Department of Agriculture, Fisheries and Forestry

Asian honey bee Transition to Management Program

Optimising Asian honey bee (*Apis cerana*) trap design and attractants

Great state. Great opportunity.

Queensland Government

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Contents

Summary

The Asian honey bee Transition to Management (AHB T2M) Program was implemented in July 2011 to identify and address gaps in current knowledge required to develop effective tools for the control of Asian honey bees (AHB; *Apis cerana* Java genotype). These tools are intended for community use following the cessation of the Program in June 2013. In this report, we investigate aspects of trap design and attractants used to lure and trap AHB in accordance with the requirements stipulated in the *Plan for Transition to Management of the Asian Honey Bee: Version 1* (Department of Agriculture, Fisheries and Forestry - Australian Government, 2011).

Biosecurity Queensland (BQ) conducted a series of trap design and attractant field trials on feral and hived AHB in the Cairns region between August and November 2012. The trap design trials investigated the capacity of standard AHB traps, sticky mat traps, pan traps, (E)-9-oxodec-2-enoic acid (9-ODA) pheromone traps and artificial flower bottle traps to trap AHB. The attractant trials explored AHB preferences for sugar concentration (0%, 25%, 35%, 55%, 60%, 75% sucrose w/w), scent (non-odorous control, orange, lavender, rose and jasmine essential oils) and colour (red, yellow, blue, black and white). Single experimental trials were also undertaken to determine whether 5-component queen mandibular pheromone (5-QMP), 5-QMP plus Eicosanol (sting venom) pheromone, and/or the *Cymbidium floribundum* orchid were successful in attracting AHB.

The key results of the AHB T2M Program's research into optimising AHB trap design and attractants were:

- The artificial flower bottle trap showed the most potential in trapping AHB, with none of the other trap types attracting any AHB.
- The number of AHB workers that were attracted to a feeding station increased as sugar concentration increased.
- AHB did not exhibit any clear scent preference, although they did appear to dislike jasmine.
- Although not statistically significant, AHB appeared to prefer the colour blue over red, yellow, black and white.
- Twice as many hived AHB were attracted to the synthetic 5-QMP compared to the synthetic 5-QMP plus Eicosanol (sting venom) pheromone.
- The orchid, *Cymbidium floribundum*, was highly attractive to hived AHB.

In conclusion, our results provide a solid foundation for further research necessary to develop an effective AHB trap and attractant. Importantly, we have shown that the artificial flower bottle trap and pheromone attractants were successful in attracting some AHB. However, the most promising attractant appears to be the orchid volatile compounds as they attracted AHB more easily than any other attractant trialled.

Introduction

The early detection and continued monitoring of an introduced species is central to its eradication (Harvey *et al.*, 2009; Ashcroft *et al.*, 2012). To achieve this, effective detection and trapping methods must first be identified, developed and refined. In May 2007, an incursion of Asian honey bees (AHB; *Apis cerana* Java genotype) was detected in Cairns (Queensland, Australia; Shield, 2007). Shortly after, Biosecurity Queensland (BQ) initiated an AHB surveillance and eradication program (Department of Agriculture, Fisheries and Forestry - Australian Government, 2011). A lack of research relating to successful detection and trapping methods for AHB (Koetz, 2013a) hampered the species' eradication, and contributed to a majority decision by the Australian Government's Australian Asian Honeybee National Management Group in 2011 that AHB were no longer eradicable in the Cairns region (Koetz, 2013a).

During the Asian honey bee Eradication Program (2007-2011), BQ employed a variety of detection methods in the Cairns region (Queensland, Australia; Table 1). Of these, Koetz (2013b) identified the most to least efficacious techniques as public reports, Rainbow bee-eater (*Merops ornatus*) crop pellet surveillance, timed floral observations, general floral observations and, lastly, standard AHB traps. Each of these approaches was restricted in their capacity to locate AHB nests – a critical consideration when attempting to eradicate or control an introduced bee species. Firstly, public reporting and floral observations were of limited use in densely-forested, inaccessible and/or remote areas (Koetz, 2013a). Secondly, while Rainbow bee-eater pellet surveillance was able to establish whether AHB were present in a given area, this method could not be used to locate AHB nests (Koetz, 2013b). Since it is not possible to destroy nests if they cannot first be detected, it was evident that further research was required to determine effective techniques to attract this species to a central bait or feeding station from which individuals could be traced back to their nest and/or the nest remotely poisoned.

Table 1: AHB trap and feeding methods used or trialled by Biosecurity Queensland (Compiled from: Shield, 2007; Koetz, 2013b; Koetz, 2013a; Koetz and Hyatt, 2013; AHB program field officers, pers. com).

