



Asian honey bee manual

Techniques for the identification, detection and
destruction of *Apis cerana*

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This Asian honey bee manual, as well as other management tools and scientific reports produced throughout the program, can be downloaded free-of-charge from www.biosecurity.qld.gov.au or www.planthealthaustralia.com.au

This publication has been compiled by Brenda Foley of the Asian honey bee Transition to Management Program (Department of Agriculture, Fisheries and Forestry) using material and reports produced throughout the program. The detection and destruction techniques described in this manual were developed during the Cairns Asian honey bee response and have been evaluated by Biosecurity Queensland. Full reports providing further detail to the suitability and efficacy of these methods can be downloaded from our website at www.biosecurity.qld.gov.au and are listed at the end of this document.

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Introduction

A significant goal of the Asian honey bee Transition to Management (AHB T2M) Program was to make available a range of safe, effective and integrated control strategies to reduce the impact of Asian honey bees (AHB; *Apis cerana*) in Australia. A key element of the plan¹ developed to guide the transition to management was to ‘validate the efficacy of detection and destruction methods and strategies as essential elements of deploying different control methods’. An essential action was to ensure that ‘detection and destruction methods and strategies were fully documented’.

The purpose of this manual is to provide biosecurity agencies and other parties involved in the management of AHB² in Australia with a reference detailing various detection and destruction techniques for use in managing or eradicating this pest bee species. It has been compiled from expert advice and experience gained during the AHB response in Cairns. The manual also provides information on other issues that need to be considered when responding

to a suspect AHB incursion, such as collecting and recording data, managing samples and selecting staff with appropriate skill sets. The detection and destruction techniques, developed during the Cairns AHB response, have been evaluated by Biosecurity Queensland. Full reports detailing the suitability and efficacy of the detection and destruction methods outlined in this document can be found at www.biosecurity.qld.gov.au

¹ Department of Agriculture, Fisheries and Forestry – Australian Government, 2011, *Plan for transition to management of the Asian honey bee: Version 1*, Department for Agriculture, Fisheries and Forestry – Australian Government (ed.), Canberra.

² Asian honey bees referred to in this manual primarily relate to the AHB T2M Program’s experience with the *Apis cerana* Java genotype. However, identification, detection and destruction techniques described in the manual are likely to be equally applicable to other strains of *A. cerana*.

Identification

Some wasps, flies and other bee species can easily be misidentified as AHB. A list of characteristics used to differentiate AHB from other similar flying insects can be found by visiting www.biosecurity.qld.gov.au and searching for 'Asian honey bee identification'. For conclusive identification, a sample should be forwarded to an appropriately qualified entomologist.

Field identification



Actual length:
Approximately 10 mm

Comparing foraging Asian honey bees with foraging European honey bees

Foraging bees, also called worker bees, collect pollen and nectar for a colony. Foraging AHB are about two-thirds the size of European honey bee (EHB; *Apis mellifera*) and have a few distinguishing features (see Figure 1):

- AHB fly quickly and erratically while feeding from floral sources; EHB forage much more slowly.
- The thorax and abdomen of AHB have less hair than those of EHB; this gives AHB a dark and shiny appearance with a black head, in difference to the golden, furry appearance of EHB.
- AHB have more prominent, evenly spaced and consistent abdominal striping, compared with EHB that tend to have uneven abdominal striping. EHB generally have thicker black stripes towards the back of the abdomen, making the abdomen appear more yellow at the front and darker at the end.



Figure 1 European honey bee (left), Asian honey bee (right)
(photograph courtesy of Paul Zborowski)

Swarms and nests

A swarm is a group of bees searching for a new nesting site and can vary in size from hundreds to thousands of bees. Swarms can be found flying or hanging from any object—such as a tree branch, house gutter or fence—as a dense cluster around a queen. Swarms may remain in place from a few hours up to one to two days while they send out scout bees to find a suitable nest site. Once a suitable site has been located, the queen will move with the swarm to the site and establish a nest. Swarming bees are not normally aggressive as they are homeless, which reduces their defensive behaviour.

A nest is a permanent colony of bees that is usually concealed within a cavity, such as a tree hollow, roof, wall vent or discarded mechanical equipment. The entrance to a nest can vary in size depending on where it is—it may be a small crack such as a building crevice or a larger hole entering a tree hollow. There will be consistent movement of bees in and out of the entrance to the nest. Nesting bees will defend their nests and queen, and are more aggressive and likely to sting if feeling threatened.

Comparing swarms and nests of Asian honey bee versus European honey bee

- AHB swarms can range in size from that of a closed hand to that of a basketball; EHB swarms are generally much larger.
- AHB swarms generally appear orderly with bees uniformly arranged and facing the same direction (see Figure 2); EHB clusters tend to be unstructured.
- AHB very rarely assemble around the nest entrance; EHB often linger in numbers at the opening platform.
- AHB approach the nest and entrance swiftly and often fly directly into the cavity when returning with pollen and nectar; EHB approach the nest at a more relaxed pace.
- AHB nest in relatively undisturbed cavities in and around human dwellings, making human encounters more likely; EHB prefer to nest in more open and less disturbed environments.



Photograph courtesy of Arthur Gibling

Figure 2 An AHB swarm

Laboratory identification and analysis techniques



Bees are of the order Hymenoptera (which includes wasps, ants and sawflies) and the suborder Apocrita. There are four main families of bees in Australia that include over 1500 native bee species:

1. Apidae
2. Colletidae
3. Halictidae
4. Megachilidae

Many wasp species, and some fly species, mimic the general yellow and black colouring of honey bees. Therefore, formal laboratory identification by a trained entomologist should be sought for any suspect bee found in the field.

Physical characteristics

AHB (*A. cerana*) and EHB (*A. mellifera*) both belong to the Apidae family of bees. A series of morphological characteristics can be used to determine if a bee belongs to the Apidae family:

The antennae must have a bend after the first long segment, like an 'elbow'.



Figure 3 Antennae shape of an Apidae family bee

All bees have two pairs of wings—the upper is the forewing and the lower is the hindwing.

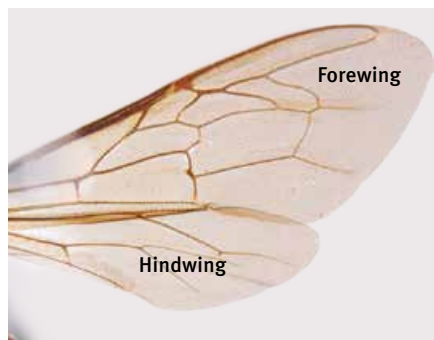


Figure 4 Forewing and hindwing of an Apidae family bee

The forewing venation pattern of all species of the Apidae family has three cells (called submarginal cells), each with the characteristic shape shown (see Figure 5).



Figure 5 Forewing venation pattern of an Apidae family bee

After determining the Apidae family sample, differentiate *A. cerana* from *A. mellifera* using wing venation as the most reliable morphological characteristic. This is particularly useful for identification of bee wings contained in rainbow bee-eater pellets (see 'Rainbow bee-eater pellet analysis' on page 6).

Following is a more detailed guide of *A. cerana* and *A. mellifera* wing venation. It should be used as a guide only; formal identification should be sought from a trained entomologist.

