Minutes AHB SAG

Meeting One of the Asian Honey Bee Scientific Advisory Group (AHB SAG)

Face-to-Face meeting held at the Novotel, Brisbane, on Wednesday 23rd November, 2011

Attendees: Rod Turner PHA (Chair), Sam Malfroy PHA (Secretariat), Colin Grant DAFF (Chair of the AHB TMG: left meeting at 1:15pm), Glynn Maynard DAFF, Denis Anderson CSIRO (left meeting at 2:15pm), Doug Somerville NSW DPI, Trevor Weatherhead AHBIC, Max Whitten FCAAA, Sharon De Wet DEEDI, Neil O'Brien DEEDI (left meeting at 1:15pm).

Apologies: Simon Barry CSIRO, Boris Baer UWA

Item 1 & 2: Welcome by the Chair and Members introduction

The Chair welcomed the Members of the Asian Honey Bee Scientific Advisory Group (AHB SAG) to the meeting. The Members introduced themselves and gave their background and experience in regards to honeybees.

Item 3: Expectations of Asian Honey Bee Transition Management Group (AHB TMG) of the SAG – Colin Grant

Dr Colin Grant, Chair of the Asian Honey Bee Management Group (AHB TMG), provided a brief background of what is expected of the SAG and how the role of the SAG fits in with the objectives of the AHB T2M program. It was discussed that the objective of the SAG is to provide technical advice to the AHB TMG on specific scientific issues as referred to it by the AHB TMG, as well as developing techniques/strategies that minimise the impact of AHB and to support the AHB T2M program. This advice would then be forwarded from the SAG to the AHB TMG for consideration.

Item 4: Terms of Reference

Terms of Reference (ToR) for the SAG were discussed and the Chair agreed to send out the drafted ToR to the SAG and AHB TMG group for comment as soon as possible.

Item 5: Conflict of Interest

There was recognition amongst all Members of the SAG to be aware of potential conflict of interests and that it is intended that Members of the SAG not be involved (directly or indirectly) in promoting personal or associated research that is being funded by the AHB T2M program.

Item 6: AHB Transition to Management Program Update – Neil O'Brien

Mr O'Brien provided an update of the current situation of AHB in the Cairns region and stated that Biosecurity Queensland will need scientifically sound data that can inform the ongoing and future management of the Asian honey bee for the people of Queensland,

industry, the environment and social amenity issues that may arise. There was agreement amongst the SAG to help provide clear guidance on the scientific and technical aspects of the AHB T2M program.

Item 7: Issues for consideration

Discussion: Communication with stakeholders and the public

The issue of how the SAG communicates with the wider public and industry members was discussed. It was agreed that the Chair would be the contact point for the SAG to the AHB TMG. The Chair noted that SAG's do not provide commentary to outside parties, and that all communication regarding the AHB T2M program will come from the spokesperson of the AHBMG.

The finalised Minutes from each meeting were suggested to be placed on the AHB website once endorsed by the AHB TMG. All Members agreed with this avenue for communication with industry and the wider public.

Discussion: Travelling to Cairns

The proposal of travelling to the Cairns region was discussed amongst the Members. It was agreed that if the SAG decided to travel to Cairns for a field visit and meeting, then SAG Members would have to pay their own way in regards to accommodation and flights.

There was agreement amongst the Members of the SAG that a visit to Cairns would be very beneficial to see the variety of landscapes and situations that AHB occurs in and should be conducted as soon as possible. The Chair proposed the dates of the 18th and 19th of January 2012 for meetings and field visits at the Cairns DPI office. All Members present tentatively agreed to these dates.

Discussion: Data analysis

All Members strongly agreed that to enable recommendations on the technical aspects of the program, analysis of the previous data needs to be undertaken. This will aid in determining the value of the previous data collected as well as guide the type of data that are gathered in future detections.

The feasibility of eradication was discussed amongst the Members, however, it was agreed by all Members that the until further data are gathered, or further information provides evidence that the likelihood success of eradication was technically feasible, then the only way forward is transition to management of AHB.

When discussing the current information regarding data collection, there was agreement amongst Members that additional details are required from Biosecurity Queensland's operational plan and strategy for conducting these operations. It was agreed details would be followed up with Biosecurity Queensland while in Cairns for the January meeting.

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Discussion: DEEDI issues for consideration

The establishment of bee free zones was discussed, however all Members agreed that establishing such areas is intensive in terms of monitoring, eradicating and the use of chemicals such as Fipronil. All Members agreed that establishing such sites would be hard to establish and maintain.

Concern was raised by some Members over the issues for consideration listed by DEEDI, specifically that not enough effort is being focused on management options that beekeepers could use to manage AHB. It was identified by Members that there was a strong need to research various management options that beekeepers could use to manage AHB.

The current state of funding and research into viruses was questioned by the Members. It was recognised that there was a need for update by Denis Anderson on the situation of virus work on the AHB population, as well as any future projects outlined for Denis Anderson's CSIRO lab.

Discussion: Next Meeting

There was agreement to hold a teleconference on the 14th of December to discuss the AHB T2M program as well as determine arrangements with the proposed trip to Cairns.

Minutes AHB SAG

Meeting Two of the Asian Honey Bee Scientific Advisory Group (AHB SAG)

Teleconference held on Wednesday 14th December 2011

Attendees: Rod Turner PHA (Chair), Sam Malfroy PHA (Secretariat), Sharyn Taylor PHA, Glynn Maynard DAFF, Denis Anderson CSIRO, Doug Somerville NSW DPI, Trevor Weatherhead AHBIC, Max Whitten FCAAA, Sharon De Wet DEEDI, Boris Baer UWA.

Apologies: Simon Barry CSIRO

Item 1: Welcome by the Chair

The Chair welcomed the Members of the Asian Honey Bee Scientific Advisory Group (AHB SAG) to the teleconference.

Item 2: Terms of Reference

The Chair stated that the Terms of Reference (ToR) will be distributed to all SAG Members for comment once approved by the Asian Honey Bee Transition Management Group (AHB TMG). The Terms of Reference will be provided for comment whilst the SAG is in Cairns.

Item 3: Members comment on the AHB T2M

All Members agreed that they could not comment on the AHB T2M without seeing the detailed operational plan. The Members agreed that there was a lot of scope regarding projects AG 2 and 3, which is likely to be the main area where comments and advice from the SAG would be directed. The Chair stated that the operational plan would be discussed while in Cairns on January 18th and 19th.

The Chair stated that the SAG's questions outlined in Meeting One relating to data collection and operational plan details had been provided to Biosecurity Queensland. It was agreed that all scientific and operational information will be provided to the SAG at the face-to-face meeting schedules for early January 2012 in Cairns.

There was discussion amongst the Members regarding various research possibilities that could be of use to the AHB T2M, as well as the appropriateness and feasibility of possible research projects that could support the AHB T2M and extensive discussion was held on the types of research that may assist the AHB T2M program.

The Chair informed the SAG that Dave Alden (RIRDC) had contacted PHA and had provided research proposals that could be of benefit to the AHB T2M. These proposals had been provided because industry money (AHBIC and FCAAA) will be managed by RIRDC. The Chair noted that this issue will be discussed at Meeting Two of the AHB TMG on December 20th. Once approved by the AHB TMG, these proposals will be distributed to the SAG for technical and scientific feedback and will be formally discussed at the meeting in Cairns in early January 2012. The Chair reiterated that if distributed, these

research proposals are confidential and not for distribution or discussion outside this group.

Item 4: Members comment on Minutes distributed from Meeting One and request for any additional information

The minutes from Meeting One of the AHB SAG were accepted as finalised by the Members of the SAG. The Chair added that the Minutes, once approved by the AHB TMG, would be placed on the website to provide an update on the situation to stakeholders and the public.

Item 5: Confirmation of dates and times for the trip to Cairns

As outlined in the minutes of Meeting One, the Chair proposed the dates of the 18th and 19th January 2012 for the SAG trip to Cairns. All Members present agreed to these dates. The first day (18th) was proposed to include a day of field visits, while the second day (19th) was for a formal meeting of the SAG to take place.

Item 6: Talking Points to be agreed upon and presented to the AHBMG

The issue of communication was raised by the Chair. It was agreed that minutes from meetings will be placed on the AHB website and these would provide the update on activities of the SAG to stakeholders and the community.

AHB SAG Minutes

Meeting Three of the Asian Honey Bee Scientific Advisory Group (AHB SAG)

Face-to-Face meeting held at the Cairns DEEDI Office on Thursday 19th January 2012

Attendees: Rod Turner PHA (Chair), Sam Malfroy PHA (Secretariat), Glynn Maynard DAFF, Denis Anderson CSIRO, Simon Barry CSIRO, Doug Somerville NSW DPI, Trevor Weatherhead AHBIC, Max Whitten FCAAA, Sharon De Wet DEEDI, Boris Baer UWA, (all following attendees joined the meeting as observers at 1:30pm) Neil O'Brien DEEDI, Rick Symons DEEDI, Russell Gilmour DEEDI, Shirin Hyatt DEEDI, Corey Bell DEEDI and Glenn Docherty DEEDI.

Apologies: Nil

Item 1: Welcome by the Chair

The Chair welcomed the Members of the Asian Honey Bee Scientific Advisory Group (AHB SAG) to the meeting and stated that DEEDI staff involved in the program would be joining the meeting at 1:30pm as observers.

Item 2: Comment and discussion

AHB T2M Program: All Members strongly agreed that research projects associated with the AHB T2M program should focus on two separate components. The first should be research conducted on pests and diseases of the existing population of Asian honey bee in Cairns to determine what it is carrying and the potential transmission of these pests and diseases to European honey bees. The second should be on improved means for detection, surveillance as well as local suppression of Asian honey bee using Fipronil remote poisoning within selected areas in the Cairns region.

RIRDC Proposals: RIRDC preliminary research proposals were tabled with the Members and discussed. All Members raised the issue that although some of the research proposals could provide benefits to achieving AHB T2M objectives, not all components of the research proposals were considered appropriate to the AHB T2M program, or specifically to the Asian honey bee in Cairns (*Apis cerana* Java strain).

The Chair proposed that PHA, on behalf of the SAG, hold preliminary discussions with RIRDC to put out a formal tender for research proposals specifically related to the AHB T2M Program and *Apis cerana* Java strain. If this course of action were to be approved by the Asian Honey Bee Transition Management Group (AHB TMG), the SAG could advise on the information outlined in the tender, as well as provide scientific advice and feedback to RIRDC and the AHB TMG on which projects would be beneficial to the AHB T2M program. All Members strongly endorsed this motion.

> **Recommendation 1:** The SAG requests that PHA discuss options with RIRDC to organise a tender process for research proposals related to specific objectives in the AHB T2M program.

Remote Poisoning Trials: The remote poisoning trials using Fipronil were discussed extensively amongst the Members and it was agreed that this chemical could provide a useful means to test and validate suppressing the Asian honey bee in specific areas, such as transport hubs or ports. The SAG expressed that they would like to be involved in experimental design of these trials.

DEEDI stated that the Fipronil remote poisoning experimental methodology would be provided to the SAG for scientific comment and feedback so that any amendments or changes can be discussed at the next meeting.

> **Recommendation 2:** The SAG requested that they be involved in experimental design and implementation of the Fipronil remote poisoning trial.
>
> **Recommendation 3:** The SAG requests that the tomato dust experiment listed in AG Project 2 be dropped and, instead, all effort should focus on using Fipronil for remote poisoning.

Surveillance: The Members discussed the scientific validity of the surveillance currently being conducted by DEEDI, and the levels of confidence of presence or absence of the Asian honey bee in each of their surveillance techniques. It was recommended that future surveillance in the outer fringes of the Asian honey bee incursion be surveyed for presence or absence of the Asian honey bee using an improved surveillance methodology. This included incorporating sweep netting, with replicable factors such as surveillance area, time surveyed and floral source surveyed which would be useful in developing techniques for management by other jurisdictions if AHB were to spread.

It was also proposed by the SAG that it would be beneficial for potential future incursions of Asian honey bees if the DEEDI staff, with their extensive experience and expertise, record and document their methods and procedures of surveillance. Techniques that should be recorded and documented include DEEDI staff conducting bee lining, inspecting bee-eater roosts, floral sweeping, destroying a nest, as well as inspecting swarm and bait traps.

> **Recommendation 4:** The SAG requests that DEEDI develop a more appropriate methodology to confirm absence/presence of AHB. The SAG stated that they would be happy to advise on experimental design.
>
> **Recommendation 5:** The SAG requests that DEEDI video and documents various methods of surveillance that DEEDI staff are currently using. This would capitalise on their expertise for the future benefit of industry and the public.

Pollen Analysis: The issue of the nectar and pollen resources being used by the Asian honey bee was discussed amongst the SAG. All Members agreed that a pollen analysis should replace the nectar analysis as listed in AG Project 2 and that funding could also be sourced from AG Project 4 "Limiting impact on natural environments" in determining the floral resources that the Asian honey bee is foraging on.

> **Recommendation 6:** The SAG requests that the stored combs from detections since 2007 be used for pollen research to understand what the bee is feeding on, and breeding on, at specific times of the year. This is to replace the nectar analysis project which is outlined in AG Project 2.

All Members of the SAG agree that analysing the pollen in the stored frozen comb of Asian honey bee nests collected since 2007 could potentially provide a tool to understand what the bee is using a protein source.

Sniffer Dog: The SAG discussed whether the sniffer dog was achieving results in the AHB T2M program and if it aided in the scientific validity of the program. The Chair stated the sniffer dog and handler and kennel costs are significant. The SAG noted that while the program costs were high, they proposed that the dog be scientifically tested with appropriate methodology in high density areas, such as port and urban environments, to determine the cost effectiveness of the dog.

Sex allele and Microsatellite Research: The possibility of conducting research to identify microsatellite markers to determine the difference between the Cairns population of *Apis cerana* Java strain, to the populations in the Solomon Islands and Papua New Guinea was discussed. It was stated that preliminary research conducted by leading scientists in DEEDI has identified 20 markers that could potentially be used to test this hypothesis. The SAG when it was stated that it would only cost $3000 to undertake preliminary trials, recommended that a preliminary research trial be conducted to see if the populations between Cairns, PNG and the Solomon's can be distinguished by these markers.

> **Recommendation 7:** The SAG agreed that DEEDI may conduct preliminary micro-satellite work to determine any differences between the Asian honey bee Australian, Solomon Islands and Papua New Guinean populations but that this was not a high priority and based on the information that the trial would only cost $3000.

Future meetings: It was proposed that the next meeting be a teleconference held on Wednesday the 8th of February 12:00pm (AEDST).

Summary and close of meeting: The Chair thanked all DEEDI staff involved with the SAG Cairns visit for their time and effort in organising a very enjoyable and worthwhile trip.

AHB SAG Minutes

Meeting Four of the Asian Honey Bee Scientific Advisory Group (AHB SAG)

Teleconference held on Wednesday 15th February 2012

Attendees: Rod Turner PHA (Chair), Sam Malfroy PHA (Secretariat), Denis Anderson CSIRO, Simon Barry CSIRO, Trevor Weatherhead AHBIC, Max Whitten FCAAA, Boris Baer UWA, (all following attendees joined the meeting as observers) Neil O'Brien DEEDI, Russell Gilmour DEEDI and Shirin Hyatt DEEDI.

Apologies: Glynn Maynard DAFF, Doug Somerville NSW DPI, Sharon De Wet DEEDI, Dave Alden RIRDC and Sharyn Taylor PHA.

Item 1: Welcome by the Chair

The Chair welcomed the Members of the Asian Honey Bee Scientific Advisory Group (AHB SAG) to the teleconference.

Item 2: Asian honey bee odour detection dog

The recently completed DEEDI review into the AHB odour detection dog was discussed amongst the SAG. There was agreement amongst the SAG that the dog had achieved good results within the AHB eradication program and had effectively demonstrated the ability to detect AHB odour and AHB nests and swarms. However, the SAG agreed with the DEEDI review recommendations and agreed that the dog did not fit within the objectives of the AHB T2M. The SAG felt that the money that was allocated to the dog and handler could achieve a better outcome for the program if it was spent on hiring field staff for the AHB T2M program.

> **Recommendation 1:** The SAG agreed with the Asian honey bee odour detection dog review conducted by DEEDI and recommended that the dog no longer be used within the AHB T2M program.

Item 3: Discussion and Comment on the DEEDI work plan

DEEDI stated that this document clearly highlights the objectives and strategies to implement the AHB T2M. It was agreed that the SAG would provide scientific comment and feedback on the document. The Chair stated that PHA would follow up with the SAG to outline the specific areas where DEEDI is requesting feedback and that this would be compiled and presented to DEEDI on behalf of the SAG.

Item 4: Discussion and comment on the draft pathway analysis

The SAG agreed that this document provides an effective summary of potential pathway analysis into Australia of pest *Apis* spp. However, it was the SAG's opinion that the document needed to be updated, specifically in relation to the Cairns incursion and to include domestic pathway analysis of the established population of *Apis cerana* Java strain. Understanding this internal pathway would be critical in allowing an effective

transition to management as well as understanding the risks of the Cairns population spreading within Australia. DEEDI stated that are collating evidence in relation to the spread, and modes of spread of the Asian honey bee in the Cairns region and that this data would help complement and improve the pathway analysis document.

In addition, the SAG recommended that the scope of the original analysis be expanded to cover other species of *Apis* spp and potential entry points in addition to PNG and New Zealand already covered in the report.

The Chair stated that PHA will follow up with the SAG in relation to specific areas of the pathway analysis that need to be included and/or updated.

> **Recommendation 2:** That the draft pathway analysis document prepared by DAFF in 2008 be updated to include a domestic pathway analysis, and a section relating to pathway analysis of other *Apis* spp.

Item 5: Discussion and comment on pollen analysis

It was the SAG's opinion that this bee is highly adaptive and has the potential to utilise a range of plants as a pollen resource, and therefore the output from a pollen resource study is essentially limited to where the bee is and what is flowering in a specific region at the time. DEEDI also stated that many of the combs collected from nests since 2007 did not contain much pollen, and instead contained brood, and therefore a retrospective pollen study would be hard to implement.

It was discussed that research is currently being conducted in conjunction with UNE and CSIRO on pollen analysis on all AHB nests collected from the start of 2012. To this date, 8 nests have been collected and provided to the researchers. The SAG requested that DEEDI continue to supply researchers with nests, and that if possible, nests are collected for pollen analysis from various regions and plant communities in the Cairns region to demonstrate that the AHB can utilise a range of pollen resources in different situations.