Type	Purpose	Status
Sugar feeding stations	Finding foragers, bee lining, remote treatment	2010 – 2012
Sugar feeding traps	Trapping foragers	2007 - 2012
Sticky traps	Trapping foragers	Deemed ineffective in the past
Sticky frames	Trapping foragers	Deemed ineffective in the past
Pheromone log traps	Trapping swarms	Deemed ineffective in the past
Bait hives	Trapping swarms	Deemed ineffective in the past
Various other traps (including Lucitraps; palm tree flowers)	Trapping foragers	Deemed ineffective in the past
Scenting (melting honeycomb)	Finding foragers	Deemed ineffective in the past
Mega Garden (AHB-preferred floral sources in a moveable trailer)	Finding foragers	Deemed ineffective in the past

The effectiveness of a bait station used to attract bees may depend on several attributes of the station and attractant including sugar concentration, scent, colour and pheromones (Koltermann, 1973; Wells and Rathore, 1994; Leong and Thorp, 1999; Gollan *et al.*, 2011; Anderson *et al.*, 2012). Importantly, bait station and attractant design characteristics must be targeted at the species of interest since the foraging strategies and pheromones of closely-related species often differ (Wells and Rathore, 1994).

No published studies specifically address the success of different coloured traps in attracting AHB. However, flower colour appears to be less critical to *A. cerana indica* flower visitation than the energetic reward offered by a particular flower (Wells and Rathore, 1994). In fact, Wells and Rathore (1994) found that *A. cerana indica* foragers tended to visit only the flower with the highest caloric reward regardless of colour when flowers offered differing rewards. By contrast, *A. mellifera* in the same study discriminated between different coloured flowers irrespective of energetic reward (Wells and Rathore, 1994). This suggests that sugar concentration may be critical to the design of an effective AHB bait station attractant and that bait station colour may be useful in reducing the attraction of non-target species, such as *A. mellifera*, to the trap.

In a series of scent choice experiments conducted by Koltermann (1973), *A. cerana indica* preferred lavender, orange, jasmine, fennel, thyme, rosewood oil, rosemary and cinnamon aromas (Koltermann, 1973). In this same study, *A. cerana indica* and *A. mellifera* both highly preferred lavender odours, while *A. mellifera* disliked orange, jasmine, fennel and thyme scents (Koltermann, 1973). Anderson *et al.* (2012) found that acetic acid, isolbutanol (odour of molasses), a mix of citral and geraniol flower odours, banana-flavoured sugar syrup and coconut-flavoured sugar syrup were no more attractive to AHB in the Solomon Islands than unscented sugar syrup.

Social insects, such as bees, use pheromones as a form of chemical communication (Koetz, 2013a). Such natural scent chemicals in bees can include queen mandibular gland pheromones, sting apparatus and venom pheromones and homing or orientation pheromones (Lacey, 1999; Koetz, 2013a). As these chemicals can be exploited to attract workers or swarms, they may aid effective bait station design (Koetz, 2013a). Multiple studies have found synthetic AHB queen mandibular gland pheromone blend to be successful in attracting hived worker bees at close (15 cm) and medium ranges (2 m; Lacey, 1999; Kuang *et al.*, 2000; Lacey, 2000). Adding small amounts of the oil and pheromone component of AHB sting venom (Eicosanol) to sugar syrup has been shown to slightly increase the solution's ability to attract AHB (Anderson *et al.*, 2012). Nasonov pheromones, used for honey bee orientation, did not elicit an aggregation response from AHB workers (Lacey, 1999).

In accordance with the Asian honey bee Transition to Management Plan, the aims of this report were to:

- determine the effectiveness of AHB bait stations, their design and attractant effectiveness, including documenting bait station use and design and validating effectiveness (AG2Biii), and to

- investigate alternative AHB control techniques and attractants, including research into pheromones used to attract and/or detect AHB in order to increase trap sensitivity (AG2Civ).

The latter project is currently being investigated by Dr. David Guez (University of Newcastle, Australia) under a Rural Industry Research and Development Corporation (RIRDC) grant and is beyond the scope of this report. However, preliminary findings will be presented here since Biosecurity Queensland scientists assisted with this work.

Methods

The methods and results are divided into two sections: trap design trials (including trap efficacy field trials and single experimental trials) and attractant trials (including sugar concentration, scent and colour preference field trials and single experimental trials).

Trap design trials

Trap efficacy field trials

Several trap designs and attractants were trialled during two rounds of trap efficacy field trials. Traps were deployed for five days each in September and October 2012 at each of the eight sites in the Cairns region (Queensland, Australia; Table 2). Traps included standard AHB traps, a sticky mat traps, and pan traps (Figure 1). Pheromone traps and *Sontax Australia Soda Bottle Wasp Traps* (herein referred to as artificial flower bottle traps) were trialled during the October field trials only (Figure 1).