Comparing the wing venation of Asian honey bees versus European honey bees

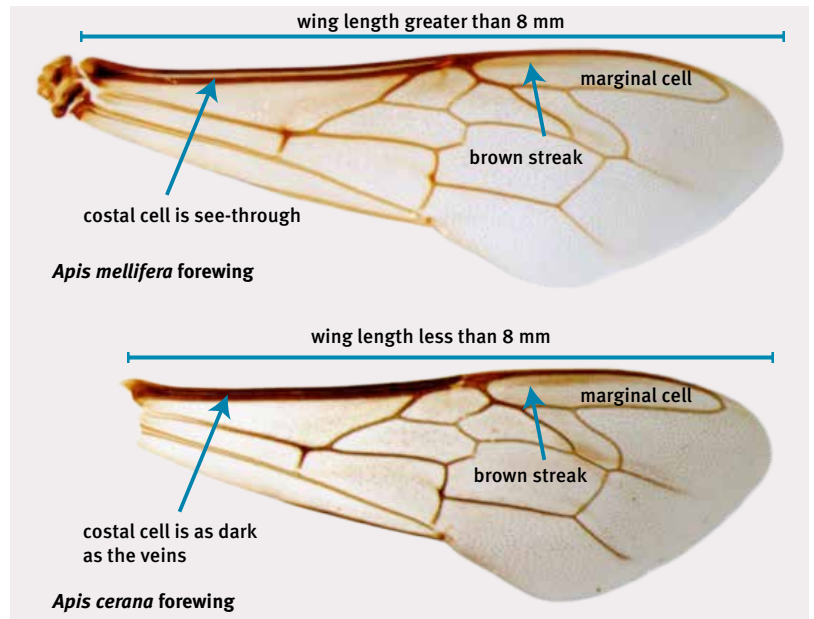
Forewing

Wing colour: *A. cerana* wings are darker, with grey-brown colouring. *A. mellifera* wings are much lighter and yellow-brown in colour.

Marginal cell: The brown streak in the marginal cell of *A. cerana* is much darker than that of *A. mellifera*.

Wing length: The wing length of *A. cerana* is less than 8 mm; *A. mellifera* wing length is greater than 8 mm.

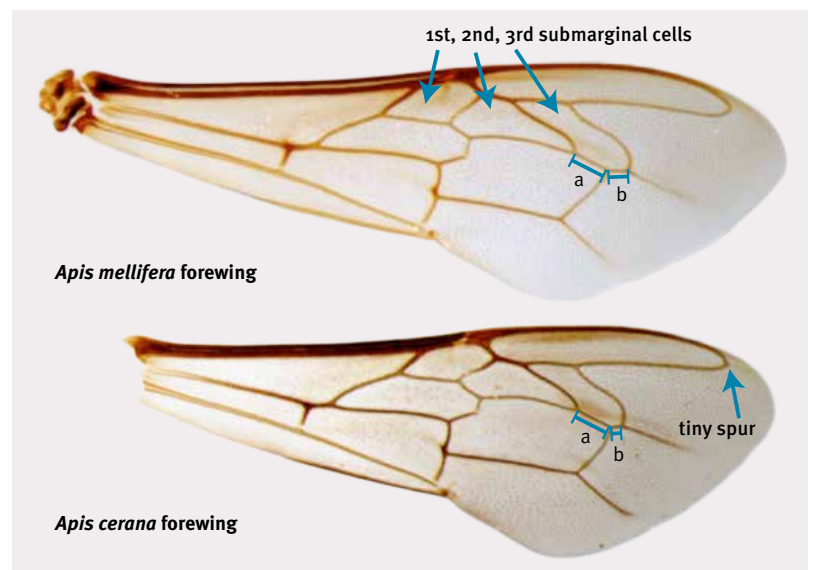
Figure 6 *A. cerana* and *A. mellifera* forewings showing the length and colouring of the costal cell and marginal cell



Submarginal cells: The fine differences in size and shape can sometimes be enough to distinguish *A. cerana* from *A. mellifera*.

The cubital index (CI) is the measurement (made using a scale eye piece) of 'A' divided by 'B'. The CI of *A. cerana* ranges from 3.1–5.1; and *A. mellifera* from 1.65–2.95.

Figure 7 *A. cerana* and *A. mellifera* forewings showing the differences in arrangement of the submarginal cells, size ratio of veins A and B.
(Wing venation photographs courtesy of www.padil.gov.au)



Hindwing

Radial vein: *A. cerana* has an extension of the radial vein of the hindwing, which in *A. mellifera* is never more than a tiny spur (see Figure 8). Some Australian native bee species also have a similar vein.

Therefore, the hindwing should not be solely relied on where the previous identifying information is not available. If at all unsure, wings should be compared by all methods of taxonomic species identification.

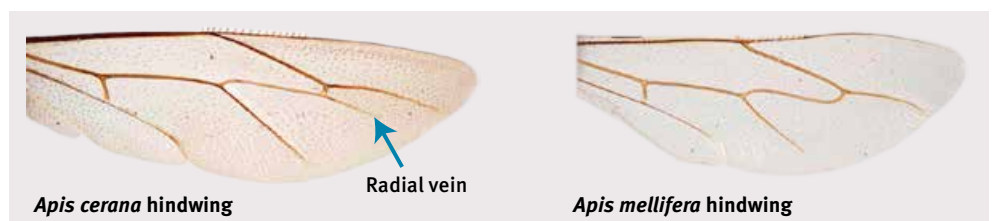


Figure 8 *A. cerana* and *A. mellifera* hindwings showing the extension of the radial vein



Rainbow bee-eater pellet analysis

Rainbow bee-eaters (*Merops ornatus*) are a migratory bird species—widely distributed throughout the Australian mainland—that display a suite of behavioural traits useful in determining the presence or absence of AHB.

Specifically, these traits include:

- feeding on bees
- regurgitating indigestible material (such as bee wings)
- routinely congregating in flocks at the same roost sites each night.

Rainbow bee-eaters expel many pellets every day (see Figure 9). The following process is used to extract any forewings and/or hindwings from a bee-eater pellet. Upon extraction, wing venation and colour can be used to identify the presence of AHB.

Figure 9 Rainbow bee-eater and two pellets prior to analysis

Extracting wings from a pellet

- Place a pellet into a small vial (50 mL–100 mL in size).
- Pour boiling water over the contents and replace the screw cap tightly, sealing the vial.
- Gently shake the vial well to loosen the material within.
- Pour the contents into a tea strainer or fine mesh sieve.
- Rinse the contents under cold water.
- Place the contents in a Petri dish and flood the material with 70% ethanol.
- Remove any bee wings from the dish.
- You can then identify the species using the characteristics previously described.

Nest identification, characteristics and age

Features of AHB comb

A unique feature used to confirm the identity of AHB nests are drone brood cells. These cells are constructed differently to the comb of any other bee species and have a raised wax cap with a distinct pore (see Figure 10).

Nest age

Determining the age of a nest may assist with determining how long the bees have been present in the area and therefore how widely the surveillance team will need to survey to delimit the AHB infestation. The colour and number of queen cells in an AHB nest can be used to determine its approximate age.

Freshly-built comb is generally white to pale yellow in colour. As each new generation of brood hatches, the cell walls darken; giving older nests a darker appearance than younger ones (see Figure 11).

Bees also build queen cells in preparation for the production of a new queen. Queen cells are usually located along the bottom edge of the comb, although they are sometimes situated in the middle or on the sides of the comb. Once hatched, empty queen cells indicate that the nest has previously swarmed.

The number of hatched queen cells can be used to determine how established the population is and how many other AHB nests may be in the area. Where multiple hatched queen cells are present and the comb colour is dark, an assumption can be made that the nest is old, has previously swarmed and that other colonies may occur in the vicinity.

