 36 times more efficacious than AHB traps (Table 16).

For some methods, a success percentage could be calculated or estimated (number of successful detections relative to the total effort). Public calls and Rainbow bee -eater surveillance were by far the most successful detection methods, followed by the AHB detection dog. Using AHB traps was the worst method (Table 16).

Table 16: **Comparison of different detection methods, showing an estimate of success, and an estimate of efficacy where available, of detecting AHB.**

| | **Estimate of success** | **Estimate of efficacy** | |
|---|---|---|---|
| **Historical data** | | | |
| Public reports | 71.2% of all detections made through public | n/a | n/a |
| Public calls | 21% of all calls & 25% of suspect bee calls result in detection | n/a | n/a |
| AHB traps | 0.14% of all traps within 17 months | 0.0025 bees/person-hr | 402.4 person-hrs to find one AHB |
| Rainbow bee-eaters | 22% of all samples contained AHB | 0.18 bees/hour for AHB<br>(~0.09 bees/person-hr) | 5.5 hours to find one AHB<br>(~11 person-hrs) |
| Detection dog | 14.8% of searches were successful | n/a | n/a |
| Bee lining | 60.7% found on the same day<br>84.1% found within the first two days | n/a | n/a |
| **Field trials** | | | |
| Overall | 37.1% of individual surveys detected AHB | | |
| Transect walks | 41.1% | 0.0056 bees/person-hr | 178.6 hrs per bee |
| Timed floral observations | 33.0% | 0.0094 bees/person-hr | 106.4 hrs per bee |

# Conclusion

Unfortunately, due to the lack of data, determination of efficacy from historical data for most methods was difficult. Nevertheless, results showed that public reports and calls ('citizen science') was of upmost importance in detecting AHB. In addition, Rainbow bee-eater surveillance appeared to be an efficacious and robust method of determining presence or absence of AHB in an area, although it could not be used to find nests.

Thus, which detection method should be used depends on the ultimate goal of detecting AHB:

To find and destroy nests in an area (e.g. high-risk areas or beyond the known edge of the KIA), nests need to be found, or foragers that can be bee lined to their nest. Both can be successfully detected through public reports/calls. In addition, timed floral observations are recommended as these will detect foragers that can then be bee lined to their nest. A detection dog may speed up bee lining in certain circumstances.

To establish AHB presence or absence in an area without the need to find and destroy nests (e.g. for proof of absence, or to track the spread of AHB), Rainbow bee-eater pellet surveillance is the recommended detection method as it is the most efficacious method.

Further research is recommended to determine the actual AHB and EHB density in different areas (habitats) in order to optimise detection methods. In addition, floral preferences appear to differ greatly between the species, with AHB utilising a very narrow range of the available flowering plants. Thus, research into the floral preferences of AHB in the presence and absence of EHB may also lead to improved detection methods. Proof of concept exists for using AHB feeding stations to detect AHB presence/absence through genetic testing of the sugar syrup. However, this technique will need to be thoroughly tested and improved. Finally, preliminary research undertaken by Dr. David Guez (University of Newcastle, Australia) and Biosecurity Queensland staff has shown the native Japanese orchid, *Cymbidium floribundum*, to be highly attractive to hived AHB from distances up to 50 metres (Commerford *et al.*, 2013). Consequently, orchid volatile compounds may provide a useful attractant to aid future AHB detection. This research is being pursued by Dr. David Guez under a Rural Industry Research and Development Corporation (RIRDC) grant.

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