Table 2: Locations of study sites showing habitat type, assumed AHB density and the number of trial sites within each study site

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

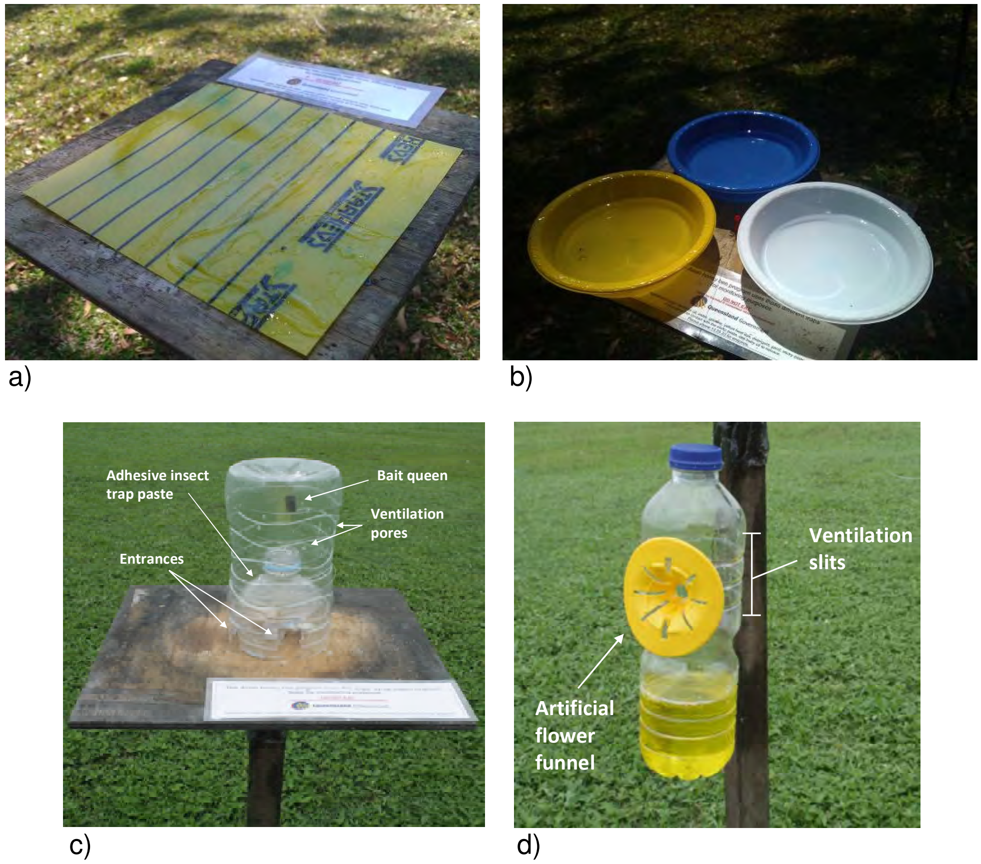

Figure 1: The experimental traps used during efficacy field trials: a) sticky mat trap; b) pan traps; c) pheromone trap; d) artificial flower bottle trap

Traps were placed on, or hung from, four timber platforms consisting of hardwood stakes with marine grade plywood top-plates attached. All platforms were approximately 1.3 m high. Timber platform stations were placed at the corners of a 2 x 2 metre square. The traps were randomly placed upon or hung from these stations (Figure 2). After placement, all traps were left *in situ* for one week prior to collecting trapped bees.

Figure 2: Setup of timber platform stations and traps during efficacy field trials; traps were randomly placed on or hung from timber stations placed at the corners of a 2 x 2 m square. Clockwise from left: sticky mat trap, pheromone trap & artificial flower bottle trap; AHB trap; pan traps & artificial flower bottle trap.

Standard AHB trap

Between 2010 and 2012, standard AHB traps were used for surveillance of AHB within high risk areas and near the edges of the Known Infested Area (KIA). 101 traps were deployed, checked and maintained fortnightly or as permitted by operational resources. The success rate of these AHB traps was found to be exceedingly low (Koetz, 2013b). Nevertheless, AHB traps were included in this trial to provide a comparison with other traps.

Standard AHB traps consisted of a round, plastic self-filling water dish of various colours (17.5 cm 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. Bulk quantities of lavender-scented sugar syrup were prepared using 2 kg of white sugar, 1 teaspoon of powdered gelatine and approximately 5 mL of lavender essential oil dissolved in 1.5 L of tap water. Gelatine was added to the syrup to make the solution more viscous, thus making it difficult for trapped bees to escape. Sufficient sugar syrup solution was poured into the well of the dish so that the beach area was exposed to bee visitors. This provided a small platform enabling bees to land and feed. The slope of the sand along the edge of the well was also saturated with syrup so that bees attempting to land there would become coated with viscous syrup, fall into the well and become trapped (Figure 3). AHB traps were placed on timber platform stations as described above.

a) b)

Figure 3: Standard AHB trap used by Biosecurity Queensland between 2010 and 2012: a) water dish showing beach, well and sugar syrup solution; b) AHB trap with hood.

Initially, an inverted soft drink bottle with excess sugar solution was 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 post 2010. Instead, traps were manually refilled by field personnel every two weeks.

To reduce evaporation of the syrup by the sun or dilution by rain, a small plastic garbage bin (hood) was modified by excising multiple rectangular entrances 4-8 cm above the rim of the upturned bin (Figure 3). This allowed bees to enter the trap while providing protection from weather conditions. 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 5-10 cm below the top-plate to prevent insects (especially ants) from carrying off trapped specimens.