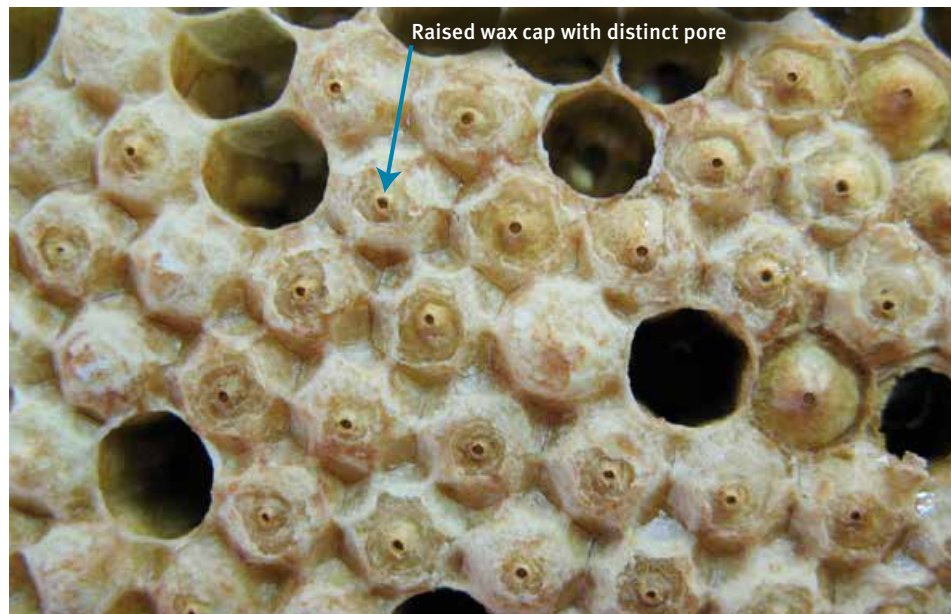


Figure 10 Drone brood cell of AHB comb
(Photograph courtesy of Ben Oldroyd, University of Sydney)

1	2	3	4	5	6	7	8
Freshly built		Young					Very old

Figure 11 Determining the age of comb by colour

Hatched and unhatched queen cells look like an elephant's trunk that usually protrudes from the outskirts of the comb. A hatched cell will have an opening at the end where the cap has been moved. An unhatched cell is one that does not have any holes (through which the queen has come out).

A queen cup looks like a stout, swollen cell protruding along the outskirts of the comb. However, these can be 'dummy cells' and may never have produced a queen.





Detection

Biosecurity Queensland used many surveillance methods to detect AHB in the Cairns region, with varying levels of success. For the full analysis and/or to download the report on the efficacy of the following detection (surveillance) techniques, visit www.biosecurity.qld.gov.au

Below, we provide a step-by-step guide, recommending when, why and how each surveillance technique should be employed.



Public reporting

Public reporting has been the most successful surveillance strategy throughout the response in Cairns. When responding to an AHB incursion, emphasis must be placed on the rapid development of a multi-faceted community engagement plan. This plan is to strategically engage the public and key stakeholder groups and help build awareness amongst the widest audience possible within the survey area.

Public reports must be managed with care and accuracy. It is also important to act in a timely manner to ensure the public understands the importance of their role in detecting and reporting targeted pest species.

A public call to action such as *Biosecurity is everyone's responsibility* can be used to encourage public reporting of targeted pest species. The 'Community engagement and public relations' section of this manual provides tips for developing a community engagement strategy and building good relations with the public and other stakeholder groups.

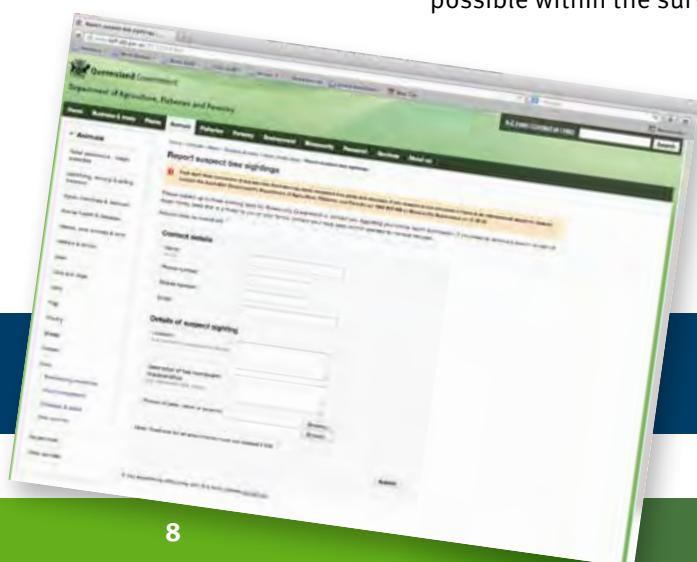


Figure 12 A toll free phone number and an online reporting tool is essential for community reporting



Phone 13 25 23

Generating public reports

Community engagement is critical to all biosecurity responses for many reasons. In terms of surveillance, effective engagement with the community can greatly increase the number of people looking for and reporting targeted pest species.

A number of methods are recommended to generate public reporting within the community, including:

- handing out information kits (including identification and sample collection tools) to the public and key stakeholder groups in the area of potential infestation
- educating the public through door-to-door visits in the immediate vicinity of a suspect incursion, ensuring people are aware of the pest and how they can help through community-assisted surveillance and reporting
- engaging multiple local media channels and other relevant outlets within the affected community (e.g. environmental clubs, schools, community groups) to deliver information about the pest, its potential impacts and how the community can assist in the detection and control of the pest
- establishing and maintaining a presence in local media by regularly inviting local radio, television and print news outlets to interview your media spokesperson; and then issuing media releases to quickly grab the community's attention
- incorporating useful information, good-quality photos and contact details in factsheets and posters, and on social media and relevant websites; this will help the public to identify the bee themselves and may reduce the number of false reports
- using Variable Messaging Signs (VMS) at strategic locations to increase public awareness and encourage those within the surveillance area to report suspect bee sightings; positioning VMS on main roads and heavy traffic thoroughfares will maximise exposure.

Receiving public reports

The swift development of an online reporting framework and toll free phone number is essential. Ensure the appropriate information is captured, as follows:

- An online reporting tool should enable the public to provide details of a suspect sighting and, where possible, upload an image of the suspect bee. Consider making this reporting tool 'smart phone friendly' and use existing pest reporting frameworks where available.
- Customer Service Centre staff should be provided with appropriate training so they have an awareness of AHB and the potential area of infestation. A set of standard questions and a call flowchart will help guide staff through enquiries and ensure calls are escalated appropriately.