The SAG agreed that at this time in the T2M that a retrospective pollen analysis is not necessary until the findings from the joint pollen analysis conducted by UNE and CSIRO is made public. It was further stated that a nectar analysis did not align with the objectives of the AHB T2M and that this should be dropped as a project.

> **Recommendation 3:** The SAG recommends that the nectar analysis be dropped from the AHB T2M program.

> **Recommendation 4:** That the nectar analysis proposed in the AHB T2M be replaced by the pollen analysis already being conducted jointly between UNE and CSIRO with specific focus on identifying pollen resources used by AHB nests collected in areas with different plant communities.

Item 6: Update on targeted commissioning for research proposals

Plant Health Australia provided an update to the SAG of the recent discussion with RIRDC regarding the RIRDC research proposals that were submitted to the SAG for comment. PHA stated that it had discussed with RIRDC which proposals the SAG considered were suitably aligned with the objectives of the AHB T2M, and others which were not considered suitable.

PHA also stated to the SAG that it had expressed to RIRDC the desire of the SAG to put in place targeted commissioning for research projects, specifically relating to attractant research, pest and disease research and inter-specific mating research. It was agreed that PHA would write up a call for research proposals, under the guidance of the SAG, and once agreed upon these would be provided to RIRDC for distribution to a range of scientists that were deemed suitable to conduct the research.

Item 7: Discussion and comment on the call for research proposals

The call for research proposals for attractant research and pest and disease research was tabled with the SAG for comment. All members of the SAG felt that the proposals provided an effective summary of the type of research aiming to be conducted in alignment with the AHB T2M. PHA agreed to follow up with RIRDC regarding the processes that would be required to send out the call for research proposals. PHA stated that they would follow up shortly with the SAG to provide an update of the research proposal situation.

The SAG stated that once proposals from researchers were received by RIRDC that the SAG would like to comment on each of the proposals received. The Chair stated that due to potential conflict of interest that this would have to be cleared through the AHB TMG.

> **Recommendation 5:** For PHA to follow up with RIRDC in regards to the approved 'call for research proposals' and for these to be distributed to relevant researchers in a timely manner.
>
> **Recommendation 6:** To seek endorsement from the Management Committee for the SAG to be allowed to comment on the research proposals received by RIRDC.

Item 8: Discussion and comment on experimental design and methodology in Fipronil remote poisoning experiment

The Fipronil remote poisoning experimental outline was discussed amongst the SAG. The SAG stated that this experiment should build upon previous work conducted in Western Australia and the Solomon Islands, however stated there are some critical aspects that need to be determined in this project for the Cairns scenario. This includes:

- The outcomes of the trial are in a statistically valid sense so that various levels of confidence in regards to destruction of nests can be achieved

- To determine the forager/colony size ratio in regards to how many foragers are required to take back the Fipronil to kill a nest of a certain size
- Testing how long it takes for Fipronil to kill a nest of a certain size
- To determine the distance from a remote poisoning station (i.e 500m) that you can be confident that you have poisoned all AHB in the radius
- To vary the concentration of Fipronil for specific scenarios of nest destruction
- The need to test between high density and low density areas, so that remote poisoning can be utilised in a potential future incursion

It was also raised by the SAG that Fipronil is widely used in both commercial and personal settings and it was requested that the introduction of the document be rewritten to include how, and where Fipronil is used to provide context for the public and stakeholders for this experiment to take place.

The SAG and DEEDI also expressed their desire to minimise the impact of this trial on potential non-target organisms. It was discussed amongst the SAG and DEEDI that there are two potential methods to determine if this possible. The first method is to conduct the trial at a time when the AHB has been trained to the bait station and at a time (i.e. dusk) that AHB are the dominant foraging species. The second method is to conduct a separate bait station trial which includes a form of excluder which would exclude other social and solitary insects from feeding on the bait station.

The SAG suggested that the Fipronil trial should start as soon as possible, and DEEDI agreed, stating that a number of nests had been located within the containment zone to start the remote poisoning trial. DEEDI stated that they would provide the SAG with a description of the nests and where they are and how they propose to destroy the nests and requested the SAG comment on the methodology of how these nests are destroyed and what variables are tested. The SAG agreed to respond as soon as possible on the methodology proposed and stated that the original trial of Fipronil remote poisoning needs to remain flexible and follow an adaptive approach, before refining the methodology with greater numbers of AHB nests throughout the Cairns region.

Recommendation 7: For the SAG to be directly involved in re-designing the Fipronil remote poisoning experiment based on the DEEDI document, including the testing of variables such as concentration of Fipronil, how many foragers are present on the bait station and distance to the hive etc.

Recommendation 8: For DEEDI to conduct experiments on minimising the impact of Fipronil remote poisoning on non-target organisms with direct scientific input from the SAG.

Recommendation 9: For the beginning of the Fipronil experimental document to be rewritten to include how and where Fipronil is used to provide context of how widely used this chemical is in order to provide information for the general public and stakeholders.

Item 9: Future Meetings

The Chair stated that PHA would follow up with all Members of the SAG shortly to arrange the next meeting.

Item 10: Summary and close of meeting

The Chair thanked all Members of the SAG for attending the teleconference and closed the meeting.

AHB SAG Minutes

Meeting Five of the Asian Honey Bee Scientific Advisory Group (AHB SAG)

Teleconference held on Wednesday 5th April 2012

Attendees: Rod Turner PHA (Chair), Sam Malfroy PHA (Secretariat), Denis Anderson CSIRO, Simon Barry CSIRO, Glynn Maynard DAFF, Doug Somerville NSW DPI, Trevor Weatherhead AHBIC, Max Whitten FCAAA, Boris Baer UWA (all following attendees joined the meeting as observers) Dave Alden RIRDC, Russell Gilmour DEEDI, Shirin Hyatt DEEDI and Anna Koetz DEEDI.

Apologies: Sharon De Wet DEEDI, Neil O'Brien DEEDI

Item 1: Welcome by the Chair

The Chair welcomed the Members of the Asian Honey Bee Scientific Advisory Group (AHB SAG) to the teleconference.

Item 2: Pest and Disease preliminary research proposals

Three preliminary research proposals (PRP's) were tabled for discussion. A conflict of interest was declared by Dr Anderson. However, all Members of the SAG and the Chair endorsed Dr Anderson to make an independent and impartial assessment of the technical aspects of the PRP's received for the pest and disease research.

The technical and scientific aspects of the three pest and disease PRP's were discussed amongst the SAG. The SAG agreed with the advice provided by the RIRDC Honeybee Committee and believed that the Roberts PRP would effectively deliver the scientific outcomes that are required for the AHB T2M within the timeline and budget that is being sought. It was discussed that the deep sequence and bioassay proposed in the PRP should be an acceptable methodology to help potentially re-establish trade in live honey bee exports. The SAG endorsed that this PRP be made into a Full Research Proposal (FRP).

Item 3: Attractants for *Apis cerana* research proposals

Two PRP's were tabled and discussed amongst the SAG and BQ. All Members agreed that the Guez proposal effectively included Cairns plants that were considered attractive to AHB, as well specific overseas orchids. The SAG believed that the Guez PRP was scientifically sound and that it would deliver an effective means to help manage AHB into the future. However, concern was raised over the 3 year timeline that was proposed for the research.

Due to the timelines of the AHB T2M, the SAG requested that the when writing a FRP, the researcher for the attractant research should cut the timeline down to 2 years and that some aspects of the project be simplified, such as the work on overseas orchids, to reflect this shortened timeline. However, the SAG requested that this extra work be

placed into an optional 3rd year of funding for research to take place if the results are promising.

The Senior Research Manager of the RIRDC Honeybee Committee, Dr Dave Alden, stated that when these proposals were considered at the last RIRDC Honeybee Committee meeting, the option of using a honey based attractant was discussed as an alternative to the PRP's received. It was stated that this research would be less risky than developing an attractant, and that honey is a known attractant to honey bees. However, members of the SAG who have had extensive experience with the Asian honey bee (Java strain) stated that this has been tried, both in the Torres Strait and the Solomon Islands without success. DEEDI added that two techniques were tried in the original Cairns incursion using these principles. The first was melting beeswax and honey with an odour flume and the second was by leaving out sticky supers. However, both methods failed to attract any AHB. All members of the SAG agreed that developing a specific attractant was the best way to proceed and strongly supported that the Guez PRP be made into a FRP, including the recommended changes.

The Chair stated that according to the contracts the $400,000 contributed by the Honey bee industry ($200,000 from both AHBIC and FCAAA) would have to be spent on projects in the AHB T2M by the 30th of June 2013 and because of this, the proposed 3 year project would be hard to fund in its entirety. Some Members of the SAG expressed frustration at the timelines proposed and stated that completing complicated research with a set date does not take into consideration many of the unexpected complications that inevitably arise with research. Members also expressed frustration that the June 2013 completion dates was always predicated by the 1st of July 2011 start date, which did not happen. Members stated that the 2 year timeline should only commence when projects begin, not from the July 2011 dates.

It was proposed that if a project was considered that had a timeline that finished past the 30th June 2013 end date, that money could come from other sources, such as the RIRDC Honeybee Committee to continue the research. The Senior Research Manager of the RIRDC Honeybee Committee, Dr Dave Alden agreed that this was a possibility.

The Chair acknowledged that he was unsure whether it would be acceptable to allocate money for projects that ran past the end date of the AHB T2M. The Chair agreed to seek clarification on this issue with the Chair of Transition Management Group (TMG) and would provide a formal response to the SAG and BQ about the level of movement that is allowed between projects timelines and money allocated to these projects.

Item 4: Consultant strategy to address Australian honey bee imports

Three PRP's were tabled for discussion. The SAG agreed with the advice provided by the RIRDC Honeybee Committee and felt that the Clarke PRP would be the most beneficial to the AHB T2M and that it would deliver these outcomes in a timely and cost effective manner.

The SAG stated that the FRP should include discussions with the DAFF biosecurity section that deals with honey bee import/export because they would be aware of concerns of countries that import Australian honey bee. The SAG added that discussions should also take place with the lead researcher of the pest and disease research project linked to the AHB T2M. Dr Alden agreed to take these comments back to the lead researcher.

Item 5: Full Research Proposals

The FRP received by Dr Ben Oldroyd regarding the interspecific mating ability between *Apis cerana* Java strain and *Apis mellifera* was tabled. The SAG agreed with the advice provided by the RIRDC Honeybee Committee and believed that this project would provide valuable answers to a potential mating problem that could arise with the honey bee industry, as well as provide knowledge that would be critical in the future management of the AHB by industry. The SAG supported that the FRP go ahead.

The FRP received by Dr Ben Oldroyd regarding the sex alleles of the *Apis cerana* Java strain was tabled. The SAG stated that work similar to this is already being conducted by DEEDI in alignment with the AHB T2M. However, the SAG felt that if money was left over within the AHB T2M then this would be a useful project to fund as it would provide answers to the invasiveness of this species which would aid future management. It was agreed to put this research on the action list and revisit in future meetings.

Item 6: Preliminary remote nest treatments conducted on 5 AHB nests by Biosecurity Queensland

The preliminary Biosecurity Queensland (BQ) report titled *"Asian Honey Bee Remote Nest Treatment: results from 5 nests"* was tabled and the results and details of the report were discussed by BQ and the SAG. The results of the preliminary remote nest treatment were promising with 5 nests so far being treated, with 2 nests being destroyed and the other 3 being suppressed to very low levels of AHB activity. BQ stated that they are continuing to the monitor the activity very closely of the three remaining AHB nests.

It was noted that some nests had been attacked by green tree ants. The SAG expressed concern about the possible residual effects of Fipronil on the green tree ant colonies when 'cleaning out' old AHB nests and stores. The SAG requested that BQ look into how serious this possible non-target effect could be, and to potentially conduct some preliminary research into the possible effects of Fipronil on green ants that 'clean out' old AHB nests and stores.

Biosecurity Queensland stated that as the dry season begins in cairns that more AHB nests will able to be trialled for remote treatment, however, stated that they were unsure about the future direction of variables that should be tested. The SAG discussed the possibility of starting to measure the effect of variables such as a lower dose rate of Fipronil, or distance from the feeding station to the nest, however, agreed that the next nests should continue to focus on determining the level of forager numbers to suppress an AHB nest of a certain size. Biosecurity Queensland agreed with this suggestion and

the Chair stated that any updates from BQ or feedback from the SAG regarding the remote nest treatments should be coordinated through PHA.

All Members of the SAG congratulated BQ for the effort in preparing a scientific and thorough research report into the use of Fipronil to remotely suppress AHB nests.

Item 9: Future Meetings

The Chair stated that PHA would follow up with all Members of SAG if there is a development in regards to the research proposals or the remote nest treatments. All Members agreed to follow up with PHA if they wished for any minor changes to be made.

Item 10: Summary and close of meeting

The Chair thanked all Members of the SAG for attending the teleconference and closed the meeting.

AHB SAG Minutes

Meeting Six of the Asian Honey Bee Scientific Advisory Group (AHB SAG)

Teleconference held on Thursday 12th July 2012

Attendees: Rod Turner PHA (Chair), Sam Malfroy PHA (Secretariat), Simon Barry CSIRO, Glynn Maynard DAFF, Trevor Weatherhead AHBIC and Boris Baer UWA (the following attendees joined the meeting as observers) Russell Gilmour DAFF Queensland and Anna Koetz DAFF Queensland.

Apologies: Sharon De Wet DAFF Queensland, Neil O'Brien DAFF Queensland, Denis Anderson Bees Down-under, Doug Somerville NSW DPI, Max Whitten FCAAA

Item 1: Welcome by the Chair

The Chair welcomed the Members of the Asian Honey Bee Scientific Advisory Group (AHB SAG) to the teleconference.

Item 2 & 3: Remote nest treatment summary and discussion

Biosecurity Queensland (BQ) provided a summary of the remote nest treatment report. The SAG stated that Fipronil bait stations used in remote nest treatment of AHB did not appear to be the 'silver bullet' that was originally thought it would be. However, the report demonstrated that Fipronil is effective in control of AHB nests and swarms, however, the relationship between the number of bees poisoned and the ultimate destruction of the nest still needed to be determined. The SAG stated that this relation is somewhere in the data, and more nests need to be treated to figure out this relation so that there could be some level of confidence from the number of bees foraging on a Fipronil bait station and the ultimate destruction of an AHB nest or swarm.

The SAG also raised some issues of concern they had with the remote nest treatment report. It was stated that some of the conclusions that were drawn in the report are not in line with what was the intended end-use of Fipronil remote nest treatment of AHB. These issues focused around statements about the value in continuing these trials and possible environmental impacts of Fipronil remote nest treatment of AHB.

The Chair stated that although Fipronil is used for a variety of reasons, the BQ researchers do need to gather the environmental and off-target impacts of these trials as well as how much of the Fipronil residue stays in the nest and where (i.e. nectar, honey, wax etc.). BQ stated that more residue work is being completed and that these results would be forthcoming in the next few weeks. The Chair stated that he will be having a meeting with the APVMA in the near future in regards to the Fipronil trials and what kind of data packages will be required for beekeepers to gain registration for Fipronil as an AHB control tool after the AHB T2M.

It was also discussed that although the trials are demonstrating the 'knock-down' effectiveness of Fipronil remote poisoning, the kill rate after 1st treatment is still quite low in comparison. The SAG proposed that BQ look at the permit that was supplied by

the APVMA and for future trials, a half rate of the current Fipronil rate be used. This would determine whether a higher numbers of foragers, making more trips, with a lower concentration of Fipronil would be better to kill an AHB nest.

BQ raised some issues in regards to the time allocated to these trials and it was accepted amongst the SAG that the time allocated to this project was not being managed effectively. The possibility of catching AHB swarms and keeping them in hives was discussed. These hives could be observation hives (Perspex / glass on the side to see through) which would eliminate the time required to find the swarms / nests of AHB for the trial and this would also allow easy extraction of the nest and in estimating colony size in relation to Fipronil exposure. BQ stated that this could be a possibility considering the beekeeping industry in Cairns are collecting AHB swarms and managing the colonies in hives for research that is being funded out of the Honey bee industry contribution to the AHB T2M. Other aspects of the trial that could be included in future experiments were also discussed, including marking bees which fed on the feeding station, as well as an analysis of the stores in the nest.

The Chair thanked BQ for the report and stated that it provided a lot of useful information for this tool to be potentially used in the future. The Chair also stated that this report highlighted the importance of developing effective attractants for AHB which could make this tool more effective. The Chair requested that the SAG provide any comments on the remote nest treatment report to PHA (i.e. potential usefulness of observation hives) so that this can be circulated amongst the SAG and then provided to BQ for consideration.

Item 4: Bee trap efficacy

BQ explained the protocol of bee traps and discussed the report that was conducted to determine their efficacy.

The SAG stated that this report demonstrated the in-effectiveness of gel traps for surveillance of AHB when compared to other techniques, such as floral sweep netting. The SAG recommended that BQ no longer continue with the bee traps and instead re-focus their efforts in developing a proper floral sweep netting methodology to determine levels of confidence / absence of AHB in area. The SAG also stated that BQ should work closely with Dr David Guez who is conducting the AHB attractant research.

Item 5: Cairns port surveillance strategy and trial

BQ provided a summary of the combined DAFF Commonwealth and DAFF Queensland

The Chair requested that the words 'high intensity surveillance' be used instead of 'bee suppression zones' which in fact refers to a different method of surveillance and different actions. BQ agreed to change the wording. The Chair also requested that BQ look into getting local beekeepers to help with beelining any nests that are detected around the

surveillance area, as this is proving to be a costly exercise for BQ staff. BQ agreed to follow this up out of session.

Dr Baer from the SAG raised the issue on whether there is a PCR test to determine whether Varroa can exhibit it's unique micro-satellite on a honey bee forager (either AHB or EHB), even if the Varroa mite was not on that specific forager. There was some discussion regarding the plausibility of this technique and how long the lag time would be for Varroa presence to be recorded (i.e. if a bee had Varroa on it in PNG and then came to Australia on a vessel, but didn't have Varroa on it, would it still record the Varroa presence?). Dr Baer agreed to follow up about this out of session.