Sticky mat trap

Sticky mat traps were made using *Starkeys Products® Sticky Trapping Pads*. These reusable pads contain a yellow adhesive designed for use in commercial insect trapping units. Original sized mats (43 x 24.5 cm) were cut in half (exposing 526.75 cm^2 of adhesive surface) and secured to the top-plate of a platform station. Half of the sticky mat was left as is; the other half was covered with a thin layer of sticky paste to increase stickiness (Figure 2a).

Pan trap

Pan traps were made using coloured (white, yellow and blue) disposable plastic dessert bowls (17.5 cm diameter). One of each coloured bowl was secured to the top-plate of a platform station using either sticky paste or *Bostik Blu-Tack* (Figure 2b).

Bowls were filled to approximately two-thirds with a solution comprising tap water and a few drops of dishwashing detergent. Detergent was added to break the surface tension of the water and promote the drowning of insects.

Pheromone trap

(E)-9-oxodec-2-enoic acid (9-ODA) is a major component of the queen mandibular pheromone (QMP) used for sexual attraction of all *Apis* drones (Brockmann *et al.*, 2006). A cigarette filter blackened using a permanent marker was used as a replica queen (bait queen) and saturated in liquid synthetic 9-ODA to attract bees to the pheromone trap (Figure 2c). 9-ODA was sourced from Prof. Ben Oldroyd (University of Sydney, Australia).

Pheromone traps were constructed using empty 1.5 L plastic water bottles. The tops of the bottles were cut off and a small hole was punctured in the centre of the base of the bottle. Small ventilation holes were punctured around the side of the bottle approximately 5 cm above the base of the bottle. The bait queen was suspended inside the bottle using clear plastic fishing line. Sticky paste was spread around the top of the bottle which was then inserted into the base of the bottle. Finally, clear plastic tape was used to secure the bottle pieces together before creating small entrances by cutting four evenly spaced squares (Figure 2c).

Artificial flower bottle trap

Two clean 600 mL plastic bottles were modelled into two artificial flower traps by inserting a single artificial flower funnel (*Sontax Australia Soda Bottle Wasp Trap*) into the side of the bottles. One yellow flower-shaped funnel (exterior diameter of 3 cm, interior diameter of 1 cm) was attached to each bottle. Three 5 cm long vertical slits were cut into the opposite side of the bottle to ventilate the attractant. One bottle trap contained 25% fruit juice cordial (orange and mango flavoured) as an attractant, while the other bottle contained a dilute honey-water solution (approximately 3:1 concentration) as an attractant (Figure 1d).

Single experimental trials

Hived AHB colonies in Edmonton (Queensland, Australia) provided an opportunity to trial the effectiveness of various trap designs when positioned in the vicinity of known AHB nest locations. Traps were randomly hung on and around a 3 x 3 m gazebo shading the seven hived AHB colonies. All traps were left *in situ* for 24 hours on 9 November 2012 before sample collection.

Bottle traps

All traps contained a 1:1 honey-water solution as an attractant. A total of five bottle traps were deployed, including:

a) Three artificial flower bottle traps (constructed as described previously)

b) One clean 1.25 L plastic soft drink bottle, adapted into a trap by inverting the top (similar to pheromone traps) and suspending the trap from the centre of the 3 x 3 m gazebo frame.

c) One clean 2 L rectangular plastic juice bottle that was transformed into a trap by simply laying it on its side with the lid removed.

Attractant trials

Attractant trials were conducted to test three AHB perception cues, specifically taste, smell and vision. Trials, conducted by James Cook University (JCU) science students, tested for preferences in sugar concentration, scent and colour. The experiments took place between August and October 2012, using hived AHB. Research has shown that AHB tend to forage earlier in the day when temperature, light intensity and solar radiation are all relatively low (Koetz, 2013). Therefore, trials were conducted between 0930 hours and 1030 hours.

All three sets of attractant trials utilised the same setup, bee training and observation methods. Treatments were altered slightly for each of the different sensory trials (sugar concentration, scent and colour). In order to conduct trials, several worker bees were trained to feed on a training dish, approximately 20 m from the hives. In order to successfully train the bees, five to ten individuals were collected at the hive entrance (one to two individuals at a time) using a specimen jar and a sponge stopper. Bees were carried in the specimen jar and placed on the training dish while still inside the jar. Once feeding was initiated, or several minutes had passed, the jar was removed so that the trained bee could return to the hive and recruitment of other bees to the training dish could occur.

For each trial, five treatments were displayed simultaneously for an hour to the bees. Treatment stations (plywood platforms raised to about 1.3 m by wooden stakes; Figure 4) were arranged in a semi-circle around the centre training station, about 1 m apart. Treatment dishes were randomly placed on these treatment stations. Dishes were made using white plastic plates (18 cm diameter) and white paper napkins with a small inverted glass jar filled with sugar syrup (Figure 5a). Sugar syrup concentrations varied dependent on trial type. Once treatment dishes had been exposed for ten minutes, the training station was removed and bee counts were conducted. Counts of the number of individual bees feeding on a treatment dish were made for each treatment, every five minutes for the duration of the trial. If the syrup level in the treatment dish began to diminish due to foraging activity, then the glass jar was lifted and additional sugar solution was added.