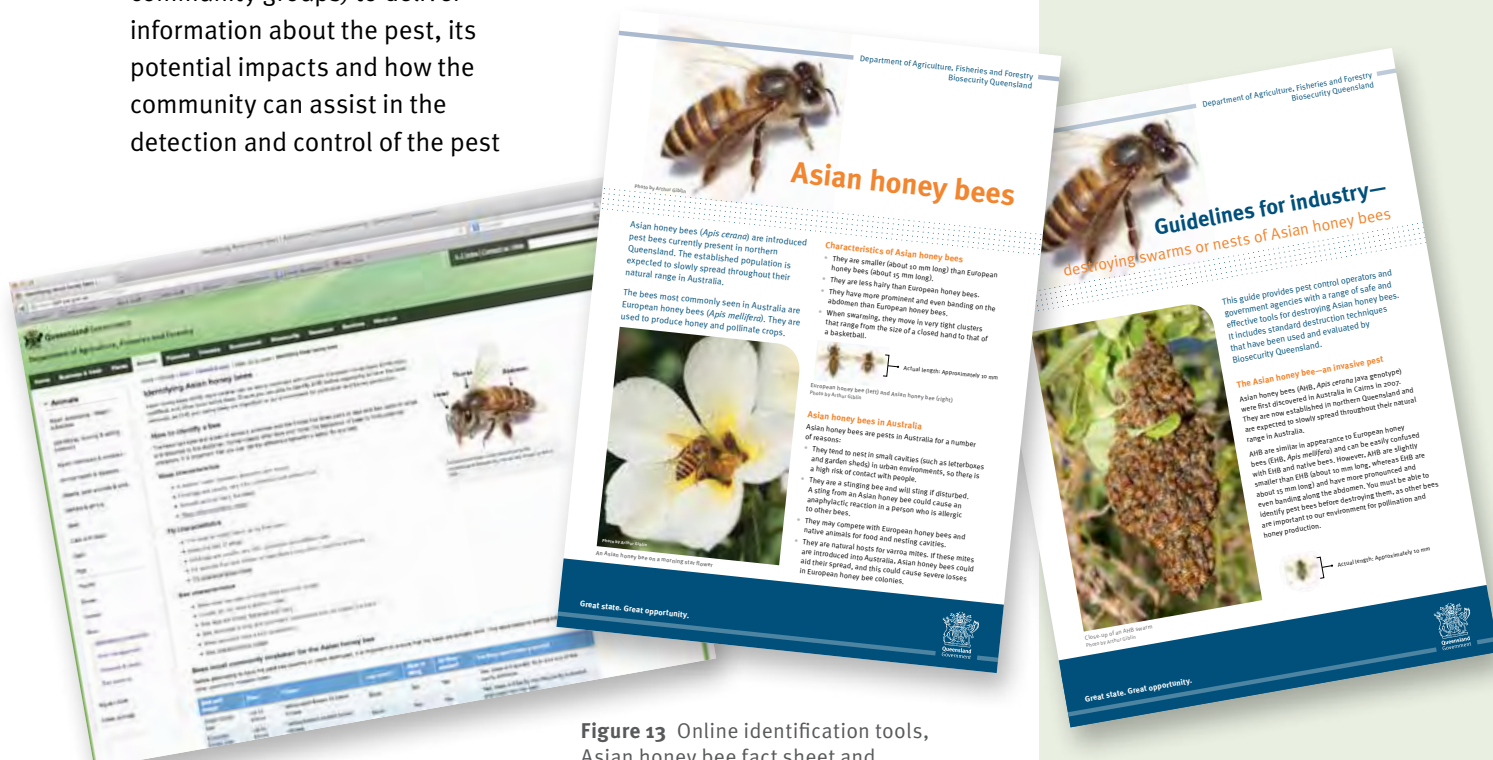


Figure 13 Online identification tools, Asian honey bee fact sheet and destruction guideline

The following is a questionnaire for a Customer Service Centre to use in capturing the required information from the public:

1. Are you familiar with what the common European honey bee and the small, black stingless bee look like?
☐ YES—Go to question 2.
☐ NO—Go to question 3.
2. Do you think the bees you saw are either of these? _____
3. Are the bees near a port?
☐ YES—Go to question 4.
☐ NO—Go to question 5.
4. What is the name of the port? (Note: Escalate this report) _____
5. How big are the bees? _____
6. Did you see an individual bee, or a number of bees, foraging (e.g. feeding on flowers)?
☐ YES—Go to question 17.
☐ NO—Go to question 7.
7. Did you see a swarm of bees (e.g. a dense cluster of bees all on top of each other)?
☐ YES—Go to questions 8–11, then to question 16.
☐ NO—Go to question 12.
8. What is the address of where you saw the swarm? _____
9. How long has the swarm been there? _____
10. How large is the swarm (e.g. size of a soccer ball, tennis ball)? _____
11. How high off the ground is the swarm? _____
☐ Go to Question 16.
12. Are the bees entering and exiting a cavity (e.g. house wall/letterbox)? _____
13. Have they recently arrived or have they been there for some time? _____
14. Can you see any sign of a nest (e.g. something that looks like honeycomb)?
☐ YES—Go to question 15.
☐ NO—Go to question 16.
15. Where is the nest located? _____
16. Is there potential for the bees to spread (e.g. by vehicle or vessel)? _____
17. Are the bees at your address/property? _____
18. What type of property are the bees at (e.g. residential, commercial, industrial)? _____
19. Where or how did you find out about the pest bees (e.g. from a factsheet, newspaper, website)? _____

Coordinating the reports

Assign a response coordinator to investigate all public reports received through the Customer Service Centre and the web-based reporting tool. Contact the person who made the report without delay, especially regarding swarms. Encourage him or her to take a photo of the suspect bee and send it via email or mobile phone.

Advise the caller to:

- leave the bees where they are
- avoid aggravating the bees as they could fly away or attack
- note the direction of flight should the bees fly away before assistance arrives.

Additional questions to ask the caller:

- Is a ladder required to reach the swarm or nest?
- Is the swarm stationary?
- Is the swarm/nest easily accessed?
- Do we have permission to access your property if you are not there?
- Are there any hazards or risks regarding safety (e.g. dangerous dog, electrical hazards)?

From the information provided, the response coordinator must decide if a physical response is necessary and complete a risk assessment considering the level of risk the bees pose and the level of response required. Biosecurity Queensland has developed two policy documents detailing the appropriate response required when receiving a report of a possible incursion of AHB or other suspect bees (see 'Further information' on page 27 for how to obtain these documents).

Before responding, contact a suitable beekeeper and/or pest controller for assistance, if required. Experience in handling bees is very important in all responses to AHB or suspect pest bees.

Always collect a sample of the suspect bees for formal laboratory identification. In addition, record all details and information regarding the possible incursion and report back to the response coordinator. The response coordinator must then ensure samples are sent for laboratory identification.

Figure 14 Bees entering a cavity may indicate a concealed nest



Floral observations

Flowering plants are key food sources for bees. Improve staff efficiency in nest detection after an incursion of exotic bees through education about local flowering plant types, as well as plants commonly visited by bees in the area.

It is recommended that floral observations are undertaken in the area of interest; being either:

- a 2 km area surrounding an incursion or report of a suspect swarm or nest
- an area needing to be established as free of AHB.

The program compiled a list of AHB-preferred floral sources in far-northern Queensland. This was used to help assist staff detect AHB nest locations of foraging bees. This list is available on the Biosecurity Queensland website (www.biosecurity.qld.gov.au) and may be used to help identify likely AHB floral sources in similar climates and urban areas. A more comprehensive discussion of AHB floral preferences in the Cairns region can be found in the report *Asian honey bee Transition to Management Program: Ecology and behaviour of Asian honey bees (Apis cerana) in Cairns, Australia* (Commerford and Koetz, 2013).



Honey bee colonies send out foraging worker bees to collect pollen and nectar for the nest. When a bee has found a good source of food or water it will return to the nest and recruit other bees to the source. Foraging bees will travel in a direct route back to the nest to deposit the food, making them easy to track.



Figure 15 A Biosecurity Qld staff member recording AHB data and collecting bee samples

Establishing surveillance using floral observations

Floral observation is the technique used for finding foragers—and finding foragers is the first step in finding nests. Follow these steps to establish a surveillance protocol to survey for and detect foraging AHB:

- Utilising existing roads or tracks in the area of the incursion, plan a path to be walked for surveillance (the transect).
- Walk slowly along the transect, scanning all floral sources for the presence of bees. To increase the rate of AHB detection, stop at a flowering plant (or a patch of flowering plants/weeds) every 50–100 m and observe it for the presence of bees for 5–10 minutes.
- Carry out observations during the early morning and mid-to-late afternoon when bees are most active. The timing of nectar flow may vary with the season and influence bee activity; local beekeepers should be able to provide advice.
- When you find honey bees foraging on a flowering plant, record the location (preferably using GPS coordinates), type of plant, time of day and date. Recording these details on printable maps will improve the efficiency of future surveillance. This will also create a catalogue and map of flowering plants in the area that are preferred by bees.
- Collect at least one sample of the suspect bee using a butterfly net. Place it in a vial containing enough 70% ethanol solution to cover the bee/s so that it can be formally identified in a laboratory.
- If foraging bees are detected on specific plants (and are formally identified as AHB by a trained entomologist) use the bee-lining technique to locate the nest (see ‘Bee-lining’ on page 15).

Traps

During the AHB T2M Program, a number of different trap designs and attractants were trialled with limited success. Until an effective attractant can be found and trap design can be greatly improved, floral observation is a much more time- and cost-effective method for determining AHB presence in a defined area (see 'Floral observations' on page 12).

The most promising trap design trialled by Biosecurity Queensland was a simple, inverted-top bottle trap (see Figure 16) containing a mixture of sterilised honey³ and water. The bees, attracted to the scent of honey, fly into the trap and drown in the liquid. (Refer to 'Further information' on page 27 for a link to the full details on the trap design and attractant trials conducted in Cairns.)