Item 6: Optimising early detection of new incursions of AHB

BQ stated that they had analysed biosecurity data and determined that the Cairns international terminal and the domestic terminal do not represent a significant risk pathway for a new *Apis cerana* incursion, however, the Cairns seaport does pose a significant risk of new *Apis cerana* detection.

BQ explained that they had formed a partnership with Australian Government – DAFF Biosecurity officers in the Cairns area to optimise early detection of any new incursions of AHB at the cairns seaport. This partnership involved BQ analysing the risks of the different areas of the seaport and using existing data to map suitable floral resources around the port, which. This was the used for fortnightly targeted sweep netting surveillance by DAFF Biosecurity Officers.

The SAG stated that BQ should continue with this work and document what was involved in setting up this surveillance strategy to optimise early detections of new incursions of AHB, as this could easily be applied in other major risk first call of port areas throughout Australia.

Item 7: Detection of Apis cerana DNA from bee eater pellets and trap liquor

BQ provided a summary of the study into extracting DNA from bee eater pellets and trap liquor. It was explained that AHB wings from the bee eater pellets have in the past been manually separated and diagnostics on the wings have been conducted manually using a microscope. However, this has proven hard when whole AHB wings are not present.

BQ explained that the aim of this experiment was to develop a quick PCR test for bee eater pellets to detect AHB wings. The experiment has been successful, but has still involved manual extraction of the wings from the bee eater pellets and then for these wings to be run through the PCR. BQ stated that there was too much genetic material / chemicals for a PCR to be run on entire bee eater pellets.

The SAG stated that considering manual extraction has to already occur (for manual diagnostics), if someone is trained in basic entomological diagnostics, then manual

diagnostics would probably be faster and simpler. However, the SAG requested that BQ continue to look at ways in which the entire bee eater pellets could be run through a PCR to test for AHB. The SAG stated it is worth to continue with this research as bee eater pellets provide a fast and reliable mode of detection of AHB.

The SAG also stated that it is worth continuing with the DNA extraction from trap liquor experiments as this will prove effective in determining the presence of AHB in a region, even if none are physically observed by field staff.

Item 8: Study of microsatellite alleles in Asian honey bees

BQ provided a summary of the study of microsatellite alleles in the Asian honey bee population in Cairns, when compared to other populations of Asian honey bee in the region.

The Chair stated that although it is interesting in determining the population dynamics of the Cairns population, this information is in fact secondary to what is aiming to be achieved by the AHB T2M. It was discussed that any new interception that is detected will be the responsibility of DAFF Biosecurity and determining where it is from comes secondary to destroying and collecting information from the interception.

The port risk assessment which will be conducted by CSIRO, as well as the revised pathway analysis will be able to provide enough information to allow DAFF Commonwealth Biosecurity to determine the risks of both the Cairns population of AHB, as well as international risks.

BQ stated that funding to continue this research is limited and that funding would have to be redirected from other projects to continue this work. There was disagreement within the SAG regarding the usefulness of this research in determining different populations of AHB and this issue was decided to be resolved out of session through feedback from the SAG when all Members are able to comment.

Item 9: Summary and close of meeting

The Chair thanked BQ for their report and also thanked the SAG for attending the teleconference and closed the meeting.

The Chair requested that Members of the SAG send comments on the BQ reports directly to PHA so that these can be compiled and presented to the AHB TMG and BQ.

AHB SAG Minutes

Meeting Seven of the Asian Honey Bee Scientific Advisory Group (AHB SAG)

Teleconference held on Thursday 27th September 2012

Attendees: Sam Malfroy (Chair), Stephen Dibley PHA, Alison Cleary PHA, Simon Barry CSIRO, Doug Somerville NSW DPI, Trevor Weatherhead AHBIC, Boris Baer UWA, Max Whitten FCAAA (the following attendees joined the meeting as observers) Neil O'Brien DAFF Queensland, Mike Ashton DAFF Queensland, Russell Gilmour DAFF Queensland and Anna Koetz DAFF Queensland.

Apologies: Rod Turner PHA, Glynn Maynard DAFF and Denis Anderson Bees Down-under

Item 1: Welcome and introduction by the Chair

The Chair welcomed the Members of the Asian Honey Bee Scientific Advisory Group (AHB SAG) to the teleconference. The Chair passed to Neil O'Brien of BQ to explain to the SAG the recent changes in management of the AHB T2M. Mr O'Brien explained that he would no longer be managing the AHB T2M in BQ, and instead the management of this program would change to Mike Ashton, Director of Plant Biosecurity and Product Integrity (DAFF Queensland), from the start of October 2012.

Mr Ashton introduced himself to the SAG and explained his past experiences in similar programs, including Citrus Canker, Sugarcane Smut and Papaya fruit fly eradication programs. Mr Ashton stated that there would be no changes to ground activities and staff of the AHB T2M.

Item 2: Remote nest treatment – recent developments

The Chair provided an explanation to the group regarding the recent developments with the remote nest treatments. The Chair stated that PHA had written a letter to both the APVMA and BASF on the 15th of August and attached the recently completed BQ Fipronil report. The letter to both of these companies requested their view of the trials currently being undertaken, as well as whether both would support the possible registration of a Fipronil based product after the AHB T2M for beekeepers to use to control AHB. The Chair stated that the APVMA provided a response that they would review the BQ document, but no further word had been received from either APVMA or BASF.

The Chair stated that PHA had been informed that BASF still control the toxilogical package to Fipronil, and their approval would be required for possible registration of any future product. Mr Weatherhead stated that he understood differently, and BASF no longer controlled the toxilogical package. There was discussion amongst the group on this issue, specifically regarding the registration of another Fipronil based product called Apithor for the honey bee industry. The Chair and Mr Weatherhead agreed to continue this discussion out of session.

There was frustration with some members of the SAG that remote nest treatments had not been conducted since July 2012. The Chair stated that BQ will not be initiating any new remote nest treatments until a response, and a position, had been received from the APVMA and BASF, or unless more information regarding who owns the toxilogical package was obtained. Once this has occurred, a SAG will be held to discuss the future of the remote nest treatments, including the variables tested, the concentration of Fipronil used as well as the design of the bait stations used.

Item 3: DAFF Queensland literature review - summary

The Chair opened up the discussion of the literature review by stating that this formed part of AG Project 3 of the AHB T2M. The Chair explained that the objectives of the literature review were to:

- Engage with apiarists in the Cairns region who have had experience with both honey bees.
- Conduct an analysis to understand what is known and not known about AHB in relation to mating, behaviour, foraging habits, weather impacts etc.
- Compare the behaviour between AHB and European honey bees to identify opportunities that support differential control methods.

The Chair stated that the literature review effectively highlighted knowledge gaps in the Java genotype, provided a context of the Java genotype within *Apis cerana* and also allowed research agencies to focus R&D efforts to areas that are not currently known. The Chair added that this literature review is a neutral document which had been compiled by peer reviewed scientific information.

Dr Koetz (BQ author of the literature review) stated that over 300 references were used in this literature review. Dr Koetz added that Professor Ben Oldroyd had reviewed the document and passed on some minor editorial comments which had been incorporated, as well as highlighting that *Apis cerana javana* is not the correct taxonomy for the bee in Cairns. Dr Koetz also mentioned that Dr David Guez was reviewing the literature and would be providing comments in the near future.

Dr Koetz also stated that comments had been received by Dr Denis Anderson out of session, also highlighting that *Apis cerana javana* is incorrect, and instead, the bee should be called *Apis cerana* Java genotype as it is not a recognised subspecies. Other comments from Dr Anderson included that BQ should work with students from nearby educational organisation to study various aspects of this bees biology, as very few references from overseas on this bee can be compared to the Cairns situation. Dr Koetz stated that BQ had already acted on this, and there were currently 3 Honours/Masters students from James Cook University conducting experiments and assisting BQ.

Dr Koetz discussed with the group that the amount of literature of *Apis cerana* Java genotype was very limited, and was either untranslatable or inaccessible. Because of this, a literature review solely focusing on *Apis cerana* Java genotype would not be possible. Dr Koetz added that placing a bee such as this within the context of *Apis*

cerana and *Apis mellifera* (which is managed in Australia) is crucial to understand it fully, as well as to understand a possible future incursion of *Apis cerana*.

Dr Koetz discussed with the SAG that if any other references were available, then she would be more than happy to include them in the literature review.

Item 4: Discussion – literature review

Dr Koetz stated she had had a few discussions with Dr Anderson about possibilities on why this bee behaves differently in the Solomon Islands and Cairns. The SAG requested that some of this information be included in the literature review as personal communication referenced material. The Chair agreed with this suggestion and stated that personal communication with Dr Anderson and Mr Annand (NSW DPI) could help build on this literature review, as very little published information is available on this bee.

Mr Weatherhead expressed serious concerns regarding some of the conclusions drawn in the literature review, and that it mislead readers in its assumptions of the Java genotype, by drawing conclusions from other *Apis cerana* subspecies. Mr Weatherhead discussed with the group that the literature review was asked to be done for *Apis cerana* Java genotype, not AHB in general, or even *Apis mellifera*. Mr Weatherhead also raised the issue that the work in PNG and the Solomon Islands was not given enough prominence in the literature review, as well as the Indonesian Master's report into this bee not being purchased or translated. In summary, Mr Weatherhead stated that as an Industry representative, that he did not support the literature review.

Mr Weatherhead also noted that that sections of the research recommendations contained in the literature review, such as the breeding *Apis cerana* Java genotype for honey production or selective breeding, would not only contravene various state Apiary Act's, but also highlighted that Industry would not want to go down this avenue. Dr Koetz stated that the literature review is not recommending this, but is merely expressing what the literature states, as similar advancements had been made with *Apis mellifera*. Mr Weatherhead also expressed that the Apis mellifera section should be completed with more relevant information. Dr Koetz stated that she would be more than happy to include other references, but these would have to be provided by Mr Weatherhead.

The Chair reiterated to the SAG what was originally requested in the project scope of AG Project 3, and that this literature review had exceeded what was requested of BQ. Some members of the SAG agreed that this was a very comprehensive literature review, and that with constructive feedback from the SAG, then this literature review could be improved. The Chair also mentioned that this literature review was an important project of AG Project 3 for Biosecurity Queensland to complete. The Chair added that it was desired that this literature review be completed with all possible feedback from the SAG, so this milestone could be achieved, and payment made to BQ within an appropriate timeframe so that other projects in the AHB T2M could be commenced and completed.

BQ stated that they would be happy to amend sections of the literature review from SAG recommendations once approved by the TMG. The Chair stated that a list of SAG recommendations would be presented to the TMG from this meeting. Dr Koetz stated she would be happy to work with the SAG to make these amendments, such as changing the title of the literature review and also including a specific section on work completed on this bee in PNG and the Solomon Islands, as well as compiling some personal communication referenced material for the literature review. BQ also stated that they would look into getting the Indonesian Master's document translated for future inclusion into the literature review.

It was acknowledged amongst the SAG and BQ that very little information is currently present on *Apis cerana* Java genotype. A suggestion was put forward to amend the current lit review and release it, but keep a copy of the literature review as a 'living document' with version control to gradually incorporate more knowledge of the bee as it comes to hand. The Chair agreed with this statement and suggested that the industry funded projects into pest and disease situation, attractants, interspecific mating and drone congregation areas would all yield valuable information that could be included in the future.

Item 5: Summary and close of meeting

The Chair thanked BQ, specifically Dr Koetz, for the literature review and also thanked the SAG for attending the teleconference and providing valuable feedback and comments.

Neil O'Brien thanked Dr Koetz on behalf of BQ for the literature review that had been conducted. Dr Koetz thanked the SAG for their comments and stated that the SAG recommendations agreed to by the AHB TMG would be easy to incorporate into a final literature review that could be released by the end of October. The Chair also thanked Mr O'Brien for the role he has played in the AHB T2M and wished him well for future projects.

The Chair requested that if Members of the SAG had any further comments, then these should be sent to PHA ASAP. The Chair closed the meeting.

AHB SAG Minutes

Meeting Eight of the Asian Honey Bee Scientific Advisory Group (AHB SAG)

Teleconference held on Tuesday 4th December 2012

Attendees: Rod Turner (Chair), Sam Malfroy (Secretariat), Glynn Maynard DAFF, Doug Somerville NSW DPI, Trevor Weatherhead AHBIC, Boris Baer UWA, Max Whitten FCAAA (the following attendees joined the meeting as observers), Mike Ashton DAFF Queensland, Russell Gilmour DAFF Queensland and Anna Koetz DAFF Queensland.

Apologies: Simon Barry CSIRO

Item 1: Welcome and introduction by the Chair

The Chair welcomed the Members of the Asian Honey Bee Scientific Advisory Group (AHB SAG) to the teleconference.

Mr Weatherhead requested that the Chair provide an explanation of the current status of Dr Denis Anderson and his involvement with the SAG. The Chair stated that PHA had received an email of resignation on the 6th of November citing personal and work related reasons, and because of this, Dr Anderson would not be involved in future SAG meetings.

Dr Somerville requested information from PHA and BQ on the status of the AHB website that was supposed to be delivered as part of the T2M. PHA stated that an AHB website had been set up at the start of the T2M, and continued to be updated with documents, reports and minutes from both the AHB SAG and the AHB TMG. Mr Gilmour stated that BQ were also in the process of developing an AHB specific website, which contained T2M documents, but had been delayed due to technical issues. Dr Somerville stated that more effort needed to be made for photo identification of AHB and European honey bees on the website to allow the general public to determine the difference between the two bees. Mr Gilmour discussed with the group that BQ were attempting to provide this and that more photos would be uploaded by the 14th of December 2012.

Item 2: Industry project update

The Chair tabled the industry project update from RIRDC for the Committees consideration. The SAG endorsed the update and had no comments.

Item 3: Remote nest treatment update

The Chair tabled the response letter from the APVMA regarding the possible registration of a Fipronil based product for beekeepers to use after the AHB T2M for AHB control.

The Chair stated some of the key concerns contained in the letter by APVMA included:

- Lack of efficacy data
- The method had not proven to be successful
- Lack of environmental data (indirect effects on wildlife and residues in dead colony wax and honey)

- Concerns regarding how this could be incorporated into apiary practices as well as avoiding exposure to European honey bees, commercial honey production and the food system.

In summary, the Chair stated that the APVMA would require comprehensive data packages and environmental packages which would come at a great cost to the Honey bee industry if they were to pursue this avenue. For these reasons, the Chair proposed that the Fipronil remote nest treatment trials be delayed until further notice, while BQ continue to work on other areas in the AHB T2M.

Mr Weatherhead and Dr Somerville rejected this conclusion and stated the trials should continue for the remainder of the 6 months of the AHB T2M with the view to achieving enough data to submit an application to the APVMA.

Dr Maynard discussed with the SAG that this extra data would most likely not assist any future registration, given that the application would need the support of a company that could not only submit the registration proposal, but monitor its use in the industry. Many businesses that are involved in the use of this chemical, such as BASF, would not be involved in this application considering the negative publicity that surrounds chemicals and major honey bee colony loss that is being experienced around the world. The Chair agreed with this statement and discussed that this would also go against many major companies International Stewardship arrangements.

Mr Weatherhead and Dr Somerville discussed that this was thought to originally be the case with the in hive Small hive beetle trap (Apithor - Fipronil) and that this had now become a major product for industry, with company backing. However, Dr Maynard stated that this trap targeted Small hive beetle, not honey bees, and hence there was a significant difference.

It was discussed amongst the SAG that even with company backing, the method, in the trials conducted to this date, had not proven to be as effective as first thought and that more trials may not resolve this issue. Dr Whitten stated that after listening to the advice, it would be beneficial to put on hold the Fipronil trials until a more effective and selective bait station is designed and trialled.

The Chair proposed that discussion move onto considering the BQ proposal before further considering the Fipronil issue.

Item 4: BQ Science projects and proposed program alternatives

Dr Koetz outlined the proposed changes to the AHB T2M which are contained in Attachment A.

Mr Ashton discussed with the SAG that the original work plan agreed upon at the start of the AHB T2M had required significantly more time and resources being invested to complete assigned projects than was initially envisaged, especially the Fipronil trials. As a result, some of the science project milestones had not yet been achieved. Mr Ashton

stated that he believed the proposed changes would provide a more complete and comprehensive outcome for this science projects and ultimately deliver greater benefits to the honey bee industry.

Mr Malfroy added that the work conducted in the proposed program amendments, as outlined in Attachment A, would also be of benefit to the National Bee Pest Surveillance Program.

The Chair proposed that the SAG endorse the recommended changes to the AHB T2M, and that Fipronil trials are revisited in March 2013 after more work is completed on other areas of the AHB T2M, specifically the development of a species specific attractant and trap design. Although there was not unanimous support, the majority of the SAG endorsed this proposal and recommended this to the TMG for approval.

The Draft spatial analysis was tabled by the Chair for the SAG's consideration. Mr Weatherhead stated that he was unhappy that this document had only been sent out one day prior to the meeting for analysis and that he did not recognise the usefulness of the spatial analysis to the T2M in the long term. The Chair stated that these documents are important for major trading partners to demonstrate the rate of spread and distribution of the AHB. The Chair discussed with the SAG that only a short time had been allowed for comment, and suggested that any comments on the spatial analysis be sent through to PHA for consideration by the 19th of December. The SAG agreed to this recommendation.

Item 5: Summary and close of meeting

The Chair thanked the SAG and BQ for attending the teleconference. The Chair proposed that the next SAG meeting be held in February/March 2013.

The Chair requested that if Members of the SAG had any further comments, then these should be sent to PHA ASAP. The Chair closed the meeting.

Queensland Government

Asian honey bee Transition to Management Science projects

Progress update November 2012

Department of **Agriculture, Fisheries and Forestry**

This publication has been compiled Dr. Anna Koetz of the Asian honey bee Transition to Management program, Department of Agriculture, fisheries and Forestry.