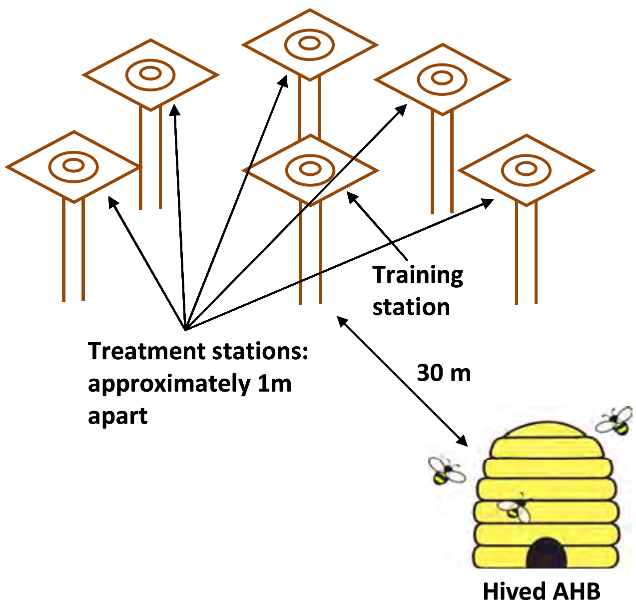

Figure 4: Training and treatment station arrangement used during attractant trials (including sugar concentration, scent, and colour preference trials).

a) b)

Figure 5: Treatment dish setups used during attractant trials a) Sugar concentration and scent preference trial apparatus b) Colour preference trial unit

Sugar concentration trials

Five treatments of different sugar concentrations were used to test preference, including concentrations of 25, 35, 55, 75% weight for weight (w/w). One dish contained water only (0% sugar). The centre training station contained a concentration of 60% w/w (Figure 5a). This experiment was replicated three times.

A Chi-square goodness of fit analysis was based on the grand mean of each treatment since the number of bees counted for each replicate may not have been independent. This lack of data independence may have resulted from bees attending the feeding station, leaving and recruiting more workers, returning to the feeding station and being counted again. Alternatively, a single bee may have been counted multiple times if it travelled between treatment dishes. The grand mean for each sugar concentration treatment was calculated by summing the number of foragers observed during each of the three replicates for each treatment, and dividing the result by the total number of replicates (i.e. 3 replicates).

Scent trials

Four treatment scents (orange, lavender, rose and jasmine essential oils) were prepared as well as a non-odorous control. Each treatment scent was contained in 60% w/w sugar syrup with one to two drops of one of the scents added. The centre training station used a non-odorous solution with a concentration of 60% w/w (Figure 5a). These experiments were replicated three times.

Scents (manufactured by 'All Garden Aromatherapy', Fitzroy, Victoria):

- Orange oil: 100% pure essential oil, *Citrus sinensis* fruit 1 mL/mL.
- Lavender oil: 100% pure essential oil, *Lavandula augustifola* 1 mL/mL.
- Rose oil: Pure essential oil dilution, 3% in Jojoba oil *Simmondsia chinensis* seed fixed oil 970 mcL/mL. *Rosa damascena* flower essential oil 30 cL/mL.
- Jasmine oil: Pure essential oil dilution, 3% in Jojoba oil *Simmondsia chinensis* seed fixed oil 970 mcL/mL. *Jasminum officianale* flower essential oil 30 cL/mL.

As explained for the *Sugar concentration trials* methods, the Chi-square analysis incorporated grand means for each scent treatment, since the bees counted for each replicate may not have been independent. The grand mean for each scent treatment (i.e. non-odorous control, orange, lavender, rose, jasmine) was calculated by totalling the number of foragers observed during each of the three replicates for each treatment, and dividing the result by the total number of replicates (i.e. 3 replicates).

Colour preference trials

The colours red, yellow, blue, black and white were used for the colour preference trials. For each treatment 80 gsm coloured paper was used to cover the base and sides of a jar containing sugar syrup jar (exposing 122.5 cm^2 of colour). Bees were trained using a feeding dish without coloured paper in order to avoid bees learning to associate the sugar syrup with a particular colour. Each of the treatment dishes rewarded the bees with a 50% w/w sugar syrup concentration, prepared in bulk using 1 kg of white sugar dissolved in 1 L of warm water (Figure 5b). Colour preference trials were replicated four times on separate days.

Single experimental trials

Pheromone attractant trial

For each disposable trap, the top of an empty, clean 600 mL plastic water bottle was cut off and inverted into the bottle base. Lures comprising medical rubber tubing saturated with one of two pheromone mixes were then suspended inside the bottle using fishing line. Each of the two *A. cerana* Java genotype queen mandibular pheromone lures (5-component and 6-component) were synthesized and supplied by Michael Lacey (www.beesdownunder.com.au). The first was 5-component from QMP (5-QMP) while the second was also 5-QMP with added Eicosanol, a pheromone derived from sting venom. Information regarding pheromone concentrations was not available from the supplier.