Figure 16 An inverted-top bottle trap



Photograph courtesy of Arthur Giblin

Figure 17 European honey bees feeding from golden cane flower

Other trap research

Proof of concept was shown for using polymerase chain reaction (PCR) genetic analysis on sugar syrup to determine whether AHB had fed on the syrup placed at feeding stations. This method requires further development to refine the procedure.

The Rural Industries Research and Development Corporation is currently funding research to develop an attractant that should significantly improve the usefulness of traps as a management tool for AHB. The results of this research will be available in the future.



Photograph courtesy of Arthur Giblin

Figure 18 Asian honey bees feeding from a station

³ Note that bee diseases can be transmitted and spread by honey; untreated honey should never be used to feed, attract or trap bees.

Rainbow bee-eater pellets

Rainbow bee-eater (*Mereops ornatus*) pellets provide an ideal tool for establishing the presence of AHB in an area. The rainbow bee-eater is distributed across most of mainland Australia. In northern Australia there are resident populations, but the birds in southern Australia migrate north during the winter months, resulting in larger populations in northern Australia between March and November. Rainbow bee-eaters eat a wide range of insects, though their diet mainly consists of bees and wasps. At night they congregate in large flocks and roost in trees. Roosting rainbow bee-eaters regurgitate non-digestible portions of their prey (such as bee wings) in the form of a pellet. These fall to the ground, making it easy to collect for analysis. Birds can be found perched on powerlines and fences during the day if evening roosting sites are difficult to locate or access.

It is more likely to find main body parts than wings within rainbow bee-eater pellets. The wings are almost exclusively forewings and are usually dry and curled up. The presence of AHB cannot be determined without analysis in the laboratory.

Finding roosting sites

The rainbow bee-eater has a distinctive, trilling call. Once you have identified this call you can track them to their roosting site. To do this, you will need to move around the area reasonably quickly, so riding a bicycle is a good idea.

- Watch for flocks of bee-eaters in the late afternoon, as they will begin grouping together in an area. They will then usually begin flying, in stages, towards their chosen roost.
- Listen for the bird calls and follow these small groups of birds until you reach the roosting site.

Rainbow bee-eaters settle down quickly for the night once at the roosting site. Sometimes there is almost no indication that the chosen tree may be hosting several hundred birds, and frequently the owner of the property is unaware of the birds.

Alternatively, ask local birdwatching groups about the roosting sites of these birds, or use local media to encourage members of the community to report any known roosting locations.



Photograph courtesy of Peter Bray

Figure 19 Rainbow bee-eater

Setting up rainbow bee-eater surveillance

- Locate a roosting site, spread a white linen sheet around the base of the identified tree and peg it out to catch the pellets. Collect pellets directly from the ground if the site is not suitable to spread a sheet, or if birds are seen regurgitating pellets during the day.
- Place samples in labelled vials and record the time, date, location and any other relevant details. Heavy rain will break down the pellets; keep them as dry as possible and do not use a tarpaulin (this will allow rainwater to pool, disintegrating any pellets). Do not refrigerate the pellets.
- The frequency of pellet collection is situation-dependent. Weekly collection will be necessary to confirm the presence of a new AHB incursion, whereas monthly pellet collection should suffice when trying to determine if an area is free of the pest bee.
- Conduct laboratory analysis (see 'Laboratory identification and analysis techniques' on page 4) to determine whether AHB are part of the rainbow bee-eater's diet, in which case AHB wings will be present in the pellets.

Bee-lining

Bee-lining is a systematic technique used to track foraging bees back to a nest. This technique is normally used when suspect bees have been detected foraging on a floral source, either through a public report or floral observations by field staff.

Bee-lining involves converting bees from the floral food source to an artificial food source (a feeding station). A floral scent (essential oil) is used in the artificial food source so that bees learn to associate that scent with a reward. The purpose of converting the bees is to encourage the foraging bee to return to, and recruit more bees to, a specific foraging location. This enables field personnel to track the flight path and subsequently locate the nest.

Other bee species (including EHB and *Tetragonula* spp.) will be attracted to the feeding station. Their presence on the feeding station should be discouraged as it may hamper the bee-lining process.

Setting up the feeding station

- Feeding stations should offer scented sugar syrup solution, an easy landing platform and something for the bees to stand on (e.g. sticks, rock, sand beach) so they don't get sticky feet. The easier it is for bees to take up the sugar solution using their proboscis, the easier it is to convert them. A cover should be placed over the platform so that the syrup is shaded from sun and protected from rain (see Figure 20).
 - Sugar syrup solution: Dissolve 1 kg of white sugar in 1.5 L of hot water, mix well and allow to cool. Add 1–2 drops of a floral essential oil to the side of the station (e.g. rose, lavender, citrus). Avoid using too much scent as it may deter the bees. Unused syrup can be stored in a plastic bottle, but will ferment if kept too long.
- Using a fine-mist spray bottle, dispense sugar syrup solution onto approximately one quarter of the floral source on which the bees are feeding.
 - Place the feeding station close to this site so that the bees will become accustomed to the new, stronger scent of the station and are attracted to it. Repeat until the bees are successfully converted.
 - Once five or more bees have returned to the station, it can be moved into an open area about 5 m away from the floral source. This will help to establish an initial bee-line that can be observed against an uncluttered skyline.
 - To distinguish individual bees, place a coloured dot (using different colours of correction fluid) on the back of each bee while it is feeding.
 - Move the feeding station slowly, in stages, in the direction of the observed flight path/bee-line. Allow a similar number of bees to feed from the station before moving it on again.
 - In dense areas (rainforest and in congested urban areas), the station can be moved up to 10 m each time.
 - In open fields (grass paddocks), the station can be moved 50 m–100 m each time, as long as you have correctly established the bee-line.
 - If bees are lost from the station or reconvert to the floral source, repeat the process.



Figure 20 A Biosecurity Qld staff member determining the flight path of bees



Photograph courtesy of Arthur Giblin

Figure 21 An Asian honey bee in flight

Tropical green ants (*Oecophylla smaragdina*) and other species will be attracted to the feeding station, which discourages bees from landing and feeding. You can apply products such as Tanglefoot or Tac-Gel to the stem of the feeding station to discourage the ants.

Taking a bearing

- Bees will generally fly in an ascending spiral as they leave the feeding station, until gaining a bearing towards the nest.
- Note a landmark (e.g. a tree) that can be seen in the direction of flight, or lay a ruler across the feeding station and align it with the line of flight and/or use a compass to mark the bearing.
- Once the same bees are returning regularly to the station they will start to take a direct route and recruit more bees. Continue to move the station in the direction of the flight path.

Tips to make bee-lining easier

- Place the station in an unobstructed and open area. Ideally, the surrounding backdrop should be free of vegetation as this can make observation difficult.
- Observe whether the bees are foraging for pollen or nectar from the floral source being targeted. Bees collecting nectar are much easier to convert to an artificial food source.
- Laying on the ground and watching for bees against the sky may make it easier to determine the bee-line.
- To estimate the distance to the nest, time the return flight of a marked bee. Very roughly, a return time of 1 minute indicates a nest distance of ~100 m, 2 minutes of ~200 m, etcetera. This is only an estimate, however, as a bee may spend varying times in the nest.
- The movement of bees (through reflection of the wing) is more noticeable when the sun is lower in the sky during the morning and afternoon.
- Good-quality binoculars or scopes are useful for studying tree hollows and detecting nests that may be too high to be seen clearly with the naked eye.
- Place a drop of the scent on the edge of the dish each time it is moved, to help the bees find the feeding station again.

Triangulation

In large open areas, two station sites can be used to triangulate the location of the nest. This method was used occasionally during the AHB T2M Program with varying degrees of success.

- Place a feeding station at Site A and record the flight path. Mark any bees feeding on the feeding station with a dot of correction fluid on the back of the bee.
- Wait for the same marked bees to locate and adjust to the new site and record their new flight path.
- The point where these two bee-lines meet should indicate the site of the nest. Draw the two flight paths on a map of the area to pinpoint the nest.
- Once a number of bees are returning to the feeding station at Site A, move the feeding station to a new site (Site B), preferably 90 degrees from the initial flight path and at least 100 m away.