Summary

The AHB Science team is late in delivering several T2M deliverables. This is due to several factors:

- No Senior Scientist was employed until April 2012
- High priority was put on the remote treatment trials as requested by SAG. These trials were more time consuming than expected due to several reasons, including, for example, the difficulty of attracting bees to the syrup stations (as outlined in the Remote Treatment Update).
- The literature review was also very time consuming as it involved reading, and summarising over 300 references to ensure as much information as possible could be reviewed.
- Difficulties in keeping boxed AHB hives slowed down some trials

All projects are well under way, but we wish to review priorities and propose new timelines and due dates for the following projects:

(1) Validate efficacy of detection and destruction methods

(2) Investigate alternative control techniques and attractants

(3) Spatial analysis

T2M Science goals to be reviewed

The following two projects have milestones and targets that overlap. These are being discussed in conjunction with each other for ease of reading:

AG2 – Developing and making available a suite of control measures for AHB

AG2 Milestone 2 – due 1/2/12 (no payment)

Validate efficacy of detection and destruction techniques available for use by commercial pest controllers and for the purposes of integrated control strategies for industry

AG2 Milestone 3 – due 15/6/12 ($175,000)

Investigate and report on alternative control measures or attractants for AHB including, but not limited to, the options provided in the AHB T2M Plan

Investigate and report optimal design of bait stations for attracting AHB

AG5 – Optimising early detection of new incursion of AHB

AG5 Milestone 2 – due 1/2/12 (no payment)

Report on assessment of optimal surveillance strategies for detection of AHB

AG5 Milestone 3 – due 15/6/12 ($100,000)

Report on assessment of optimal surveillance strategies and risk based surveillance for detection of AHB, including efficacy of detection methods

Progress, issues & proposed timeline

Items in red indicate deliverables that are overdue or falling behind.

T2M Deliverable	Progress	Issues	Plan
AG2 B(i) Validate efficacy of detection and destruction methods AG5 A Conduct differential sensitivity testing to determine the comparative effectiveness of all available detection methods e.g. sentinel hive strategy vs. strategic sampling of surveillance traps (due 31/12/12) AG5 B(ii) Determine efficacy of surveillance strategies and techniques to determine likely detection rate around ports in the context of established AHB populations (due 31/12/12) AG5 B(iii) Develop an appropriate scientific methodology for floral sweep netting in the outer areas of the containment area to gain greater confidence of absence or presence of AHB	<u>Efficacy of detection:</u> • Efficacy of AHB traps – report submitted • Efficacy of public reports, bee lining and bee eater pellet surveillance – data sourced from BioSIRT, and spreadsheet available for analysis • Efficacy of floral observations & targeted floral sweeping – Experimental design for trials developed by Senior Scientist – Monthly trials underway (two rounds completed) <u>Efficacy of destruction</u> • Different destruction methods documented • Survey for consultation with field staff and pest controllers using different methods compiled	No Senior Scientist was available until April 2012. Following employment of the Senior Scientist, fipronil trials and literature review were high priority. This resulted in a substantial delay in delivering a report for AG2 B(i). The experimental design for the <u>detection efficacy</u> trials involves monthly rounds of trials, wherein two different detection methods and a number of different traps are trialled. Three to four trials/replications are essential to get an idea of variability in the data. However, to account for any seasonal variation in the resulting data, the trials should be continued for as long as possible, i.e. March 2013. <u>Destruction efficacy</u> will be based on product information and consultation with AHB field staff and pest controllers. Surveys are being conducted. Results need to be summarised and reported. Remote poisoning efficacy was previously reported.	PREFERRED • Analyse historical data (public reports, beelining and bee eater pellet surveillance), interpret and write report by March 2013 • With the help of CE, conduct, analyse and report on destruction efficacy by March 2013 • Continue the field trials until March 2013; analyse data and deliver report by June 2013 ALTERNATIVE • Complete two more rounds of field trials (Nov & Dec); analyse data and summarise. • Other analyses as above, deliver report by March 2013.

AG2 B(iii) Determine effectiveness of bait stations, their design and attractant effectiveness. • Document bait station use and design (30/03/12) • Validate effectiveness (31/12/12)	• Bait station use and design has been documented. • AHB trap effectiveness was analysed and reported on. • Dr. David Guez assisting Science team with ideas for improved bait station designs & attractants	No issues.	PREFERRED • Progress report by December 2012 • Continue trials until March 2013 • Final report by June 2013. ALTERNATIVE Do not conduct any further trials; final report by March 2013.
AG2 C Investigate alternative control techniques and attractants	<u>Alternative control techniques</u> • Trials conducted by JCU students & Snr Scientist/ Scientist to optimise/find design & attractant: – Different scents (trial in progress) – Different sugar concentrations (trial to be completed) – Different bait station colours (field trial finished) – Different bait station designs (trial in progress) – Different trap attractants & designs (trial in progress)	Bees had to be moved from our premises as they were interfering with AQIS' surveillance trial and were in close proximity to a transport company. Hive boxes are now a 15-minute drive from the offices, i.e. time to conduct trials will increase. We had a period of 2 weeks in September when our hives either absconded or died. As we are still acquiring knowledge of keeping Asian honey bees, losses do occur, slowing down the trials. One of the three JCU students dropped out of the specific subject that the trials were done for – her trial (on scent preferences) needs to be concluded.	PREFERRED • Progress report by December 2012 • Continue all trials until March 2013 • Final report by June 2013 ALTERNATIVE • Finish all trials by December 2012 • Final report by March 2012

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AG2 C(iv) Research into pheromone use to attract and/or detect AHB in order to increase trap sensitivity	• Assisting Dr. David Guez with his RIRDC-funded project (exchange of ideas; Snr Scientist caring for orchids; sourcing and supervising two Masters students) • David assisting Science team with ideas for improved bait station designs & attractants • *Cerana* queen pheromone ordered and received from Mike Lacey (CSIRO)	Dr. David Guez's trial will be conducted after June 2013, so results will not be available for the T2M final report. Trials conducted by AHB Science team are done in conjunction with project C (see above)	Continued collaboration with Dr. David Guez until June 2013. Timeline for design and attractant trials – see Project C above

The following two projects have milestones and targets that overlap. These are being discussed in conjunction with each other for ease of reading:

AG3 – Limiting impact on honey production

Milestone 2 – due 1/2/12 (no payment)

Literature review and report from apiarists on the impact of AHB on commercial honey production within AHB infested areas

Report on behaviour of AHB compared to European honey bee to identify opportunities for control

Milestone 4 – due 31/12/12 ($87,500)

Report on modelling of AHB population dynamics and drivers of spread

Report on PCR tools for detection of AHB

Report on the outcomes of Project 2 for field application for industry

QG2 – Improving operational efficiency and effectiveness

QG2 E

Undertake technical analysis of all nests and honeycomb to guide the spread and spatial analysis

Progress, issues & proposed timeline

Items in red indicate deliverables that are overdue or falling behind.

T2M Deliverable	Progress	Issues	Plan
AG3 A(i) • Literature review (incl. gaps in knowledge and AHB/EHB comparison) (due 31/03/12) • Engagement with apiarists in the Cairns area who have had experience with both honey bees - also A(iii) (due 30/06/12)	• Literature review completed, awaiting further comments before approved by TMG • Noted down AHB/EHB interactions as observed by local beekeepers • Survey written; needs to be conducted, results analysed and summarised (Science & CE collaboration)	<u>Literature review</u> Awaiting further comments before final edits. <u>Apiarists</u> Very few observations by beekeepers (two reported interactions) Previous survey was of low quality with very low participation, and was not analysed/summarised. New survey is essential.	<u>Literature review</u> Comments by industry by 9th November, changes assessed & incorporated and review sent to TMG members by 23rd November. <u>Apiarists</u> Survey to be distributed to local beekeepers by December 2012; analysed and report by March 2013
AG3 C & QG2 C-D • Model the population dynamics and drivers of spread as they impact on the management of EHB hives (AG3 C) • Undertake spatial analysis of current AHB infestation to guide to future surveillance activities (QG2 C) • Undertake spread	<u>Spatial analysis:</u> • Before leaving, mapping officer compiled all necessary data and created required maps. • Data analysis is underway. <u>Population spread modelling</u> • Before leaving, mapping officer provided data to BQ Intell Unit and created maps from preliminary modelling results. • Awaiting further results to improve first, preliminary model.	These projects have been summarised into two parts that will be combined into one report: 1. Model the population dynamics and drivers of spread and 2. Undertake spatial analysis of current AHB infestation Spatial analysis well underway and on track. Spread modelling has been hampered due to changes in the AHB team and Biosecurity Queensland. The computer model was provided to the Snr Scientist who is unlikely to have time, unless this is high priority and other projects will be put on hold.	• Continue spatial analysis, report by December 2012. • Use available preliminary modelling data and maps (albeit very rudimentary) and report on by December 2012. Note: due to general lack of data on *Apis cerana* Java genotype, and difficulty determining whether *A. cerana* nests

analysis of current AHB infestation to guide future management strategies (QG2 D)			inside rainforest, outcomes of the spatial model will be unreliable, and any further modelling/analysis will not improve the spatial model.
QG2 E • Undertake technical analysis of all nests and honeycomb to guide the spread and spatial analysis	Technical analysis prior to IP500 needs to be sourced. Analysis post IP500 is available for all extracted nests and collected swarms.	Prior to IP500: Data is not in analysable format (papercopy only or inappropriate file format) and needs to be sourced and entered.	• Continue to analyse all extractable nests and swarms until March 2013; • Report by June 2013

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AHB SAG Minutes

Meeting Nine of the Asian Honey Bee Scientific Advisory Group (AHB SAG)

Teleconference held on Tuesday 27th May 2013

Attendees: Rod Turner (Chair), Sam Malfroy (Secretariat), Glynn Maynard DAFF, Simon Barry CSIRO, Trevor Weatherhead AHBIC, Max Whitten FCAAA (the following attendees joined the meeting as observers), Mike Ashton DAFF Queensland, Russell Gilmour DAFF Queensland, Anna Koetz DAFF Queensland and Melanie Commerford DAFF Queensland.

Apologies: Doug Somerville NSW DPI and Boris Baer UWA

Item 1: Welcome and introduction by the Chair

The Chair welcomed the Members of the Asian Honey Bee Scientific Advisory Group (AHB SAG) to the teleconference.

The Chair tabled the apologies of both Dr Somerville and Dr Baer, and stated that although they could not join the teleconference, they had both expressed an interest in commenting on the variety of reports which had been tabled by DAFF Queensland.

Item 2: Industry project update

Mr Malfroy tabled the industry project update from RIRDC for the SAG's consideration. This summary has been attached as Attachment A.

The Committee discussed the preliminary results from the attractant and bait design project. Dr Maynard suggested that the project seemed to be deviating from its original intention of testing the semio-chemical response from the attractiveness of *Cymbidium floribundum* and AHB attractive plants in the Cairns region. Dr Whitten stated that he had held discussions with the lead researcher, Dr Guez, and this deviation in the project had been because the arrival of the synthesised chemical of this orchid from Russia and the USA had been delayed considerably. The committee agreed that although this had been delayed, the current research was worthwhile to determine the effectiveness in attracting AHB to different concentrations of glucose, sucrose and fructose syrup in a variety of bait stations.

The pest and disease analysis of *Apis cerana* and *Apis mellifera* populations conducted by CSIRO was also discussed. Mr Malfroy stated that the final report of this project will be handed in to RIRDC by the end of June, and that once this had occurred, a final version would be distributed to the SAG and DAFF Queensland.

The SAG endorsed the update and had no further comments.

Item 3: AHB T2M – Science Project Update

Dr Koetz and Mr Gilmour tabled and discussed the below DRAFT reports with the SAG:

- Detection of Asian honey bee (*Apis cerana*) in Cairns, Australia
- Destruction of Asian honey bee (*Apis cerana*) in Cairns, Australia
- Ecology and Behaviour of Asian honey bee (*Apis cerana*) in Cairns, Australia
- Optimising AHB bait/feeding station design and attractants

These DRAFT reports were discussed by the SAG, with some suggestions and changes being put forward by some committee members. The Chair requested that any comments or changes should be forwarded to PHA by the 7th of June for inclusion in the Final versions. The Chair added that the comment period for Dr Somerville and Dr Baer would be extended till the 14th of June.

Dr Koetz and Mr Gilmour tabled the below Final reports:

- North Queensland apiarist survey report (Attachment B)
- AHB remote nest treatment (Attachment C)

The SAG endorsed these Final reports.

Mr Gilmour also stated that an Asian honey bee manual, which focused on identification, detection and destruction, was nearing the printing stage. The Chair questioned why this manual had not been seen by the SAG. Mr Gilmour stated that DAFF, NAQS and AHBIC had all provided comment on the manual. The Chair stated that PHA, as administrator the AHB T2M, would also like to provide comments on this manual, considering the manual's end use for other important programs such as the National Bee Pest Surveillance Program. It was agreed that PHA would provide comments on this manual by the 31st of May.

Item 4: Future R&D Recommendations from the AHB SAG

The Chair outlined to the SAG that the AHB T2M would be finalised on 30th June 2013, and because of this, it was important to capture the thoughts of SAG members in proposing research areas where industry R&D should be focused on in the future.

Mr Whitten discussed that it would be critical to go back to the Full Research Proposals submitted with RIRDC to look at what is expected to be delivered as part of the Industry funded projects, considering they will continue for another 1-2 years after the end date of 30th June 2013. Mr Malfroy agreed and stated that these R&D recommendations would instead focus on shortcomings experienced in the AHB T2M, or other areas that would be of interest and be of benefit, such as sex allele research, or hive biology and behaviour research. Mr Weatherhead added that remote nest treatments should also be included in this statement.

The Chair stated that PHA would prepare a draft Future R&D Recommendations report and would distribute to the SAG in the near future for comment.

Item 5: Summary and close of meeting

The Chair stated that if required, another SAG meeting would be held in late June to discuss any final scientific reports produced as part of the program. It was proposed that this teleconference be held before the final AHB TMG meeting which is scheduled for the 25th of June.

The Chair reiterated that if Members of the SAG had any further comments on the reports provided by DAFF Queensland that these would need to be provided to PHA by the 7th of June. An extension would be provided to Dr Somerville and Dr Baer until the 14th of June to provide any comments. It was also agreed that PHA would distribute a draft R&D Future Recommendations in the near future for the SAG to provide comments.

The Chair thanked the SAG and DAFF Queensland for attending the teleconference and closed the meeting.

Progress Report on the Industry-funded, RIRDC-managed, Asian Honey Bee – Transition to Management projects as at 20 May 2013

Please Note:
Total funding for the below projects equals $377,000 of the $400,000 honey bee industry (AHBIC and FCAAA) contribution to the AHB T2M. AHBIC is currently seeking a RTO to prepare a quote for a training course for beekeepers in the Cairns region, to spend the remaining $23,000 in accordance with objectives of the AHB T2M.

Organisation – The University of Newcastle
Principal Researcher – David Guez
Project Title – Develop an attractant specific to *A. cerana* Java strain
Timeframe – (31/07/2012 – 1/12/2014)
Industry Funding – $131, 000
Project Summary – This project will determine whether or not it is possible to improve the spontaneous visit rate of feeding stations by giving them flower like characteristics and to use this new feeding system to attempt exclusion of *Apis mellifera* and native bees. The researchers will determine if the use of odorants from Lychee, Mad hatter and Coral Vine can improve spontaneous visitation and recruitment to the bait station. The researchers will also determine the attractiveness of Cymbidium species and to see whether the chemical composition of the semio-chemical produced exclusively attracts *Apis cerana*.

Research in Progress report (at 1 May 2013)
As indicated in my previous report, two master students (Nicholas Wall and Dylan Stolzenhein) have been recruited to perform experiment pertaining to the project. Despite administrative delays the students have been trained in Newcastle prior to going to Cairn permanently to perform the experiments.
Student training:
Since I will only be able to make relatively brief trip to Cairn the rational behind training the student in Newcastle is two- fold:

- Insure that the student can confidently and safely manipulate bees.
- Insure that the students have acquired basic skill necessary to the conduct of the experiments (e.g. experimental rigor, and consistency).

Skill training:
The students have been trained to perform both free flying experiment and Proboscis extension reflex. Although, it is anticipated that most if not all experiment performed in Cairn will be free flying experiment, training in PER experiment (Sugar threshold determination and Olfactory conditioning experiment) were also perform because the allowed me to evaluate their capacity to manipulate in such manner that their results were reproducible (independently to weather consideration).
I needed also the student to learn the importance of good experimental record, something that I believe can only be achieved by facing your own error and avoiding them in the future. I believe that was best achieved on experimental work that had no bearing on the studies that need to be performed for this grant. It also allowed me to provide for a progressive learning curve in the difficulty of the experiment to be performed.

Current short term Experimental plan:
The first experiment to be conducted in the next few weeks is to address the issue of sucrose concentration preference. In other words which sucrose concentration induce the best (in term of magnitude and rate) recruitment possible.
The second experiment will address colour and shape preference to enhance spontaneous feeder visitation. For this experiment bees will be trained to a scented feeder (sucrose concentration to be determined from previous experiment). Once bee number on the feeder have stabilized, the feeder

will be removed and replaced by a choice array of artificial flowers differing only by their respective colour (all containing the same sucrose solution and carrying the same scent than the original feeder). Spontaneous landing on the various colour will be recorded. Shape preference will be evaluated in the same manner.

The result of these two experiments will then be used to test the relative attractiveness of different chemical and flower scent extract by measuring the rate recruitment on scented feeders. In parallel trapping experiment will evaluate the spontaneous attractiveness of each scent or chemical.

Brief protocol for experiment 1

Experiment 1: Sucrose content preference (The methods is the same for evaluating fructose or Glucose content)

1. Prepare identification tag for each feeder indicating scent, sugar content, date, location and experimental repetition (to be placed on the top of the feeder for easy photo identification).
2. Test solution: 20%, 30%, 40%, 50% and 60% (weight/weight) sucrose solution.
3. Scent: Lemon, Almond, Rose, peppermint, vanilla (Test solution and scent association will be counterbalanced. See table 1)

Dilution (w/w)	Repetition 1	Repetition 2	Repetition 3	Repetition 4	Repetition 5
20%	Lemon	Vanilla	Peppermint	Rose	Almond
30%	Almond	Lemon	Vanilla	Peppermint	Rose
40%	Rose	Almond	Lemon	Vanilla	Peppermint
50%	Peppermint	Rose	Almond	Lemon	Vanilla
60%	Vanilla	Peppermint	Rose	Almond	Lemon

Table 1: scent counterbalancing

Note: Each repetition will be performed at different site or if not practical with at least a week interval between each repetition if a site need to be reused.

4. Collect forager bee from flower in tubes (5 bees per tubes).
5. Place bee on feeders and release the bees while they are feeding.
6. Record bee population on each feeder every (5 min) by taking a photo (manual count to be done from photos)

Note: It may be interesting to run the same experiment using fructose solution since fructose solution do not seems to induce recruitment in Apis mellifera (to be tested concurrently).