Two traps, one for each lure type, were affixed to opposite corners of a 3 x 3 m gazebo shading the AHB research hives. The research hives were within 1.5 metres of the pheromone traps. Each pheromone trap was observed by a separate observer, and the number of bees that approached the lures (closer than approximately 30 cm) for a total of 30 minutes was recorded. The location of the traps was swapped after 15 minutes.

Orchid trial

Cymbidium floribundum is a native Japanese orchid that attracts swarms of Japanese AHB to its flowers (Sugahara, 2006; Sugahara *et al*., 2013) and has been used to lure swarms into hive boxes in Japan (Koetz, 2013a). Flowering *C. floribundum* orchids provided by Dr. David Guez (University of Newcastle, Australia) were used to determine attractiveness to AHB in Cairns. The plants were enclosed in a mesh netting to protect the delicate flowers and placed within five to ten metres of hived AHB and observed.

Results

Trap design trials

Trap efficacy trials

The standard AHB trap, sticky mat trap, pan trap, *A. mellifera* 9-ODA pheromone trap and artificial flower bottle trap trialled as part of the efficacy field trial in September and October 2012 all failed to trap any AHB.

Single experimental trials

Bottle traps

The artificial flower bottle traps were more successful in collecting AHB than the rectangular bottle on its side and the inverted bottle trap (Table 3). On average, each artificial flower bottle trap collected 4 AHB (n = 3), while the rectangular bottle collected 3 AHB and the inverted bottle trap collected no AHB (Table 3). EHB as well as other insects were also trapped in most bottle traps (Table 3).

Table 3: Results of the bottle trap design trials, including artificial bottle traps, rectangular bottle on its side and inverted bottle trap.

Trap	# AHB	# EHB	Other species captured
Artificial flower bottle trap 1	6	9	1 wasp
Artificial flower bottle trap 2	5	16	2 *Trigona* sp. bees 2 wasps
Artificial flower bottle trap 3	1	1	3 green ants (*Oecophylla smaragdina*) 11 *Campanotus* sp. ants
Rectangular bottle on its side (lid removed; 2.00 L)	3	2	nil
Inverted bottle trap (1.25 L)	0	2	1 earwig

Attractant trials

Sugar concentration

If bees did not exhibit a sugar concentration preference then the means of all treatments (sugar concentrations) would receive the same or similar visitor numbers by chance. A Chi-square goodness of fit test showed that the grand means were significantly different to that expected by chance (X^2 = 67.842, d.f. = 4, $p < 0.05$), i.e. mean numbers of foragers increased with increasing sugar concentration (Figure 6). The water treatment displayed the lowest mean (1.02 bees) and 75% w/w concentration displayed the highest mean (49.31 bees; Figure 6).

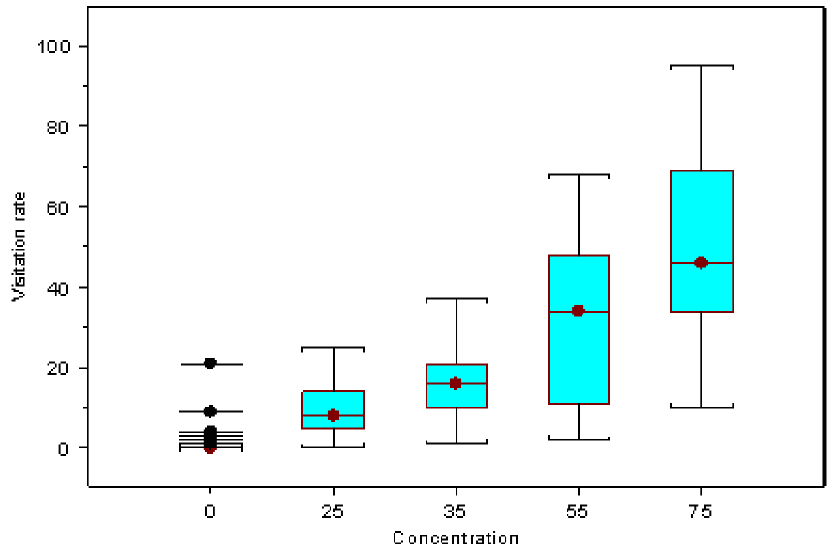

Figure 6: Boxplot of the number of bees attracted to different treatments (sugar concentrations: 0. 25, 35, 55 and 75% w/w) per 5 minute increment over three one-hour long trials.

Scent preference

Data for the first 30 minutes of each of the two trials were used for this analysis. If bees did not exhibit a preference for any scent then the means of all treatments (scents) would receive the same or similar forager bee numbers by chance. A Chi-square goodness of fit test showed that the grand means were significantly different to that expected by chance ($X^2 = 20.42$, d.f. = 4, $p < 0.05$), i.e. mean numbers of foragers differed between different scents (Figure 7). Specifically, Jasmine attracted significantly lower number of foragers, whereas all other scents and the control attracted similar numbers of foragers (Figure 7).