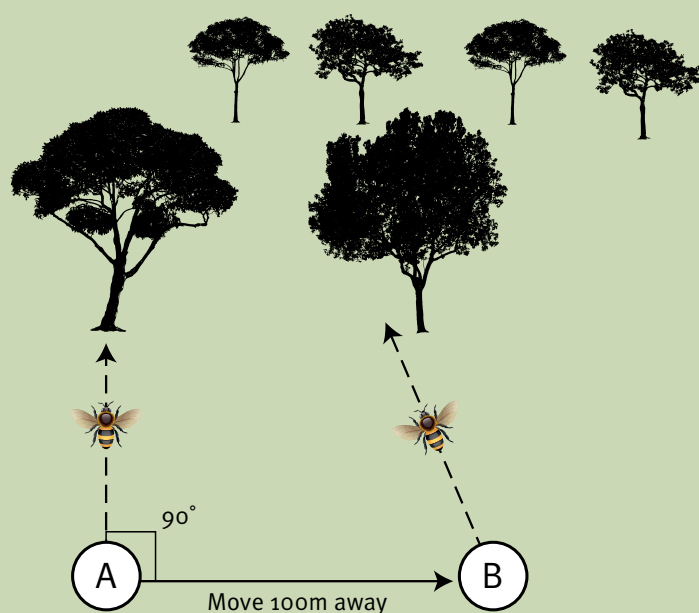


Figure 22 Schematic of triangulation process

Detector dog

During the response in Cairns, a detector dog was trialled to find swarms and nests of AHB.

Suggested situations where the detector dog may be effective include:

- where a new incursion is suspected and the swarm/nest of bees still appears to be restricted to the vicinity of a port area
- when working together with field personnel (e.g. once an approximate distance and bee-line had been established, the dog could detect an odour at ~50 m from the site and quickly homed in on the scent)
- areas that are difficult for people to visualise or access (e.g. ports, ships, container storage areas and transport hubs)
- areas that are high-risk (e.g. barges)
- where the dog did not have to cover large areas of terrain
- where the environment was unsuitable for multiple field staff to enter.



Conditions where the detector dog may not be efficient or cost-effective include:

- situations that could severely challenge the nose of the dog (e.g. where a nest is positioned high above the ground in a hot, windless environment and protected within a cavity of a tree)
- urban areas with heavy road traffic and narrow streets bordered by high buildings that funnel the wind
- very large search areas in which an indication of direction and distance could not be provided to the handler
- environments of high heat and very high humidity that affect the dog's stamina and create the need for additional rest time.

A detector dog needs to let its nose rest after 20 minutes of searching for a scent. For efficacy data, a GPS tracker that logs the search area covered and the duration should be attached to the detector dog. Daily records should also be kept for the purpose of reviewing performance and success.

Exotic bee incursions occur frequently in northern Australian ports that receive cargo from overseas. Any *Apis* species introduced into Australia could be harbouring a range of exotic mites and/or bee diseases. Using dogs trained in the detection of honey and bees may be useful in these situations.



Figure 23 An Asian honey bee nest built outside of a cavity



Destruction

The efficacy of the following destruction products and techniques were assessed during the course of the AHB T2M Program. For the full analysis and/or to download the reports, visit www.biosecurity.qld.gov.au

The different destruction techniques used by the AHB T2M Program are also captured in the *Guidelines for industry—destroying swarms or nests of Asian honey bees* and in a short YouTube video showing application of all three products in different situations. Both of these tools can be found at www.biosecurity.qld.gov.au



It is recommended that you refer to the relevant legislation applicable to your state regarding the use of certain insecticides. In Queensland, insecticides must be used in strict accordance with the approved label. Approval from the

Australian Pesticides and Veterinary Medicines Authority (APVMA) is required where products are to be used for purposes other than specified on the label.

Assessing risk before destroying a swarm or nest of bees

Bee swarms and nests can be found in a variety of environments. Each will behave differently when approached and requires a slightly different method of treatment. Situational site risk assessments are essential to reduce potential hazards to buildings and personnel. It is important to ensure that:

- the product and technique you employ is suitable for the situation
- action is taken to eliminate potential bee escape routes from a nesting cavity
- additional products are available on standby.

When attempting to destroy bees, a minimum of two people should be present to:

- ensure each other's safety
- keep onlookers at bay
- provide help in the case of emergency.

Inexperienced bee handlers should engage the services of a local beekeeper to assist with identification. Similarly, a commercial pest controller should assist with the destruction of bee swarms or nests. Consider seeking additional specialised assistance if:

- the swarm or nest is too high or unsafe to reach (consult a tree lopper accredited with a Working Safely at Heights certificate)
- you will need to remove roofing panels (consult a roofing contractor)
- you will be working around live electrical wires (consult an electrician)
- you will be working in an area with pedestrians or vehicular traffic (consult a traffic controller)
- you will be working on an industrial site that requires specialised personal protective equipment (PPE).

All personnel involved in detecting and destroying bees should wear appropriate PPE as listed in the Material Safety Data Sheet (MSDS) for the product(s) used. The minimum PPE recommended includes an apiarist veil, elbow-length gloves, full body disposable overalls, sturdy enclosed footwear, a dust mask and safety eyewear (see Figure 24).

You can buy personal protective equipment from suppliers listed under 'apiarist supplies' or 'beekeeping supplies' in the phone directory or online.

When working around bees, keep well-maintained medical kits, that have the capacity to treat severe allergic reactions (anaphylaxis), within reach.



Figure 24 Personal protective equipment

Products recommended for different situations

Swarms and nests		Nest only	
	Flying insect spray	High-output liquid insect killer spray	Permethrin dust
Ideal for	• a swarm that can be captured in a bag • cavities in domestic and natural areas • small or medium-sized cavities • a swarm or nest that is accessible	• a swarm that cannot be captured in a bag • external nests • nests that are inaccessible but visible (≤ 4.5 m away) • cavities with electrical wiring	• cavities that are inaccessible • nests that are not visible • cavities with multiple exits that can be plugged/sealed • cavities with electrical wiring • large cavities (≤ 2 m)
Avoid use	• near electrical wires and sources of ignition • when ventilation is poor	• on very large swarms or nests • on nests that are inaccessible, not visible, or ≥ 4.5 m away	• in very large cavities (≥ 2 m) • in cavities with exits that cannot be plugged

Flying insect spray (aerosol)

This product is commonly found in most households for the control of flies, mosquitoes and other house-dwelling insects. It is readily available for purchase in many convenience, grocery and hardware stores.

High-output liquid insect killer spray (aerosol)

This product is a fast-acting insecticide liquid spray (often called ‘wasp spray’) that is intended to control a variety of flying and crawling insects, including feral bees. This product has a few distinct advantages, i.e. it is safe to use in and around areas with electrical wires and equipment (e.g. wall cavities); it can be sprayed upwards at a 15 degree angle; and it can be sprayed over distances of up to 3.0 to 4.5 m. It is publicly available for purchase in most shops that stock industrial supplies.

Permethrin (dust)

This is used to control a variety of insects, including feral honey bees. It is a fine, lightweight and residual product (due to its hydrophobic properties), with a heightened capacity to penetrate inaccessible spaces, making it ideal for inaccessible spaces such as wall cavities and block wall crevices. This chemical is readily available to the public in many hardware stores or professional pest control chemical supply stores.

Always use products in strict accordance with the label. Ensure that the label does not state that it should not be used around bees.



Destruction techniques

Swarms

Flying insect spray and a large plastic zip lock or garbage bag is the most effective technique to contain and kill an accessible swarm.