Organisation – The University of Sydney

Principal Researcher – Ben Oldroyd

Project Title – Inter-specific matings between *A. cerana* and *A. mellifera*?

Timeframe – 2 (29/05/2012-15/05/2014)

Industry Funding – $56, 230

Project Summary: This project will quantify the threats to the Australian honey bee industry associated with interspecific matings by the following experiments: 1) In Cairns, the researchers will perform reciprocal artificial inseminations of *A. cerana* and *A. mellifera*. The researchers will study the eggs of the queens to determine if there is embryogenesis. The researchers will allow some brood to emerge in an incubator to quantify the proportion of offspring that are haploid males, inviable hybrids and thelytokous females. The researchers will use microsatellites to confirm the maternity and (lack of) paternity in the offspring. 2) In the Solomon Islands where there are extremely dense populations of the Java strain of *A. cerana*, the researchers will determine the drone flight time of the males of both species to see if there is overlap. If logistically feasible, the researchers will determine the location of DCAs of *A. mellifera* and *A cerana*. Finally, the researchers will examine the offspring of *A. mellifera* queens that we allow to naturally mate with *A. cerana* males.

Research in Progress report (at 1 May 2013)
20% of a sample of Mellifera queens from China had Cerana semen in their spermatheca. We are waiting on a shipment of A. mellifera queens mated in Cairns, which should arrive next week.
We artificially inseminated 2 Mellifera queens with Cerana sperm in Cairns. We found no evidence of thelytokous reproduction. We DID induce thelytoky in a control queen inseminated with saline. Thus it may be manipulation of the genital tract, rather than heterospecific matings that induces thelytoky. The finding that thelytoky can be induced by inseminating with saline is exciting, with potential applications in breeding and importation.
20 Cerana queens from DAFF collections did not show any heterospecific semen. Thus we suspect that Cerana males can mate with Mellifera queens but not vice versa.
We determined that there is overlap between the drone flight times of Cerana and Mellifera in Cairns. Drone trapping attempts suggested that the congregation areas overlap (i.e. we saw but did not trap).
Bruce White has been unable to contact any beekeeper in the Solomons. I feel that it would be better to devote the funds to working on the Cairns population rather than an attempt to set up work in the Solomons from scratch. [RIRDC has approved this approach.]

Organisation – AgEconPlus Pty Ltd
Principal Researcher – Michael Clarke
Project Title – A strategy to address concerns of countries that import Australian honeybees
Timeframe – (1/6/2012 – 28/09/2012)
Industry Funding – $30, 000

Completed
Final report provided to the AHB T2M Management Group on 4 October 2012.

Organisation – CSIRO
Principal Researcher – John Roberts
Project Title – Establishing the disease status of *A. cerana* Java strain in the Cairns region
Timeframe – (1/06/2012-4/06/2013)
Industry Funding – $109, 212
Project Summary – The objectives for this study are to establish the disease status of the Asian honeybee and the European honeybee in the Cairns region. With this information the researchers will aim to identify the possible transferability of pathogens from the Asian honeybee to the European honeybee in the Cairns region. Identification of honeybee pathogens will involve a two-pronged approach. One approach (1) will engage metagenomic sequencing while the other approach (2) will use standard laboratory procedures as described by Anderson (1990, J. Apic. Res. Vol 29: 53-59) and Chen (2004, J. Inv. Path. Vol 87: 84-93). Metagenomic sequencing of DNA and RNA from pooled samples of *A. cerana and A. mellifera* will be performed at the Biomedical Research Facility based at the Australian National University. Genomic sequence data will be analysed and compared with public sequence databases to assemble partial genomes and identify known and unknown pathogens. The second approach will use bioassays involving the injection of honeybee extracts into pupae and adults of both *A. cerana* and *A. mellifera* to propagate viruses. PCR and serology techniques will be used to identify known viruses. Injected bees that show signs of disease, but are negative in PCR and serology tests, will be further tested to isolate novel pathogens.

Annual Progress Report (as at October 2012)
Bee samples were collected from the Cairns region between 16-7-12 and 27-7-12. Several hundred adult bees were collected from 7 Apis cerana and 14 Apis mellifera colonies, and larvae were

collected from 3 and 5 of these colonies, respectively. An additional 10 adult A. cerana samples (10-20 bees each) were provided by DEEDI. Brood comb was inspected from 4 A. cerana and 14 A. mellifera colonies and samples taken of suspected diseased brood. Chalkbrood and possibly American foulbrood and sacbrood disease were detected in A. cerana colonies, while chalkbrood and possibly European foulbrood and sacbrood disease were detected in A. mellifera colonies. Small hive beetle was also found in A. mellifera colonies and from one dead A. cerana colony provided by a local beekeeper. In addition, adult A. cerana have been tested microscopically for Nosema, tracheal mites and malpighamoeba, with low levels of Nosema found in 4 A. cerana colonies.

Bioassays to propagate viruses in pupae were conducted in Cairns using adult and larval extracts from 3 A. cerana and 5 A. mellifera colonies. All injected pupae and extracts have been tested against available antisera, which detected Kashmir Bee Virus in A. cerana and A. mellifera samples and Black Queen Cell Virus in A. mellifera samples. Positive samples have been confirmed by diagnostic PCR.
Extracts of viral RNA from adult A. cerana and A. mellifera are currently being prepared for deep sequencing using Illumina sequencing technology.

Collection of bee samples from the Cairns region is completed, although it was hoped that more A. cerana colonies with brood would be available. Access to live brood was essential for the bioassays to propagate viruses and for brood comb inspections. Despite attempts to have several suitable A. cerana colonies identified by DEEDI prior to arriving in Cairns, only one colony was available. The other 6 colonies collected were found through public reports during our trip and only 3 colonies were suitable for a bioassay. However, the distribution of these samples across the Cairns restricted area will be suitable to meet the project objectives.

Bioassays and testing against antisera has been completed for all A. cerana and A. mellifera samples. Only few positive samples to common viruses (Kashmir Bee Virus and Black Queen Cell Virus) have been detected with available antisera despite signs of viral infection in many injected pupae. More sensitive testing of these samples through virus purification and deep sequencing is expected to reveal the presence of any undetected viruses.

The planned research was presented at the Queensland Beekeeper's Association at Bribie Island, QLD in June 2012.

Organisation: CSIRO
Principal Researcher – Simon Barry
Project Title – Risk assessment of ports for bee pests and pest bees
Timeframe – (15/06/2012-1/10/2013)
Industry Funding – $50, 600
Project Summary – This project will estimate the relative likelihood of establishment of pest bees and/or bee pests at Australian ports based on the best available information. The researchers will do this by combining likelihood of entry with likelihood of establishment. To estimate likelihood of entry, the researchers will analyse shipping records and combine this with available interception data. The researchers will develop a species distribution model for A*. mellifera* and *A. cerana* to underpin estimates of likelihood of establishment across Australia's ports. This project directly links with other research conducted on this topic, including the recently completed ABARES report 'A benefit-cost framework for responding to an incursion of Varroa destructor'.

Research in Progress report (at 1 May 2013)

The project is in full swing now after key staff have delivered on other contractual commitments.

Accessing data on port layout:

The desktop nature of the study precludes site visits to no more than a few Australian ports of first call, we have subscribed to the IHS Maritime Sea-Web Ports Online module to facilitate access to the physical layout (berthing locations etc.) of all the ports or interest.

Building species distribution models:

From each state and territory we have contacted both an industry and government stakeholder to start an exchange of information on the distribution of feral bees in their state as means of calibrating better species distribution models for A. mellifera and A. cerana. We have also organised spatial GIS layers to underpin this modelling.

Assessing risk models:

We have had discussions with AQIS staff to gather information factors relating to commodities, countries and ports that influence the risk of pest bees or bee pests successfully mounting an incursion.

Analysing shipping data:

We have undertaken preliminary analysis of the arrival pressure of shipping containers by port. We note that industry belief regarding is highly context specific, and this may not be reflected in broadscale analysis of ship movements. For example, cargo from NZ is a particular concern for an introduction of Varroa on A. mellifera, yet small scale supply vessels operating out of Northern Australia are considered a major risk for introduction of A. cerana and Varroa.

Department of Agriculture, Fisheries and Forestry

Asian honey bee Transition to Management Program

North Queensland apiarist survey report

This publication has been compiled by Brett Ross-Reid, Brenda Foley and Dr. Anna Koetz of Biosecurity Queensland, Department of Agriculture, Fisheries and Forestry.

Contents

Summary

Officers from the Asian honey bee Transition to Management (AHB T2M) Program have engaged the beekeeping industry through attendance at monthly local beekeeper club meetings, facilitating local industry liaison meetings, participation in teleconferences on both state and national levels, conducting AHB T2M workshops to distribute detection and destruction tools to beekeepers, and a joint and united approach during the far north Queensland show circuit in 2012.

An early survey of apiarists/beekeepers was attempted in January and February 2012. However, this survey suffered from poor question design and a lack of respondents. The extension of the AHB T2M Community Engagement team in 2012 enabled scientific research outcomes to be converted into tools for industry to use in the management of Asian honey bees (AHB). A new survey was developed and conducted during October and November 2012 to encourage beekeepers to provide feedback on whether the information and tools currently available were comprehensive and sufficient for their needs, or whether additional tools were needed to help them manage the impacts of AHB.

North Queensland beekeepers were given the opportunity to participate in the survey and suggest what they thought was important in the development of additional management tools. Comments received indicate the industry felt strongly that the development of a specific AHB trap is necessary to assist the industry to manage the pest bee. However, the majority of respondents gave no response when asked to suggest tools that could assist and support their industry in the management of AHB.

In general, it was found that beekeepers in north Queensland (Cairns and Townsville) are most concerned about a new incursion of AHB introducing Varroa mites or exotic bee diseases.

Introduction

Following the detection of the Asian honey bee (AHB; *Apis cerana* Java genotype) in Cairns, Queensland, in May 2007, the beekeeping industry raised concerns about the negative impact AHB may have on honey production. These concerns included possible competition for resources, robbing of European honey bee (EHB) hives and the introduction of unspecified bee pests and diseases.

An apiarist/beekeeper survey (herein referred to as 'survey') was conducted with north Queensland apiarists/beekeepers and responses were collected. The target audience ranged from beginner to professional beekeepers from within the Known Infested Area (KIA) (Appendix 1) and from Townsville, outside the KIA. The survey aimed to evaluate and assist the future delivery of several AHB T2M deliverables, particularly those that required stakeholder engagement and collaboration:

AG2Bi - Develop integrated control strategies for different industries to minimise impacts of AHB, including identifying any off-target impacts (especially the balance between AHB and commercial EHB in the same environment to minimise impact and honey production)

AG2Bii – Develop the timing of implementing these methods and strategies to maximise effectiveness of control methods

AG3Bi – Development of management strategies. Based on outcomes of project 2, develop and test management strategies targeted at limiting impact of AHB on honey production in areas where AHB are established

AG3D – Develop technology to assist industry to mitigate AHB impacts. Stakeholder and industry engagement to identify needs and priorities for technology development

AG3F – Develop approaches with the honey industry for adoption and implementation of management strategies

This survey was designed to determine what additional tools or information the honey bee industry requires to manage the impacts of AHB.

Survey methods

Development

The survey was designed to encourage succinct answers and was aimed at gathering information from beekeepers in the Cairns and Townsville regions (referred to as north Queensland) about their observed and perceived impacts of AHB. The survey clearly identified that it was developed by the Department of Agriculture Fisheries, and Forestry and was conducted as a paper-based questionnaire (refer Appendix 2).

Questions

The survey consisted of 16 questions, some of which were subdivided. Most questions were closed questions, where participants could only choose answers from the options available (e.g. “Yes/No” or “tick one box or multiple boxes”). Some closed (Yes/No) style questions also gave participants the option to specify “other” and provide relevant details. Closed questions were used to assist with analysis, so that each answer could be given a numerical value and be analysed or graphed. One question in the survey (Question 16) was an open style question, which gave participants the opportunity to respond with their own thoughts and opinions (refer Appendix 2).

Delivery

Cairns participants were given a number of opportunities to participate in the survey. The survey was made available at:

- a specially organised monthly meeting where AHB T2M officers presented Program outcomes, explained the purpose and importance of the survey, and distributed copies of the survey;
- monthly meetings following the specially organised meeting;
- the premises of the president of the Cairns Beekeeping Club.

Participants could either complete the survey *in situ* and place it in a labelled survey box, or they could take the survey away and return it to the president of the club or to the AHB T2M Program’s main office in Redden Street, Cairns. AHB T2M officers reminded club members about the return of survey forms on a number of occasions.

Townsville members were given the opportunity to complete the survey during a specially organised monthly meeting, held by the Townsville Beekeeping Club, at which AHB T2M Program officers attended and presented. At the end of the Townsville meeting, club members were asked if they could assist the AHB T2M Program by filling out the survey and placing it inside a labelled survey box.

Analysis

All closed style questions were analysed by calculating the percentage of respondents who answered ‘Yes’ or ‘No’ and results were displayed on a graph. The survey data was analysed as a whole for Questions 1 and 14 as well as comparisons made between Cairns and Townsville respondents. Questions 9, 10, and 11 were all analysed by counting the total number of responses against the number of participants surveyed. Question 16 of the survey was an open question and respondents’ suggestions were summarised in the survey results section of this report.

Survey results

Questions 1-3

86% of participants from both Cairns and Townsville correctly identified the picture of the Asian honey bee (Image A) when it was compared to the European honey bee. 14% of Cairns respondents incorrectly marked Image B as AHB, with 7% of incorrect answers from Townsville respondents. 7% of respondents from Townsville did not answer the question (Figure 1).

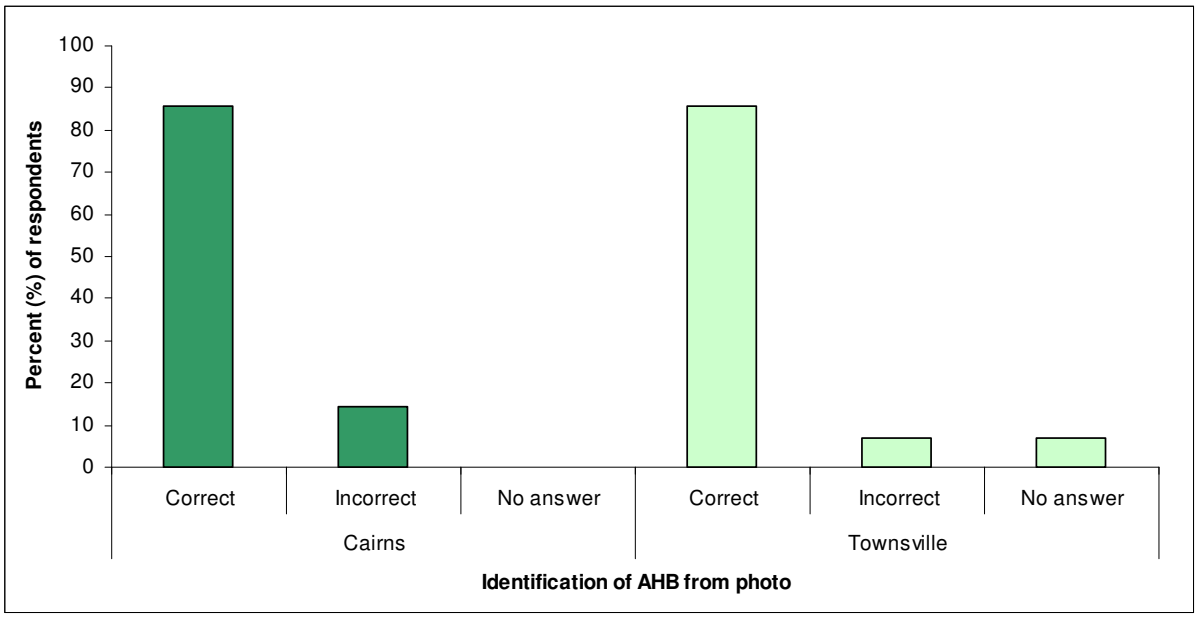


Figure 1: Comparison of correct identification of AHB from two photos showing AHB (photo A) and EHB (photo B). Percentage of respondents answering correctly, incorrectly and giving no answer shown for Cairns respondents (dark green) and Townsville respondents (light green).

Question 2 of the survey questioned participants' awareness that the current AHB Program is in a transition to management (T2M) phase. The results illustrate that both regions have knowledge of the Program's status. 86% of Cairns beekeepers answered 'yes' (indicating awareness of T2M) and the remainder of the respondents gave no response. Similarly, 93% of Townsville beekeepers answered 'yes' (indicating awareness of T2M) and 7% answering no. Overall 90% of respondents surveyed answered 'yes' (indicating awareness of T2M) with 5% answering 'no' or not responding (Figure 2).

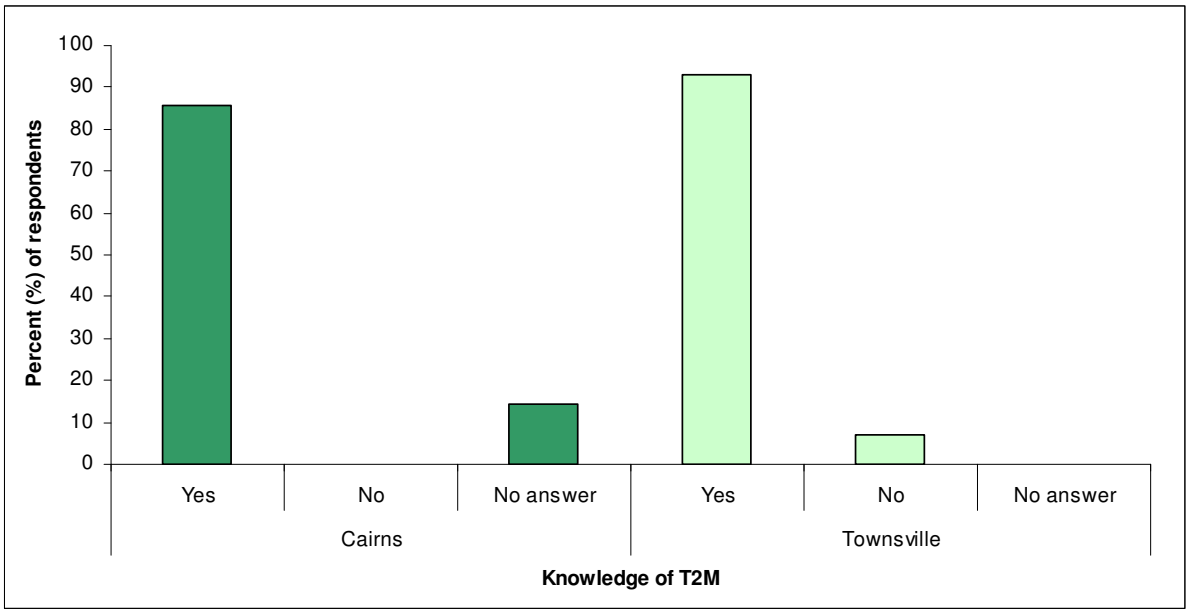


Figure 2: Knowledge of Program status – Percentage of respondents answering 'yes' (having knowledge of Program status), 'no' (not having knowledge of Program status) and giving no answer for Cairns respondents (dark green) and Townsville respondents (light green).