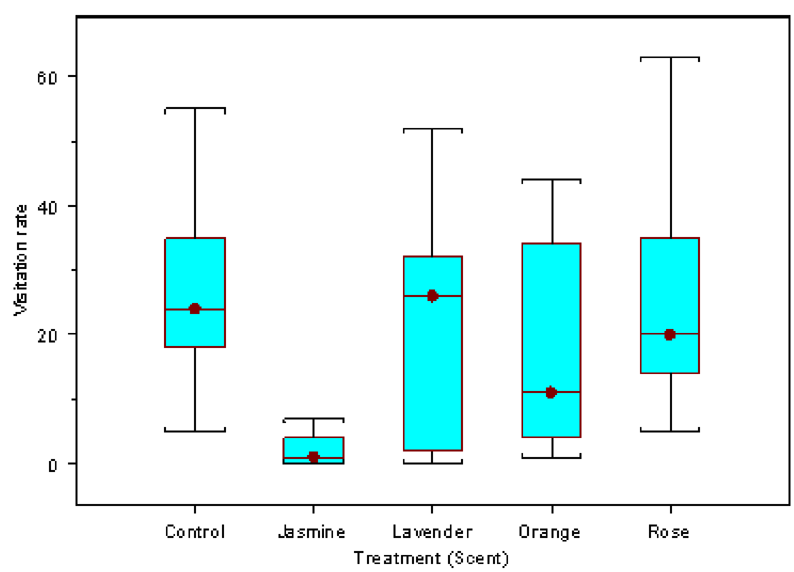

Figure 7: Boxplot of the number of bees attracted to different treatments (scents: control, jasmine, lavender, orange and rose) per 5 minute increment over three half-hour long trials.

Colour preference

Only the first 30 minutes of data collected during both trials were used for analysis. The remaining data was disregarded because statistical analysis of this data showed that after 30 minutes, bees no longer discriminated between colours, probably because bees had learned that the reward from each treatment was the same.

If bees did not exhibit preference then the means of all colours would be the same by chance. A Chi-square goodness of fit test showed that grand means were not significantly different to that expected by chance ($X^2 = 2.642$, d.f. = 4, $p = 0.62$), i.e. neither colour was preferred over another. However, blue colour tended to attract slightly more bees than any other colour (Figure 8).

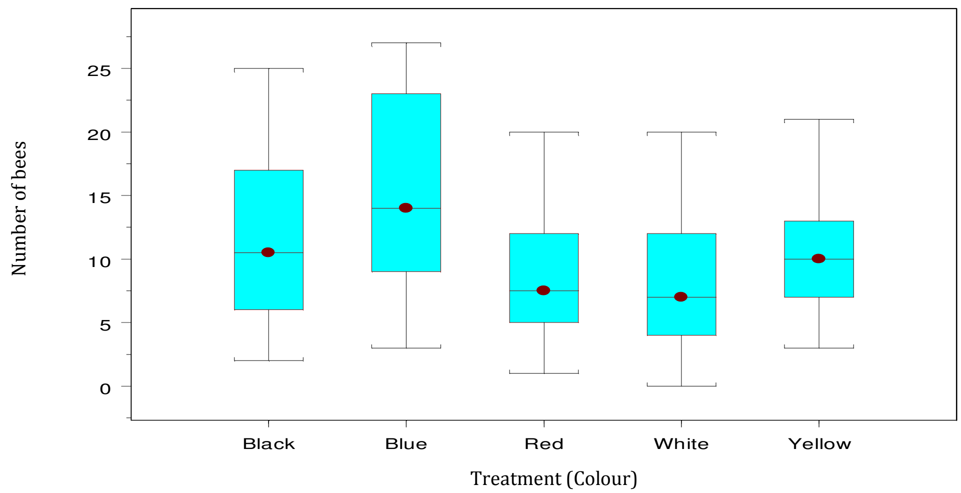

Figure 8: Boxplot of the number of bees attracted to different treatments (Colour: black, blue, red, white and yellow) per 5 minute increment over two one-hour long trials.

Single experimental trials

Pheromone trial

The number of AHB that approached the synthetic 5-QMP lure was approximately double (n = 33) the number that approached the 5-QMP plus Eicanosol lure (n = 17) over the cumulative 30-minute observation period (Table 4).

Table 4: The number of AHB attracted to the (i) 5-QMP and (ii) 5-QMP plus Eicanosol pheromone lures over two consecutive 15-minute observation periods.

15-minute interval	5-QMP lure	5-QMP plus Eicanosol lure
1	22	11
2	11	6
TOTAL	**33**	**17**

Orchid trial

AHB were strongly attracted to the orchid as soon as it was removed from the car. Between five and 20 AHB tried to gain access to the orchid flowers as long as it was exposed (Figure 9).