- Dress in the appropriate PPE for bee removal.
- Assess the position of the swarm and its attachment to the structure. Taking care not to disturb the bees, remove any objects that may hamper the bag being placed around the bee mass.
- Check that the bag is large enough to hold all of the bees, then in one slow, relaxed, smooth motion enclose the bees with the bag and bring your hands together at the top to seal them in.
- Gently shake the swarm mass until it detaches and drops into the bag.
- Tie the bag and place it inside another bag to ensure that no bees escape.
- If unsuccessful in capturing the majority of the swarm and the queen, repeat the process when the bees have resettled.
- Spray any flying bees and the area where the swarm was hanging to discourage any uncaptured bees from returning (note that a swarm of bees requires a queen to survive and if removed, remaining bees will die).
- Once capture is complete, place the bag containing the swarm of bees into a freezer or pierce a small hole in the bag and spray insecticide through it. Either of these will kill the bees.



If you cannot place a bag around the swarm of bees, cover the whole swarm and structure that it is clinging to with a large sheet. Bees will naturally try to escape by flying upwards, so create a teepee (by pulling up the middle of the sheet, but without lifting the sheet off the ground) to trap the bees. Then spray aerosol chemical under the edges of the sheet to kill the swarm; have a number of people help with this.

High-output liquid insect killer can be used to effectively kill swarms that are at an unreachable height or near electrical wiring, or for situations where bagging or using a sheet is not suitable. The aerosol can be attached to an extension pole applicator to increase the reach of the liquid jet. The chemical should be sprayed onto the bees, starting at the top of the mass and working your way down as quickly as possible while ensuring plenty of chemical is applied. Depending on the size of the swarm, you can use an aerosol can in each hand for greater coverage.

The bees may become disturbed and start dispersing prior to destruction or as destruction begins. If this occurs, the swarm of flying bees can be tracked until they resettle at another location, often nearby.

Occasionally swarms must be tracked over hundreds of metres in complicated environments, so additional help is recommended. Once bees have resettled, choose the most appropriate technique and recommence destruction. Note that bees may disperse to a new nesting cavity. If the swarm is observed entering a cavity, use the following methods for nest destruction.



Figure 25 Asian honey bee swarm up high in a tree

Nests

After destroying a swarm or nest of bees (especially for suspect new incursions), it is vital to confirm that the queen bee has been captured. If you cannot find the queen amongst the dead bees, it is possible she may have escaped with enough worker bees to produce a viable colony elsewhere. Immediately conduct surveillance activities in the surrounding area in an attempt to locate the bees and/or confirm that the area is free of the pest bee (see 'Detection' on page 8).

Dead bees and treated comb should be removed from the cavity, correctly labelled and retained for laboratory analysis (see 'Sample management' on page 26). This will also minimise the risk to off-target animals that are attracted to the food source.

Aerosol sprays and permethrin dust are suitable for destroying nests of bees. Decide which chemical product will best suit the situation and ensure appropriate PPE is worn. The amount of chemical required to kill a nest of bees depends on the nest size and cavity location. Occasionally, multiple products may be required to successfully destroy a nest.

Examine the nest entrance and check for possible escape routes. Plug all of these holes with wet paper towel (or another suitable material). If the main nest entrance is large and there is potential for bees to escape through it during destruction, either plug the main hole and leave a small entry point, or have several people available to spray the main entrance at once.

If you cannot see the nest inside the cavity, listen for the high-pitched hum of the bee colony to locate it (the inability to see a nest is called a blind kill).

- Dispense the chemical into the nest entrance, covering as much area as possible within the cavity. Try to apply the chemical directly onto the comb and bees.
- Seal the main entrance, after applying enough chemical, and leave it for a few minutes. The fumes from the chemical should circulate within the cavity and kill the trapped bees.

- Spray any foraging bees that try to return to the nest.
- Listen for changes in sound from the nest; as the nest of bees dies, the high-pitched hum will decrease to a very low and dull hum. When the audible humming sound ceases, the nest can be presumed dead.
- Always ensure that the nest remains plugged for as long as possible to avoid off-target impacts. To ensure the success of the kill, check the nesting site 24 hours after destruction.

Tips for destroying nests

- When permethrin dust needs to diffuse around corners (e.g. in wall cavities), an electrical pump applicator can be used to spread chemical further, making it more likely to reach bees and comb deep within cavities.
- To safely destroy a nest located near electrical equipment use high-output liquid insect killer (e.g. a nest within a fuse or metre box). The high-output 'jet' spray applies chemical to targets up to 4.5 m away, in a 'knock down' effect.
- Two or more people, and several aerosol cans, may be needed for adequate chemical coverage of large nests.
- Where possible, spray nests late in the afternoon or very early in the morning, when the nests are less active and most of the foraging bees are present.





Other considerations



Figure 26 Biosecurity Qld staff member removing comb and honey from a destroyed nest

Recruiting a competent surveillance team

Consider the following when building a team for AHB surveillance and destruction:

- Seek help from an experienced bee handler who is keen to pass on his or her skills to the team. This bee handler should provide practical technical knowledge and advice on the skills required to handle bees in the field.
- When recruiting staff, consider each applicant's eyesight, literacy skills, communication skills and cultural sensitivity, problem-solving proficiencies and known bee allergies. Due to long working hours in outdoor conditions, only physically capable personnel should be engaged.
- Consider if contractors and staff are certified to work at heights and use a chainsaw.
- If necessary, engage a recruitment agency to assist with recruiting and selecting suitable candidates. In preparation for an emergency bee response, maintain a list of possible candidates to be assessed and recruited urgently when required.

The plans and procedures below are integral in building an effective new incursion response team:

- PLANTPLAN (under the Emergency Plant Pest Response Deed) is the agreed technical response plan used by biosecurity agencies and industry when responding to an Emergency Plant Pest incident. It outlines the phases of an incursion (investigation, alert, operational and stand down), as well as the key roles and responsibilities of industry and government during each of these phases. PLANTPLAN is available at www.planthealthaustralia.com.au
- Nationally agreed Standard Operating Procedures have been developed for use by authorities during responses to animal incidents and emergencies. You can find these documents on the Animal Health Australia website, www.animalhealthaustralia.com.au



Figure 27 Asian honey bee with full pollen sacks returning to the nest

Community engagement and public relations

Community engagement specialists are critical to ensuring the community understands, and is receptive to, any biosecurity response. It is essential that the community engagement plan includes strategies to both inform and empower the community. Clear and transparent engagement objectives must:

- Identify and collaborate with critical stakeholders – Some groups are critical in ensuring a successful response and will need a high level of engagement (e.g. local apiarists, local environmental and government staff, pest controllers). Establish a process that keeps these groups informed, enabling them to contribute to decisions that could impact the effectiveness of the response.
- Include a range of specific briefings with stakeholders and the community in both the affected and unaffected areas – Use a call to action such as *‘Biosecurity is everyone’s responsibility; each person can help by being alert and reporting suspect bees’*.
- Appoint an appropriate media spokesperson(s) – Consider the demographics of the affected area, including foreign languages or cultural groups. Advise the public of the presence and intent of the field staff to allay any community concerns.
- Brief field personnel regularly about engagement material and provide these staff with appropriate public relations training and strategies for answering difficult questions – Engaging and communicating with the public is vital and staff enthusiasm in this should be encouraged.
- Review the effectiveness of your engagement strategy to ensure the objective is providing both proactive and reactive results – A strategy needs to be flexible and adaptable to change during different phases. Evaluate and evolve your engagement tools to reach all demographics (e.g. use social media to engage more technically savvy community members).

A contained, live bee display is an excellent engagement tool that boosts community awareness of the pest bee. It also provides an opportunity for the community to distinguish between AHB, EHB and other bee species. Live bee displays are particularly effective at major community events (e.g. local shows).

Where it’s necessary to transport AHB to an uncontaminated zone, it is imperative that precautions are in place to negate the risk of spreading the pest, such as removing the queen and any brood from the display. Safety measures should also include a route plan, escape procedure, and a spill procedure in the event of a breach of containment during transportation. Consult a skilled beekeeper for advice during these activities. All bees should be contained in a separate section of the vehicle (such as in the sealed canopy of a utility vehicle) and not in the main section of the car. Ensure the vehicle is equipped with a destruction kit and complete sets of PPE.