Question 3 of the survey asked participants if they had seen AHB in the 'wild' (i.e. in the environment). Results differed dramatically for this question as many beekeepers from Townsville have not travelled to and/or observed AHB in the KIA. 43% of Cairns respondents answered 'yes', compared to 7% from the Townsville respondents'. 57% of the Cairns respondents had not seen AHB in the environment, compared to 86% of the Townsville respondents. All respondents from Cairns answered Question 3, while 7% of the participants from Townsville gave no response (Figure 3).

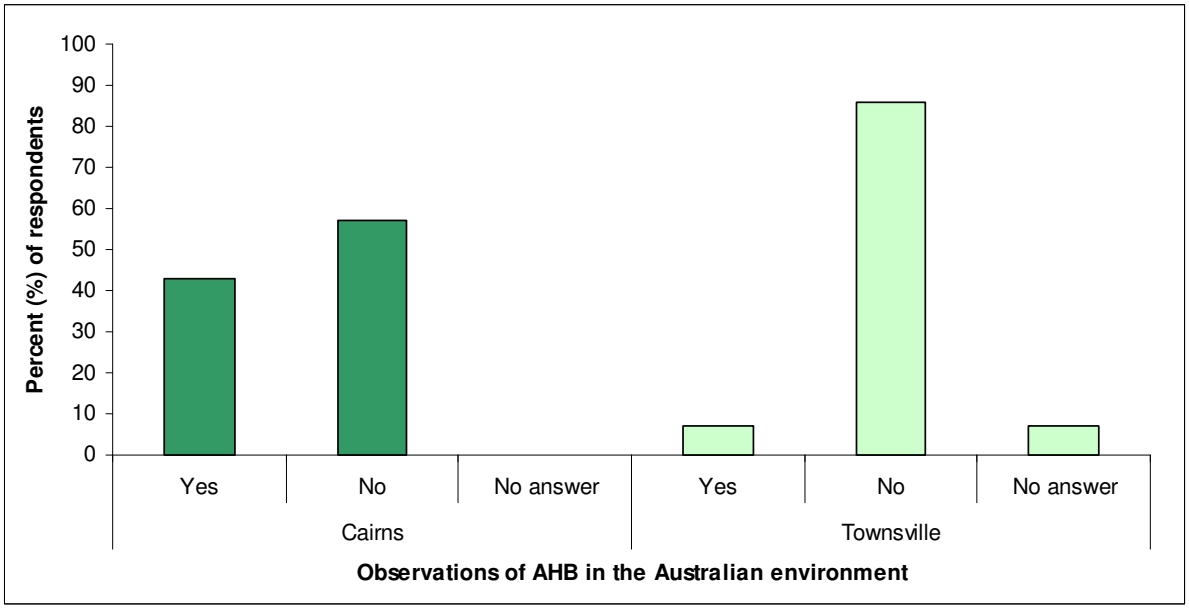


Figure 3: Observations of AHB in the Australian environment – Percentage of respondents answering 'yes' (having observed AHB in the environment), 'no' (not having observed AHB in the environment) and giving no answer for Cairns respondents (dark green) and Townsville respondents (light green).

Questions 4 - 7

Questions 4 to 7 relate to several different examples of AHB observations. In all of these questions, no Townsville respondents gave an answer of 'yes' for observing AHB around their hives, competing for food, or displaying aggressive behaviour between the two species. This is not surprising given AHB are yet to establish in the area.

As a result, the following graphs only display results from Cairns beekeepers' observations. Results from Question 4 showed that 14% of Cairns beekeepers had observed AHB in, on or around EHB hives. 43% of Cairns respondents answered 'no observations' and an equal percentage gave no response to this question (Figure 4). 64% of Townsville respondents answered 'no observations' of AHB near EHB hives, along with 36% giving no response.

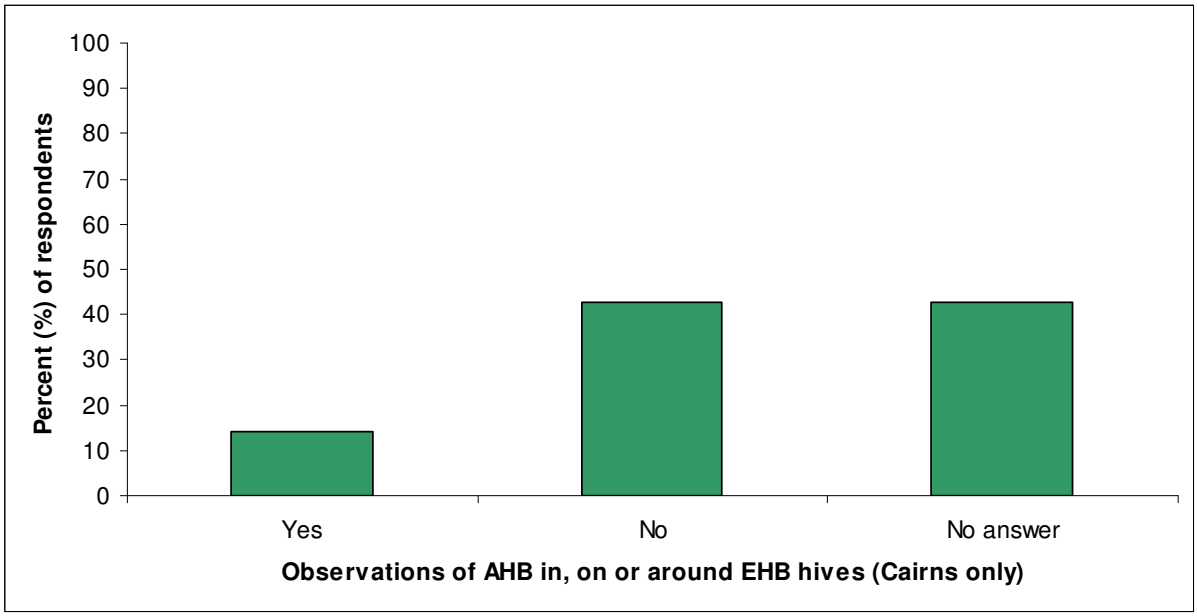


Figure 4: Hive observations from Cairns beekeepers – Percentage of respondents answering 'yes' (having observed AHB in, on or around EHB hives), 'no' (not having observed AHB in, on or around EHB hives) and giving no answer for Cairns respondents.

Question 5 of the survey asked whether competition for food had been observed between EHB and AHB. Results show that 29% of Cairns respondents had observed competition for a food source, whereas 29% had not observed competition for a food source. 43% of respondents from Cairns gave no response (Figure 5). 64% of Townsville respondents marked 'no observations' and the remaining 36% gave no response.

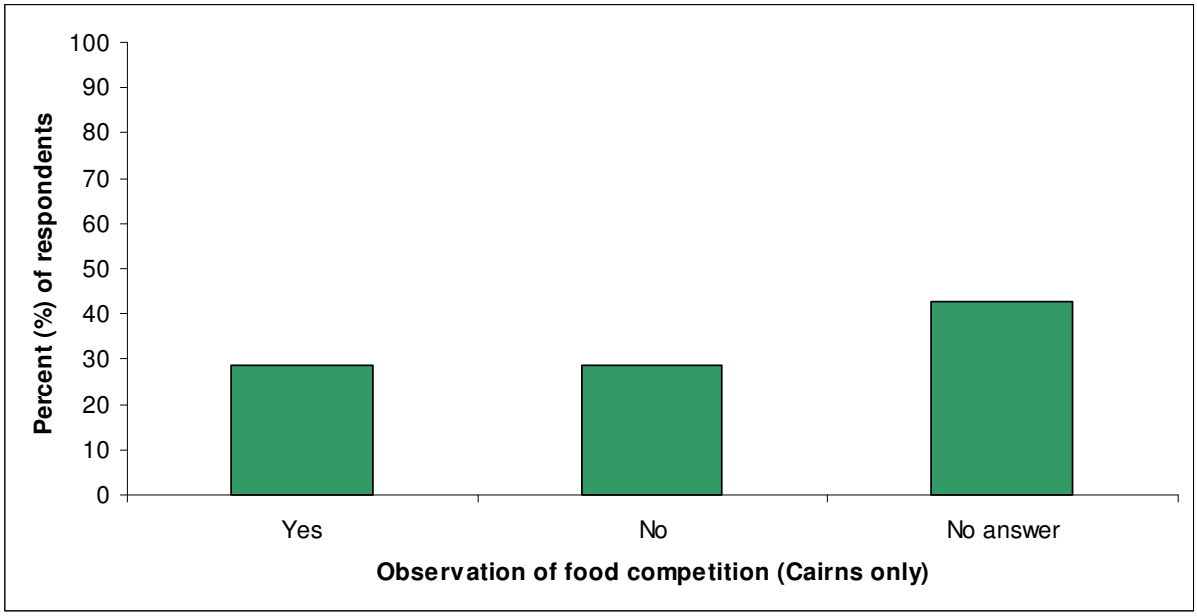


Figure 5: Food competition observations from Cairns Beekeepers between EHB and AHB – Percentage of respondents answering 'yes' (having observed food competition), 'no' (not having observed food competition) and giving no answer for Cairns respondents.

Questions 6 and 7 asked beekeepers if they had observed aggressive behaviour of AHB towards EHB (Question 6) and vice versa (Question 7). The results were the same for both questions. 14% of Cairns respondents observed aggressive behaviour in AHB towards EHB as well as in EHB towards AHB, whereas 43% had not observed any aggressive behaviour and the remaining 43% gave no response (Figure 6). 64% of the Townsville respondents marked 'no' and the remainder gave no response to Questions 6 and 7.

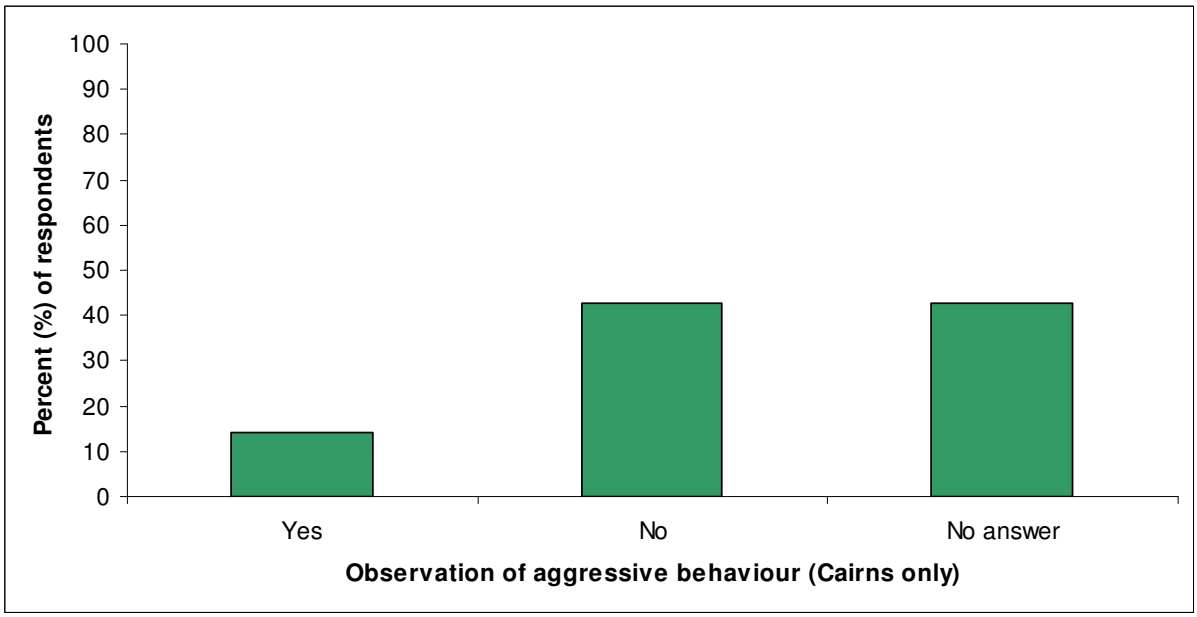


Figure 6: Observations of aggressive behaviour between AHB and EHB from Cairns Beekeepers – Percentage of respondents answering 'yes' (having observed aggressive behaviour), 'no' (not having observed aggressive behaviour) and giving no answer for Cairns respondents.

Questions 8 - 11

Question 8 asked beekeepers if they had observed AHB demonstrating any of the following behaviour: AHB robbing of EHB hives; AHB fighting EHB; AHB taking over EHB hives; and/or AHB entering or exiting EHB hives.

Most of the participants from both Cairns (86%) and Townsville (71%) gave no response to Question 8. However, 14% of Cairns respondents indicated that they had observed the above behaviours, and 29% of Townsville respondents answered 'no' (i.e. they had not observed any such behaviour).

Question 9 asked participants if they had seen any negative impacts of AHB on honey production or foraging behaviour of EHB. There were no observed impacts on honey production or foraging behaviour of EHB by any of the participants from the survey. Only four people out of 21 answered this question. However, these four respondents answered the question incorrectly by writing 'no' instead of ticking one or two of the two available boxes. Therefore, it can be concluded that none of the respondents had observed a negative impact on honey production or foraging behaviour of EHB.

Question 10 asked 'what impacts do you perceive the AHB will have?' Respondents indicated that they thought AHB could have an impact on the following: robbing EHB hives; fighting EHB; reduced honey production; taking over EHB hives; competition for native fauna and flora; and introducing new diseases. Of these options, introduction of new diseases was identified as the number one perceived impact to industry with 16 out of 21 respondents ticking this box.

Question 11 was similar to Question 10 in that respondents were given the opportunity to indicate what they believed to be the biggest perceived threat to the honey bee industry. Results indicated that respondents were more concerned about Varroa mites being introduced to Australia than they were about the current infestation of AHB in North Queensland (which have no Varroa mites). 18 of the 21 beekeepers from both Cairns and Townsville believe that new infestations of AHB carrying Varroa mites would be the honey industry's biggest threat. 15 beekeepers believed that EHB carrying Varroa mites into the country would be the biggest threat to their industry. Only three out 21 respondents believe that the current AHB incursion will be the biggest threat.

Questions 12a - d Tools for industry

12 out of 21 respondents answered 'yes' to Questions 12a and 12b, indicating that they felt they needed tools to manage the impacts of AHB. They indicated that the recently developed *Guideline for industry destroying swarms and nests of AHB* provides adequate information to manage some of these impacts. Only one person out of the 21 answered 'no' with the remainder of the respondents giving no response.

14 out of 21 respondents answered 'yes' to Question 12c, indicating that a video depicting AHB destruction techniques would be a helpful tool for industry. Only one person out of the 21 answered 'no' with the rest of the respondents giving no response. When respondents were asked to specify what additional tools they believe industry may require in relation to managing AHB, minimal responses were given. Three respondents answered "specific trap", "public need more awareness" and "recent information on spread impact".

Questions 13-14 How do beekeepers access information?

Questions 13 to 14 were included in the survey to establish by what means north Queensland beekeepers access information on the established population of AHB. These questions do not contribute to the AHB T2M deliverables stated in the introduction. However, the information collected was used to assist the Program with provision of further AHB information to regional beekeeper clubs in north Queensland. The results from Questions 14 show that overall, 57% of all respondents surveyed had sourced AHB information from the Biosecurity Queensland (BQ) website, with 33% sourcing information elsewhere and 10% giving no answer.

Questions 15a –g Awareness of the tools and information available

North Queensland beekeepers were asked to indicate their knowledge of AHB information and tools available to the industry, through the BQ website, by marking 'yes' to seven items (AHB factsheet, AHB destruction guideline for industry, KIA map, scientific reports, identification tools, images, online reporting tools). The results were then illustrated in two pie charts (Figure 7).

When asked if the Cairns respondents were aware that printable information such as the AHB factsheet, AHB destruction guideline for industry and the KIA map were available online, 57% answered 'yes' to the first two items and 71% answered 'yes' to the latter. However, Townsville respondents appeared to have greater awareness with 64% knowing of the factsheet and 71% answering 'yes' to the guideline and the map. Similarly, only 14% of the Cairns respondents compared to 50% of Townsville participants knew that they could report AHB sightings using an online reporting tool.

Regarding awareness that scientific reports completed by the Program were available online, Cairns respondents showed less knowledge than those in Townsville with 43% and 64%, respectively.

Again, Cairns respondents fell behind on the knowledge stakes regarding the improved online species identification tools and images of nests and swarms on the website with 28% and 29% answering 'yes', respectively. However, Townsville showed a far greater awareness in general with 64% answering yes to both questions (Figure 7).