Figure 9: Aggregating AHB on a mesh covered *C. floribundum* orchid in Edmonton (Queensland, Australia)

Discussion

Main results

Trap design

None of the traps trialled in the efficacy field trials (standard AHB traps, sticky mat trap, pan traps, 9-ODA pheromone traps or artificial flower bottle traps) trapped any AHB. Therefore, it was decided to trial traps and attractants in close proximity to hived AHB. This was considered to be a sound approach since innate and learned choices are known to be based on the interaction between visual stimuli and olfactory cues for flower visitors at closer distances (i.e. within 1 m of a floral source; Lunau and Maier, 1995).

The most promising trap design to effectively trap bees was the artificial flower bottle trap (as discussed in the *Trap design trials - Single experimental trials* section). Since artificial flower bottle traps were used in both the *Trap efficacy field trials* and *Single experimental trials*, the concentration of the sugar attractant must have been responsible for this result. Specifically, sweeter honey-water solution (1:1 concentration) was more effective in attracting AHB than 25% fruit juice cordial (orange and mango flavoured) or dilute honey-water solution (3:1 concentration). Our attractant trial results as well as those of Wells and Rathore (1994) further support this conclusion.

Attractants

The synthetic 5-QMP lure was twice as attractive to hived AHB as the synthetic 5-QMP plus Eicosanol lure. It has been shown previously that low levels of Eicosanol can have an attractant function, whereas high levels of Eicosanol can have the opposite effect (Anderson *et al*., 2012). The lower success of the 5-QMP plus Eicosanol lure may be due to higher levels of Eicosanol. Unfortunately, concentrations of the 5-QMP and 5-QMP plus Eicosanol lures were not revealed by the supplier.

The orchid, *C. floribundum*, was highly attractive to hived AHB. This was immediately apparent as the orchid was removed from the car. Foragers continued to be attracted to the orchid flowers even at distances up to 50 metres. *Cymbidium floribundum* is an orchid that thrives in temperate climates but does not do well in the tropics. This was clear as flowering orchids started to drop flowers soon after arrival. Therefore, synthesising the volatile component of this orchid would be preferred. Further research is being conducted by Dr. David Guez through a RIRDC funded project.

Of the trialled attractants, AHB showed a clear preference for higher sugar concentrations (near saturation), but only showed a slight preference for the colour blue and no preference for any essential oil-based scents, with the exception that AHB disliked Jasmine scent. Our results are consistent with Wells and Rathore (1994) who found that AHB tend to select flowers that maximise the energetic reward obtained regardless of colour. The finding that AHB disliked Jasmine in the Cairns region differed to that observed by Koltermann (1973) for *A. cerana indica*. Exploration of the reasons for this dislike, and further trials using other scents are recommended. Additionally, future colour trials may be enhanced by replacing the coloured paper used in this study with ultraviolet paint of a known wavelength.

Although lavender was used in all traps and feeding stations through the AHB Program, AHB showed no preference for this scent. This may be explained by the fact that tropical AHB (Java genotype) would never have been exposed to lavender in the tropics (i.e. Australia or Indonesia) and so would never have learnt to associate lavender with a sugar reward. It may be useful to trial other scents, specifically those that tropical AHB may associate with a reward in the wild, e.g. palm flower or citrus scent or any scent of flowers that AHB are observed to forage on.

Preferring near-saturation sugar syrup may be influenced by the proximity of the feeding station to the hive. Higher sugar syrup concentrations may be easy to carry for short distances, but lower sugar concentrations may be preferred over longer distances (Dr. David Guez, pers. com.). In addition, it may be important which sugar type is used. In our trials we used sucrose. However, AHB may prefer fructose or glucose (Dr. David Guez, pers. com.). These options should be explored in future research.

In addition, our trials explored the foraging behaviour of AHB. Physiological responses (e.g. proboscis extension trials) to any of these attractants also need to be explored (Dr. David Guez, pers. com.; e.g. Menzel and Muller, 1996).

It is important to note that bees have to learn to associate colour and scent with a specific reward. It needs to be explored how useful attractants such as these are, if training is necessary. On the other hand, pheromones elicit an innate response without the need to train bees. It appears that the orchid is a true attractant, attracting feral AHB exclusively without having to train them. This is likely to be due to the orchid exuding a smell that mimics AHB queen pheromone. In our trials, a number of workers were attracted to the orchid but no swarm appeared. According to Dr. David Guez, this was due to the fact that the workers belonged to a working hive with their own queen, whom they would not abandon.

Conclusion

Two main conclusions can be drawn from the results presented in this report. Firstly, artificial flower bottle traps were the only trap type that have been successful in trapping AHB, and consequently provide a basis for further AHB trap design research. Secondly, although 5-QMP and 5-QMP plus Eicosanol were able to attract AHB at closer distances (1-2 metres), the orchid (*C. floribundum*) was able to attract AHB from up to 50 metres. Given this, orchid volatile compounds appear to offer a novel avenue for further research. This opportunity is currently being pursued by Dr. David Guez of the University of Newcastle (Australia) under a RIRDC grant.

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