Figure 28 Asian honey bee feeding on a mad hatter flower

Data collection and recording

Thorough data collection and recording is imperative for reporting, planning, consistency and analytical research. It is important to consider future program directions (e.g. determining an area that is free of the pest bee, or moving from eradication to management). The data collected should be useful for any of these purposes. Data is invaluable in:

- measuring the efficacy of all methods employed to find and destroy bees (or any other pest)
- determining the comparative sensitivity of different methods of detecting bees
- tracing where samples have come from, which is important for accountability, genetic testing (if applicable) and determination of the biology and ecology of the bee/pest species
- clarifying which trials/experiments have been conducted, how they were conducted and with what outcome.

Data collection needs to include as much detail as possible, for example:

- contact details (name address, email, phone numbers)
- situational information (maps, GPS details, location descriptions)
- photographs
- height of the nest or swarm and the tree/house/structure containing nest/swarm
- type of structure, or the type/species of tree/plant on which the nest or swarm was found
- the chemical used to destroy the swarm or nest
- date, time of day, duration
- weather information
- any additional comments relevant to the situation.

Design datasheets well to ensure that relevant information is recorded easily and accurately. Quality control of data, checking for errors, and providing staff feedback, will ensure accurate data collection. Also, ensure staff keep logbooks detailing who did what and when to determine the efficacy of any methods being trialled in the field.

Advise field staff to record all information, and complete forms and logbooks, in a timely manner—recalling details after an event can be difficult and create inconsistencies and errors in data. Also, make staff aware that while the paperwork may not feel important at the time, it is critical for the efficient and effective operation of the program.

Science should be an integral part of the program prior to, during, and throughout the program. A suitably qualified scientist (e.g. biologist, ecologist, zoologist or entomologist) should source as much information about the biology and ecology of the bee/pest species as possible and provide advice and guidance for detection, destruction and other relevant aspects of the program.

For the safety of laboratory staff, always record the chemical used to destroy a nest or swarm.

Figure 29 Physical characteristics of an Asian honey bee



Photograph courtesy of Arthur Giblin

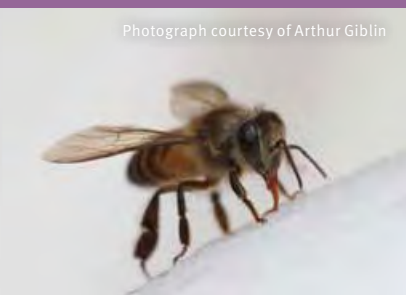


Figure 30 An adult *Varroa* mite

Figure 31 Both adult (brown) and immature stages (white nymphal mites) of *Varroa* mites can remain within the comb cells

Sample management

Exotic bees and suspect bee samples **must** be examined for pests and diseases, and most importantly, for bee mites such as *Varroa destructor*, *Varroa jacobsoni*, *Tropilaelaps clareae*, *Tropilaelaps mercedesae*, and *Acarapis woodi*. Where possible, abandoned nests should also be examined for mites—the preferred habitat for the *Varroa* mite is within the drone cell (see Figure 31). A full list of exotic pests and diseases that should be tested for, including notifiable established pests and diseases, can be found by visiting www.planthealthaustralia.com.au

Bee samples (e.g. comb) should be properly secured and contained in a labelled plastic bag or container. Douse the bees with insecticide spray or powder to kill them before entering any building. Ensure the samples are correctly labelled and that sample submission forms are completed before placing the sample into a freezer for a minimum of 24 hours.

Handle samples in ways that will not compromise their identification by laboratory staff:

- Equip vehicles with the means for keeping samples cool, if not frozen, during transportation.
- Store samples in a freezer, refrigerator, or on ice, to slow deterioration once at the laboratory.
- Clearly label samples with the type of chemical used to destroy the nest.
- Provide samples for laboratory identification as soon as possible.

Things to consider during shipping are:

- Samples can turn rancid during lengthy transportation.
- Samples can leak excess liquid (e.g. honey or ethanol).
- Exercise caution when handling dead bees to prevent accidental stings.





Figure 32 Asian honey bee queen (in the middle) with worker bees

Further information

The following reports capture the research outcomes of the Transition to Management Program and can be found by visiting our website at www.biosecurity.qld.gov.au

Commerford, MM & Koetz, AH 2013, *Ecology and behaviour of Asian honey bees (Apis cerana) in Cairns, Australia*, Department of Agriculture, Fisheries and Forestry (Queensland).

Commerford, MM, Wittmeier, N, Froemmcke, L & Koetz, AH 2013, *Optimising Asian honey bee (Apis cerana) trap design and attractants*, Department of Agriculture, Fisheries and Forestry (Queensland).

Hyatt, S 2011, *Asian honey bee (Apis cerana javana) in Cairns, Far North Queensland: Foraging, nesting and swarming behaviour—Report of field observations April 2007–September 2011*, Department of Employment, Economic Development and Innovation (Queensland).

Koetz, AH 2013, *Asian honey bee (Apis cerana) detection efficacy*, Department of Agriculture, Fisheries and Forestry (Queensland).

Koetz, AH 2013, *Spread of Apis cerana in Australia, 2007–2012*, Department of Agriculture, Fisheries and Forestry (Queensland).

Koetz, AH 2013, *The Asian honey bee (Apis cerana) and its strains—with special focus on Apis cerana Java genotype – Literature Review*, Department of Agriculture, Fisheries and Forestry (Queensland).

Koetz, AH & Hyatt, S 2013, *Asian honey bee (Apis cerana) remote nest treatment*, Department of Agriculture, Fisheries and Forestry (Queensland).

Koetz, AH & Scanlan, JC 2013, *Asian honey bee (Apis cerana) spread modelling*, Department of Agriculture, Fisheries and Forestry (Queensland).

Wittmeier, N & Hyatt, S 2013, *Asian honey bee (Apis cerana) destruction efficacy*, Department of Agriculture, Fisheries and Forestry (Queensland).

Unpublished reference material

Durkan, M 2010, 'Field guide to plant hosts of *Apis cerana* including declared plants pests within the AHB restricted area as potential hosts', Department of Employment, Economic Development and Innovation, Cairns.

Shield, J 2007, 'The Asian Honey Bee: Report of an incursion in Cairns 2007—Technical aspects of the response', Department of Primary Industries and Fisheries, Brisbane.

Zborowski, P, Shield, J, Doherty, B, Royer J and Bellis, GA 2008, 'The Asian Honey Bee: a guide to identification', Department of Primary Industries and Fisheries, Brisbane.

Other Biosecurity Queensland documents available upon request

Gilmour, R, Bell, C and Docherty, G 2012, 'Asian honey bee odour detection dog review', Department of Employment, Economic Development and Innovation, Cairns

'Responding to notifications of pest bees other than AHB and bee pests'

'Responding to public notifications of Asian honey bee'

To request documents, contact the Customer Service Centre on 13 25 23.

Biosecurity Queensland's Asian honey bee form

Additional comments for scientific use

OFFICE USE ONLY

Identified by		Signature	
Sample Identified as		Date	/ / 20

OFFICE USE ONLY – sending samples

Name of institution sample was sent		Date	/ / 20	Initial	
If sample was not sent provide reason					
Results received by (name)		Date	/ / 20	Initial	
Data entered into bioSIRT by (name)		Date	/ / 20	Initial	

OFFICE USE ONLY – summary of results

Positive identification	Yes / No	Comments	
Disease present	Yes / No	Comments	
Parasite present	Yes / No	Comments	

Key

Detected by	(PR) public report	(FS) floral sweeping	(BL) bee-lining	(Trap) bee trap
Destruction or extraction by	(BQ) Biosecurity Queensland	(C) contractor	PC(pest controller	(BK) beekeeper

Call 13 25 23 or +61 7 3404 6999
Visit www.biosecurity.qld.gov.au