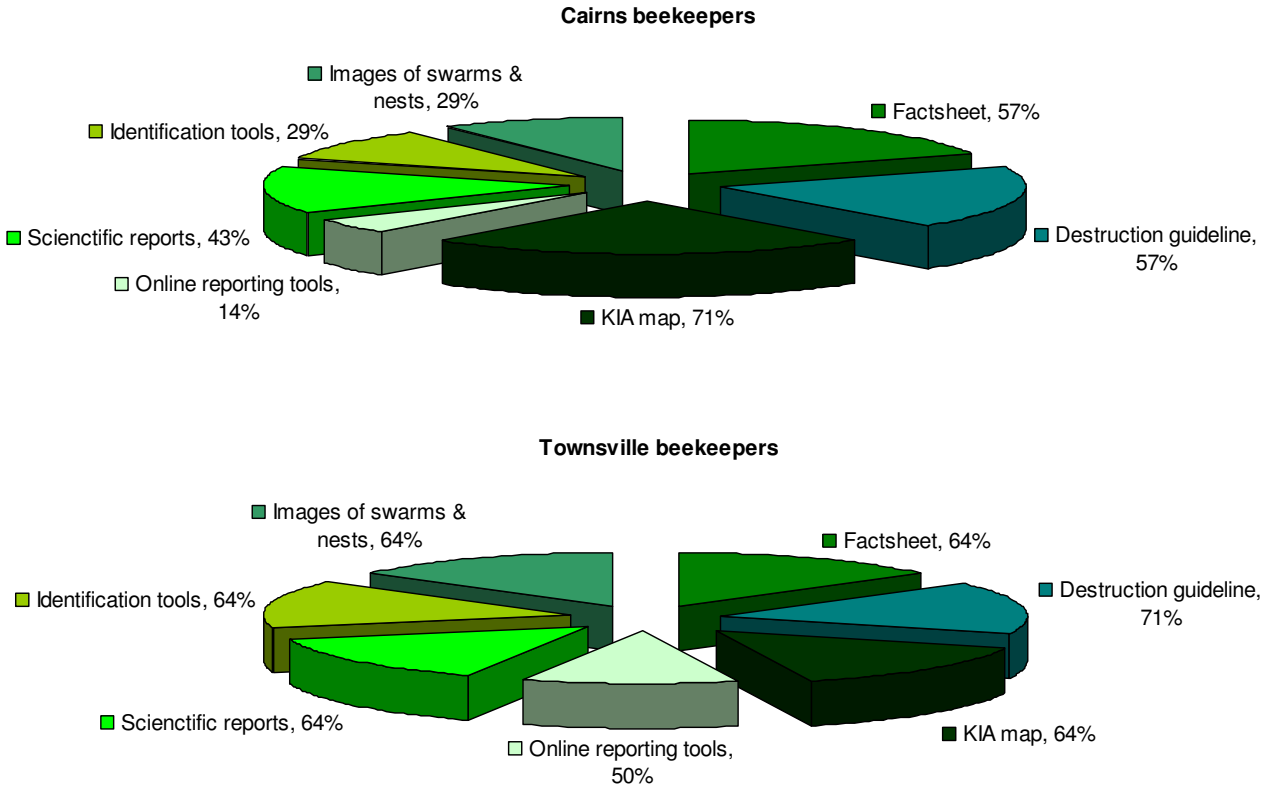


Figure 7: The percentage of participants that responded 'yes' to having knowledge of online information and tools, including AHB factsheets, AHB destruction guidelines for industry, KIA maps, scientific reports, identification tools, images, online reporting tools.

Questions 16 Industry suggestions

Question 16 asked participants for suggestions that could help with the current infestation. The majority of the Townsville participants (71%) answered this question, whereas only 28% of Cairns participants responded to this question. The following responses were provided:

"Interaction and cooperation with as many areas as possible"

"Keep it going"

"Destroy all AHB Hives"

"Be aware; keep watching - just the bleeding obvious"

"Wipe them out by whatever means"

"Haven't got a hive of EHB at the moment, just getting started"

"If you see a swarm of AHB, report to the president of Townsville beekeeper association"

“The same time spray with poison, kill them”

“Information given quickly to members”

“Education”

“Stay alert and keep records of sightings”

“Carry on, good work”

“Better public awareness”

Discussion

It is noteworthy that Cairns and Townsville beekeepers have reasonable identification skills regarding AHB and a good understanding that the current AHB Program is in a transitioning to management phase.

The new identification tools available on the BQ website (including comparative images of different bee species) will improve beekeepers', other stakeholders' and the community's ability to detect and report suspect AHB. Further, beekeepers from both regions have been made aware of the various tools that are available online to assist them in the detection and reporting of AHB should there be a new incursion or the pest bee spreads to their area.

Beekeepers from both regions appeared satisfied with the current management of the AHB infestation. Townsville, in general, appeared to have a greater understanding of the AHB T2M Program and what is available to their industry in regard to online tools for AHB management. This could be due to recent detections and publicity in the area and wanting to be proactive against any bee related threats to their region.

Cairns has a much greater understanding of AHB and the KIA, which is understandable considering it was Cairns where AHB were first detected and community engagement activities have been concentrated in this area since May 2007. Another key factor is the efforts of the AHB T2M Program though a range of activities including liaison with an AHB-specific industry committee, presence at regular monthly beekeeper club meetings, the legislative requirements regarding movement of bees, and beekeepers in the Cairns region being subjected to more intensive interactive displays at the 2012 far north Queensland show circuit.

Findings from the observational questions in the survey show that beekeepers still do have concerns regarding possible negative impacts from the pest bee but are generally more fearful of a new incursion bringing exotic mites and bee diseases into the country. A low number of respondents indicated that they had observed aggressive behaviour of AHB to EHB and vice versa. This may be explained by the fact that some beekeepers reported verbally (during monthly meetings) that they had observed AHB going into EHB hives, but EHB successfully defending their hive. This is consistent with research overseas that showed that fights between AHB and EHB were always won by the much larger and stronger of the two species: EHB (reviewed in Koetz, 2013).

It should also be noted that Cairns participants have been exposed to AHB for six years prior to the survey, but the bee is yet to establish in the Townsville region. Therefore, only Cairns beekeepers' responses regarding AHB behaviour are relevant here.

The majority of respondents who answered the questions regarding the need for tools to manage or minimise the impact of AHB found the newly developed *Guideline for industry destroying swarms and nests of AHB* to be adequate for their needs. Industry do encourage and support the idea of a video depicting AHB destruction techniques being developed and this is currently on track to be available on the BQ website this year (2013).

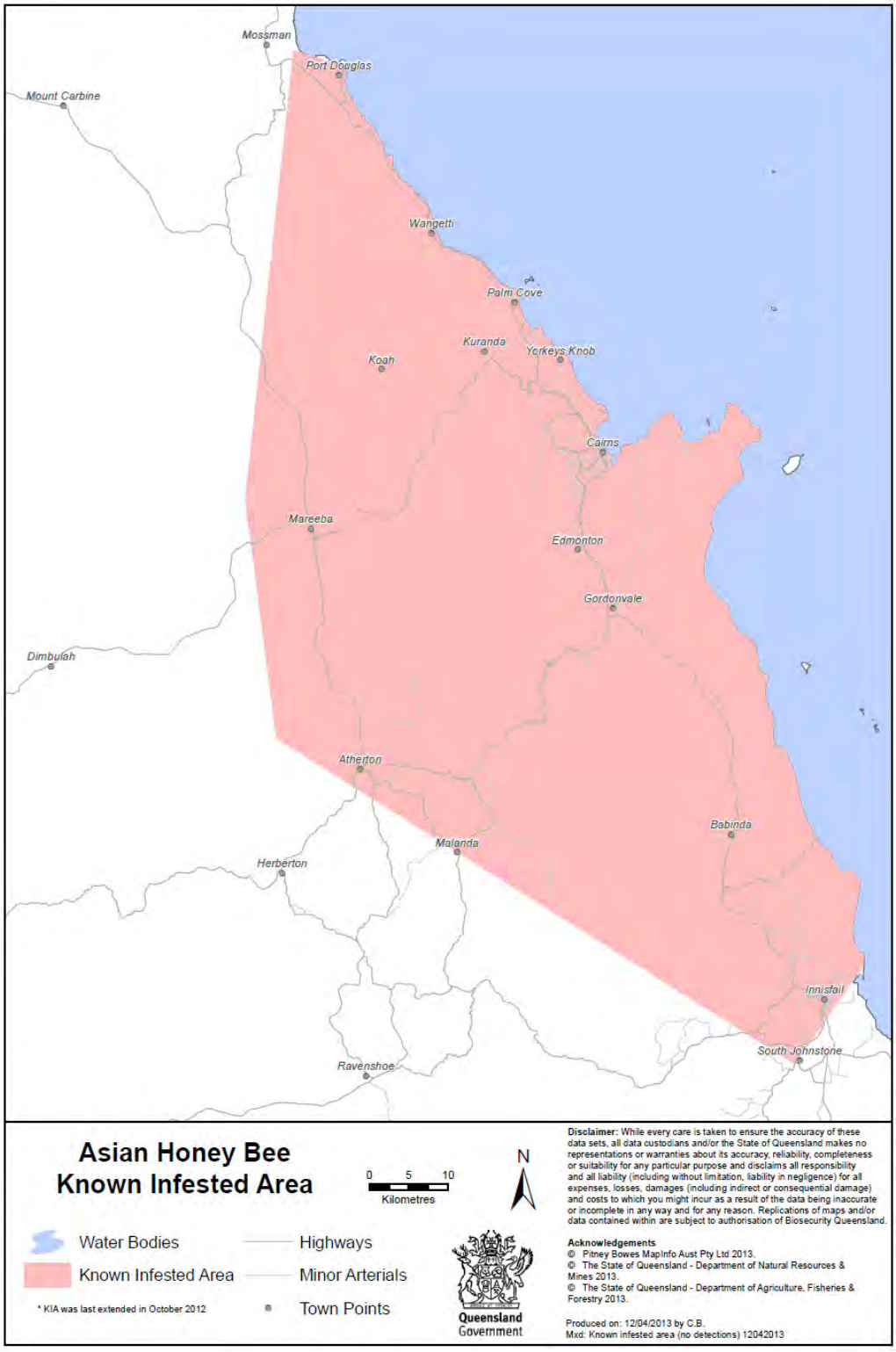
Beekeepers that responded to the survey did indicate their ongoing desire for development of an AHB specific trap to support their industry. However, the majority of respondents gave no response when asked to suggest additional tools that could assist and support their industry in the management of AHB.

As can be seen from the comments provided for Question 16, most respondents are happy with AHB T2M Program's efforts with comments including "Carry on the good work". No negative comments were recorded for this question.

Interestingly, Townsville beekeepers were more aware of where and what tools are available on the BQ website than those in the Cairns region. This may reflect their geographical proximity to the established population and their apprehensions regarding the pest bee and to what extent it might impact their industry. Their willingness to educate themselves may also be due to the low level of face-to-face contact the club has had with the AHB T2M Program. Finally, the style of the survey proved appropriate to the audience (closed or multiple choice questions) as most closed questions were rewarded with an answer, while very few participants took the opportunity to provide comments or more detail when given the opportunity.

In conclusion, the survey targeting north Queensland beekeepers and conducted by AHB T2M Program staff fulfilled its objective of gathering important information from beekeepers to assist with some of the deliverables of the AHB T2M Program. Ideally, the sample size of the survey would have been larger and more representative of the whole of the beekeeping community to give the findings of this report more substance. However, the information provided is an invaluable insight into what attitudes and knowledge beekeepers in both the Cairns and Townsville regions have regarding the AHB T2M Program.

Appendix 1 Asian honey bee Known Infested Area (KIA)



Appendix 2 Survey for apiarists/beekeepers

Department of Agriculture, Forestry and Fisheries

Apiarists/beekeeper Survey

Queensland Government

* Please circle your answer to the following questions

1. Which bee would you identify as the Asian honey bee?	A / B
2. Are you aware the AHB program is Transition to Management?	YES / NO
3. Have you seen AHB in Australia in the wild? (if no, go to Q10)	YES / NO
4. Have you seen AHB in/on/around your European hives?	YES / NO
5. Have you observed competition for nectar/pollen between European Honey bees (EHB) and AHB?	YES / NO
6. Have you observed aggressive behaviour from AHB to EHB?	YES / NO
7. Have you observed aggressive behaviour from EHB to AHB?	YES / NO

* Please tick one or multiple boxes for the following questions

8. Have you observed AHB;

Robbing EHB hives	☐	Taking over EHB hives	☐
Fighting EHB	☐	Entering or exiting EHB hives	☐

9. Have you seen any negative impact on;

Honey production	☐	Foraging behaviour of EHB	☐

Other (specify)_____

10. With the most recent information extended from the program, what impacts do you perceive the Asian honey bees will have;

Robbing EHB hives	☐	Taking over EHB hives	☐
Fighting EHB	☐	Bringing new diseases	☐
Reduced honey production	☐	Native fauna and flora	☐

Please turn over the page and continue

Thank you for filling out this survey.

For more information regarding Asian honey bees in Australia please visit www.biosecurity.qld.gov.au

Department of Agriculture, Forestry and Fisheries

Queensland Government

* Please tick one or multiple boxes for the following questions

11. What do you perceive as the biggest threat to your industry?

a) The current infestation of AHB	☐
b) A new infestation of AHB carrying varroa	☐
c) A new infestation of EHB carrying varroa	☐

Other (specify) _____

* Please circle your answer to the following questions

12. Do you think that:	
a) You need tools to manage, control or minimise the impact of AHB on the commercial honey industry?	YES / NO
b) The "Guideline for industry destroying swarms and nest of AHB" provides adequate information to destroy colonies?	YES / NO
c) A video depicting destruction techniques be helpful?	YES / NO
d) Are there additional tools the industry requires (specify)	
13. Do you attend local beekeeping meetings?	YES / NO
14. Do you access information about AHB from the Biosecurity Queensland website?	YES / NO
If no, where do you access information about AHB (specify)	
15. Did you know that the following tools are available on our website?	
a) An Asian honey bee Fact Sheet	YES / NO
b) A Guideline for industry destroying swarms and nests of AHB	YES / NO
c) A 'Known Infested Area' map	YES / NO
d) Access to completed AHB scientific reports	YES / NO
e) Online identification tools	YES / NO
f) An online reporting tool to help track the spread of the bee	YES / NO
g) Images of Asian honey bees/swarms and nests.	YES / NO

16. What suggestions can you make to help with managing the current infestation?

_____

_____

References

Koetz A.H., 2013. The Asian honey bee (*Apis cerana*) and its strains - with special focus on *Apis cerana* Java genotype - Literature Review. Asian honey bee Transition to Management Program, Department of Agriculture, Fisheries and Forestry (DAFF), Cairns

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Queensland Government

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

Asian honey bee Transition to Management Program

This publication has been compiled by Dr. Anna Koetz and Shirin Hyatt of the Asian honey bee Transition to Management program, Department of Agriculture, Fisheries and Forestry.

Contents

Summary

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Introduction

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

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

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

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

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

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

Methods

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

Prerequisites for treatments

Treatments commenced when:

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

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

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

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

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

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

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

Feeding station

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

Nest Entrance & Foraging activity

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

Fipronil treatment

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

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

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

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

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

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

Second treatments

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

Target number of bees

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

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

Data analysis

Nest size versus nest entrance activity

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

Treatment success

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

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

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

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

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

Treatment effectiveness/efficacy

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

Effects on off-target species

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

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

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

Results

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

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

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

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

Nest size

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

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

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

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

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

*A second treatment was conducted on these nests.

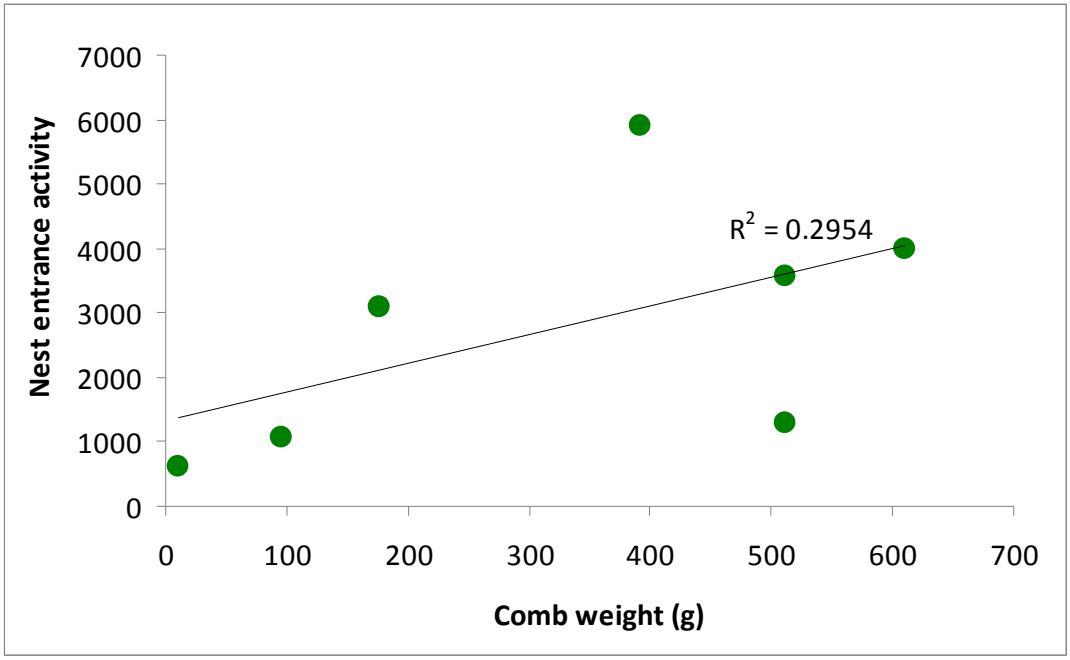

Figure 1: Nest entrance activity versus nest size (comb weight, g) for six remotely treated *A. cerana* nests. One nest was treated twice – it is represented twice in this graph. The second nest that was treated twice (see Table 1) had an unknown nest activity for its first treatment and is only represented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

Nest & feeding station suppression

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

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

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

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

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

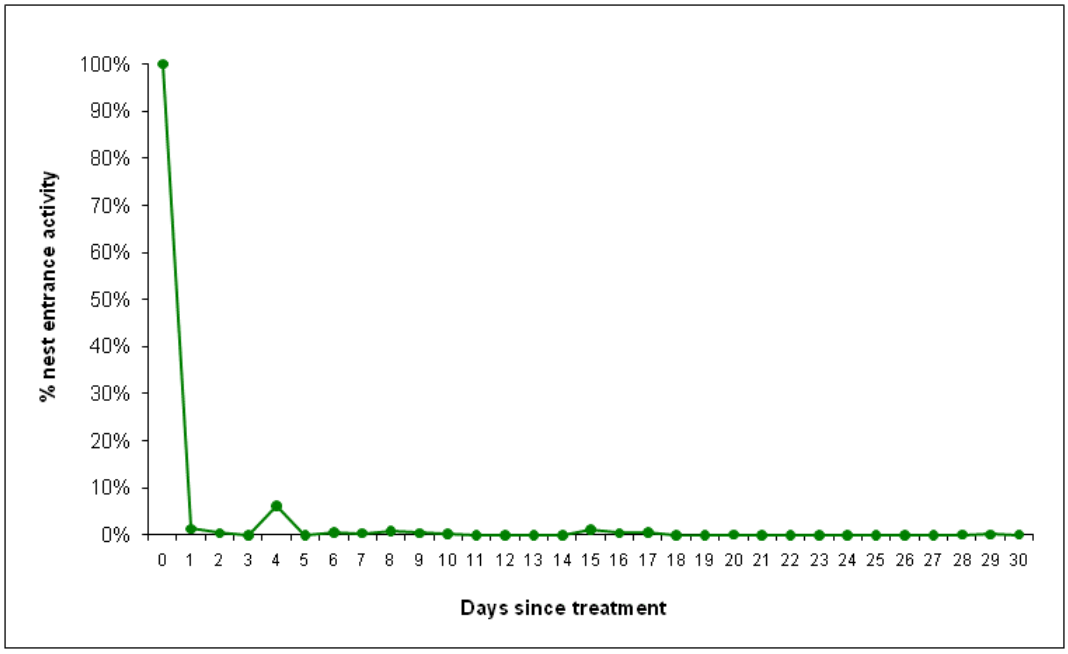

Figure 2: Average *Apis cerana* nest entrance activity of successfully treated nests (N = 11) in the 30 days following remote treatment using fipronil

Predicting treatment success

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

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

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

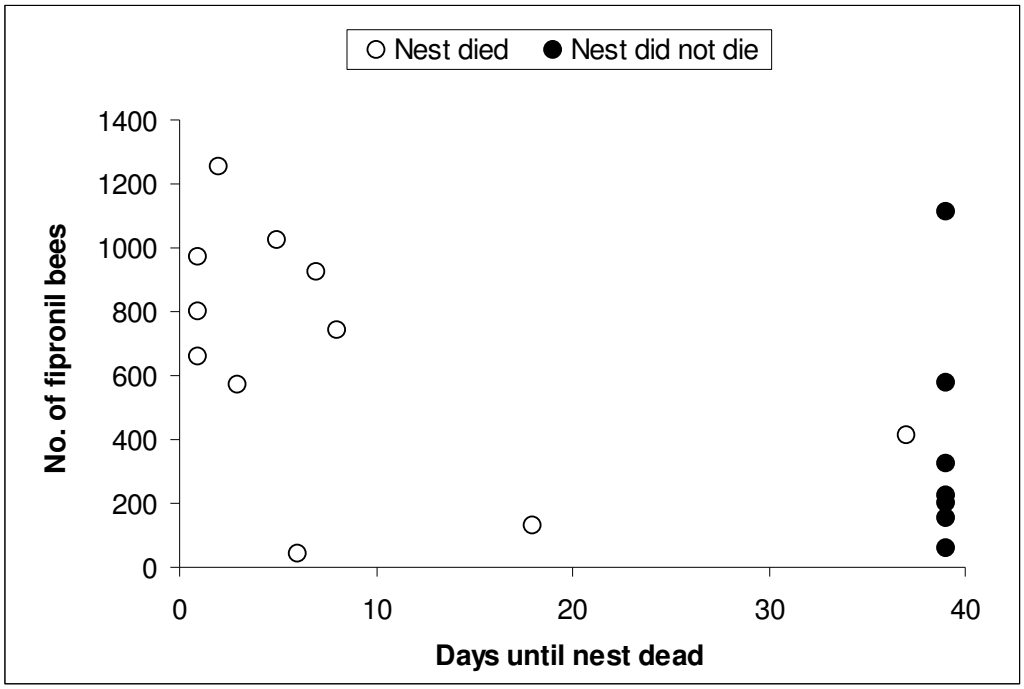

Figure 3: **Number of days until *A. cerana* nests died (or did not die) after a certain number of bees foraged on a fipronil-bated station. Nests that died are depicted as clear circles, whereas nests that did not die after treatment are depicted as black circles.**

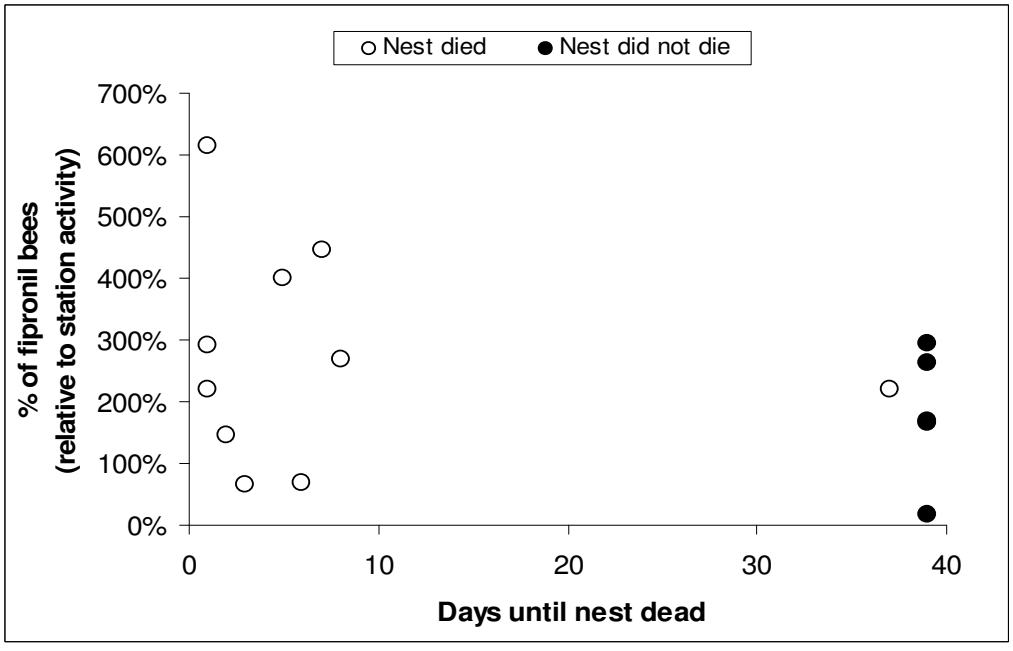

Figure 4: **Number of days until *A. cerana* nests were dead after a certain percentage of bees (relative to feeding station activity prior to treatment) forage on a fipronil-bated station. Nests that died are depicted as clear circles, whereas nests that did not die after treatment are depicted as black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

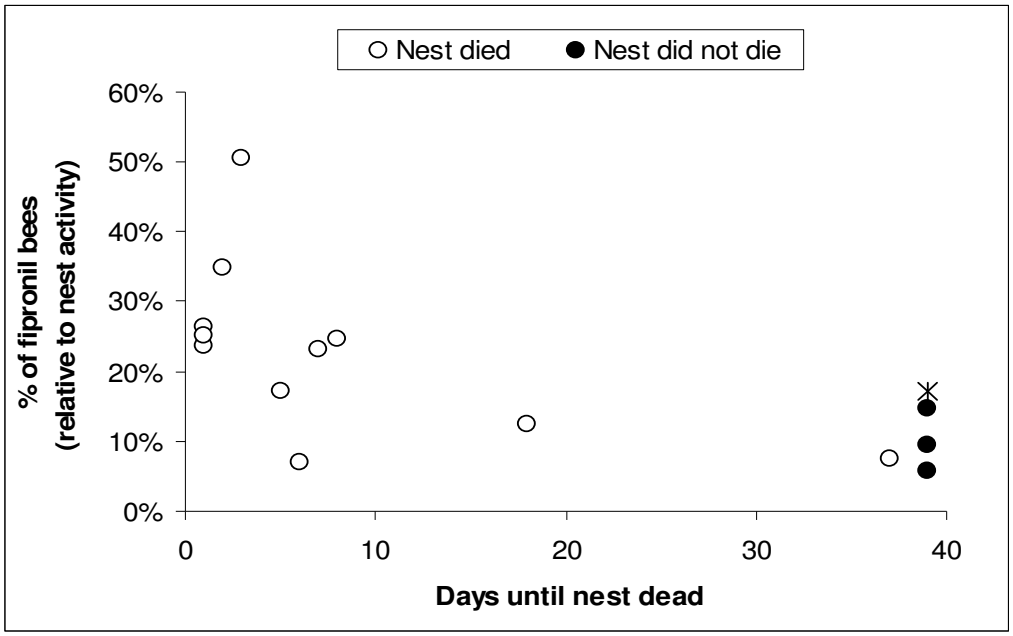

Figure 5: Number of days until *A. cerana* nests were dead after a certain percentage of bees (relative to nest entrance activity prior to treatment) forage on a fipronil-bated station. Nests that died are depicted as clear circles, whereas nests that did not die after treatment are depicted as black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

Treatment Efficacy

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

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

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

Non-target species

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

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

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

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

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

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

Discussion

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

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

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

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

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

Off-target species

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

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

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

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

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

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

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

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

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

Treatment Efficacy & Difficulties

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

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

Conclusion

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

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

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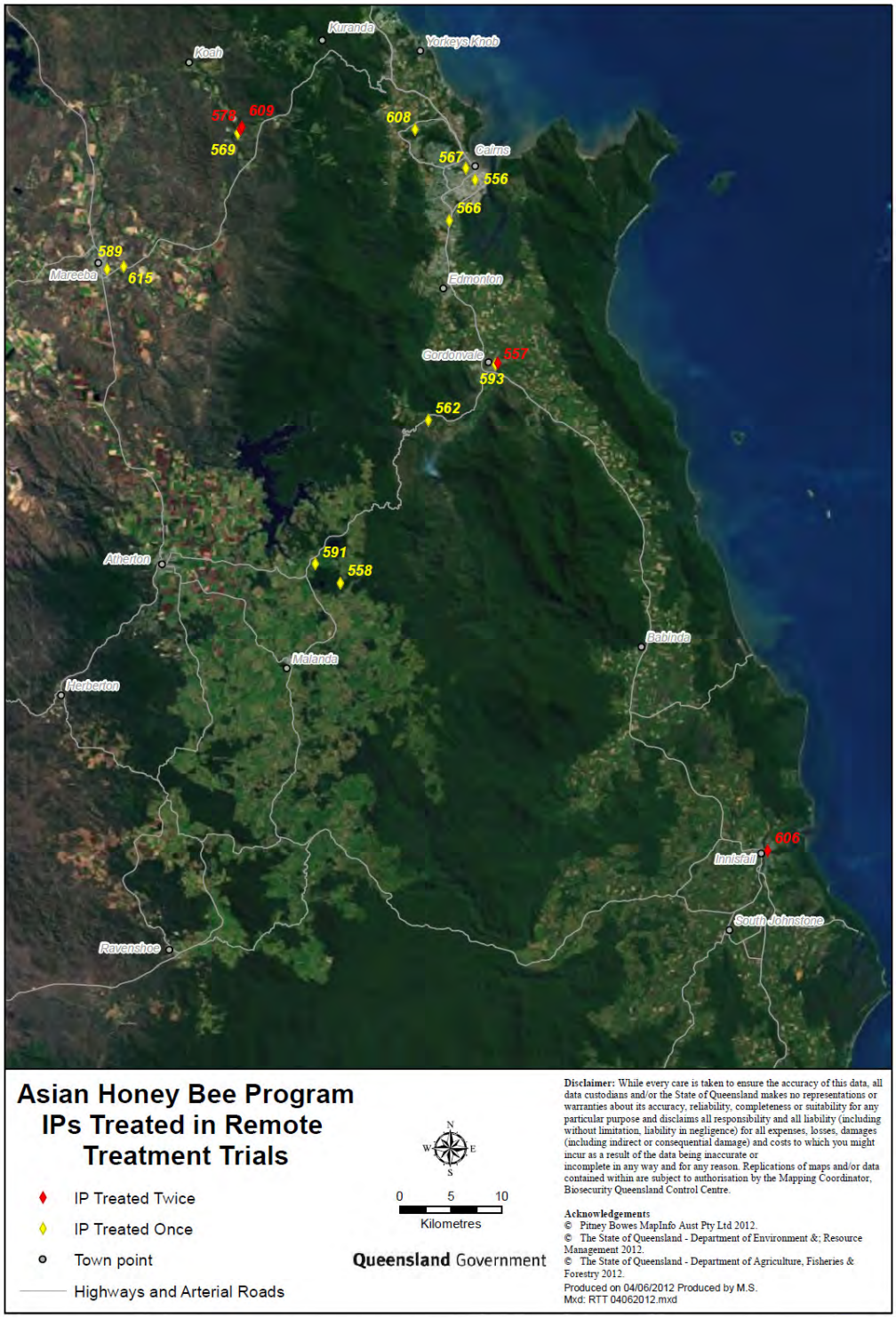
Appendix 1 Map showing locations of nests used for trials



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AHB SAG Minutes

Meeting Ten of the Asian Honey Bee Scientific Advisory Group (AHB SAG)

Teleconference held on Tuesday 25th June 2013

Attendees: Rod Turner (Chair), Sam Malfroy (Secretariat), Glynn Maynard DAFF, Doug Somerville NSW DPI, Boris Baer UWA, Trevor Weatherhead AHBIC, Max Whitten FCAAA (the following attendees joined the meeting as observers), Mike Ashton DAFF Queensland, Russell Gilmour DAFF Queensland, Anna Koetz DAFF Queensland and Melanie Commerford DAFF Queensland.

Apologies: Simon Barry CSIRO

Item 1: Welcome and introduction by the Chair

The Chair welcomed the Members of the Asian Honey Bee Scientific Advisory Group (AHB SAG) to the teleconference.

Item 2: Industry project update

Mr Malfroy tabled CSIRO's final report into the pest and disease status of *Apis cerana* and *Apis mellifera* in the Cairns region. Mr Malfroy stated that this was an industry funded project and the results demonstrated that no new viruses and no parasitic mites were observed. Mr Malfroy stated that RIRDC wished to publish the report in the near future, and that any comments on the report should be forwarded to PHA and RIRDC by the 28th of June.

Mr Weatherhead discussed that he had some technical issues with the report and the Chair requested that Mr Weatherhead pass these onto PHA and RIRDC so that the author of the report can consider these comments.

Mr Malfroy also tabled the preliminary proposal which was submitted by AHBIC to RIRDC to hold a 2-day AHB training workshop in August in the Cairns region. Mr Weatherhead stated that Dr Denis Anderson and Professor Ben Oldroyd would be conducting the training and that this would form part of an accredited and competency based training program. Mr Weatherhead added that he would also attend the workshop. The Chair stated that this would be presented at the TMG meeting scheduled for the afternoon (25 June 2013) for endorsement.

Item 3: AHB T2M – Final science project reports from DAFF Queensland

Dr Koetz and Mr Gilmour tabled and discussed the below FINAL reports with the SAG:

- AHB Manual
- AHB Fact sheet
- AHB – Guidelines for destroying nests or swarms
- AHB destruction video
- North Queensland Apiarist Survey Report

- Optimising AHB trap design and attractants
- Destruction efficacy of AHB
- Detection efficacy of AHB
- Ecology and Behaviour of AHB
- AHB Remote Nest Treatment
- AHB Spread Modelling

The SAG discussed the reports and approved their finalisation. The Chair stated that it was obvious that the most recently completed report, the spread modelling report, had not been circulated for a long period of time, and therefore, granted the SAG until COB on Wednesday 26th of June to provide any comments.

Mr Turner stated that all of the reports produced as part of the AHB T2M would be placed on the Asian honey bee website and that PHA would maintain this website into the future. Mr Turner also stated that PHA had been commissioned by RIRDC to develop a honey bee and pollination website and some of this information would also be used for that website. It was discussed that PHA could also re-develop and amend some of the reports into the future to meet the needs of particular programs, such as the National Bee Pest Surveillance Program.

The Chair deemed all reports final and stated that they would be presented at the AHB TMG meeting for approval.

Item 4: Future R&D Recommendations from the AHB SAG

The Chair outlined to the SAG that the AHB T2M would be finalised on 30th June 2013, and because of this, it was important to capture the thoughts of SAG members in proposing research areas where industry R&D should be focused on in the future. The Chair tabled the DRAFT Future R&D Recommendations Report for the SAG to provide comments.

Mr Weatherhead discussed that he would like changes made to R&D Priority area 1 which included studies on the hive biology and beekeeping methods of AHB. Mr Malfroy disagreed, and stated that a major impediment to research being conducted was the inability to collect and maintain hives for brood samples which arose from the lack of knowledge of the biology of this species of bee, hence the requirement of this project. Mr Weatherhead also added he would like recognition that Dr David Guez is currently conducting R&D into floral preferences of AHB and that any future work conducted in this area is dependent on this research. Mr Weatherhead also requested that Fipronil strength and concentration be included in R&D Priority area 3.

Mr Weatherhead requested that an update on Professor Carolyn Gross pollen analysis work is provided as well as the microsatellite work. Mr Gilmour stated that it was agreed in a previous SAG meeting that microsatellite would not go ahead. The Chair stated that they would investigate the pollen analysis research and possibly include in the R&D statement. The Chair accepted these recommendations and discussed that any further recommendations be forwarded to PHA by the end of the week.

Item 5: Next steps for the AHB T2M

Mr Ashton informed the SAG that members of staff currently working on AHB as part of the AHB T2M in DAFF Queensland would be leaving their roles by 30th June and that management of the AHB in Qld would pass to the Animal Biosecurity and Welfare program in DAFF Queensland. Mr Ashton stated that responsibility for responding to reports of suspect AHB would rest with the apiary officers in DAFF Qld. Only reports of AHB in high risk areas such as around ports or transport hubs, or in new areas where the bee had not previously been detected, would be responded to. Reports of AHB in the known infested area (KIA) around Cairns would be the responsibility of the community to manage and, in the future, only reports that are deemed potential new incursions would be responded to in the KIA.

Item 6: Summary and close of meeting

The Chair informed the SAG that this would be the last meeting of the AHB SAG, however, the AHB TMG would could continue to meet over the next year to discuss topical issues such as the RA and trade issues, as well as the progression of the industry funded research projects, to help keep communication channels open between governments and industry.

The Chair thanked the SAG for their extensive input into the AHB T2M and DAFF Queensland for attending the teleconferences and closed the meeting.