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2 Executive summary

The exotic Asian honeybee (*Apis cerana*) was discovered in 2003 on Guadalcanal and Savo, Solomon Islands, coinciding with the demise of almost all managed European honeybees (*Apis mellifera*) and cessation of honey production. An assessment in 2004 concluded that the newly arrived bees had become well established, could not be eradicated, and would eventually spread. The losses of managed honeybees were attributed to robbing by the Asian honeybees and increased competition for floral resources. This project was initiated in 2007, following a request from the Solomon Islands Government, with the over-arching aims of (a) obtaining more information on the Asian honeybee in the Solomon Islands and (b) developing methods that would assist Solomon Islands beekeepers to reduce the negative impacts of the Asian honeybee.

The strain of Asian honeybee established in Solomon Islands is prone to swarming, produces very little honey, and has not been successfully domesticated elsewhere. Since permanent eradication was judged impossible, the project focussed on temporary suppression of the Asian honeybees. Based on a method previously used elsewhere to control feral European honeybees, the broad-spectrum insecticide fipronil was offered to foraging bees at 'bait-stations', allowing the lured bees to return to their hives where the poison is dispersed and destroys the colonies. Initially, for 4-7 days between 10.00 a.m. and mid-day, foraging Asian honeybees within the designated area are lured to bait-stations (500 metres apart) offering sugar-syrup rewards (60% sucrose in water). Once large numbers of bees are visiting (>500 arriving simultaneously), the sugar-syrup rewards are replaced at each station for a 1-hour period (11.00 a.m. - 12.00 noon) with fresh sugar-syrup containing 0.05% fipronil (TERMIDOR®). The bait-stations are then removed and any remaining poison bait buried. Before using the method, all managed European honeybees must be relocated (> 5.5 km from the nearest bait-station) and kept away for 4-6 weeks. A single treatment destroys most of the feral Asian honeybees within a designated area. The method is cheap, effective and had no observable negative effects. Used in conjunction with a modified hive that restricts entry to robbing Asian honeybees, this method will allow the development of beekeeping in the Solomon Islands to be resumed. However, beekeepers will need ongoing assistance in adopting these methods and in other means to upgrade their skills and beekeeping technology.

DNA fingerprinting shows that the bees invading Solomon Islands are of the same Java 'haplotype' of *A. cerana* that is invasive in Papua New Guinea and northern Australia, following its intentional introduction into Indonesian Papua during the 1970s. It is now well established on the Islands of San Cristobal (Makira Province), Guadalcanal (Guadalcanal Province), Savo, Florida (Central Province), New Georgia and Kolombangara (Western Province). Surveillance was established elsewhere.

Asian honeybees in Solomon Islands were found to carry the microsporidian pathogen *Nosema ceranae*, Kashmir bee virus and a Java strain of the parasitic mite *Varroa jacobsoni*. European honeybees were found to be relatively healthy, carrying sacbrood and chronic bee paralysis viruses, but being free of *Ascosphaera apis*, *Melissococcus plutonius* and *Paenibacillus larvae* (the cause of American foulbrood disease). They do carry *N. ceranae*, probably acquired from the introduced Asian honeybee, so trade in live European honeybees and used equipment should not be permitted from islands with Asian honeybees to those without. Neither Asian nor European honeybees in the Solomon Islands host any other species of parasitic mite (e.g. *V. underwoodi*, *Acarapis woodi* or *Tropilaelaps* spp.) but on islands currently inhabited by both European and Asian honeybees, the European honeybee colonies are invaded by low numbers of adult female *V. jacobsoni*. These mites do not currently cause serious harm to the colonies, lacking the ability to reproduce on the European honeybee brood; however, this situation will need to be continually monitored in case the mite develops this ability, as recently observed in Papua New Guinea.

3 Background

This project addresses a problem affecting European honeybees (*Apis mellifera*) in the Solomon Islands, the origins of which can be traced to neighbouring New Guinea. During the 1970s several hived colonies of the Asian honeybee (*Apis cerana*) were intentionally introduced into the Indonesian province of Papua (the western region of New Guinea formerly known as Irian Jaya) from Java (Anderson, 1994). The strain of bee introduced (a Java type) is difficult to manage, is a poor honey producer, swarms a lot and can negatively impact on managed European honeybees when the two bees are sympatric (Saleu, 2009).

Once in Papua, the Asian bees swarmed, multiplied in the wild and became invasive. They gradually became established throughout Papua, including on the offshore islands of Biak and Yapen. They then spread into neighbouring Papua New Guinea (PNG), where they were first detected at the northwest coastal town of Vanimo in 1986 (Delfinado-Baker and Aggarwal, 1987). By the late 1990s they had become established throughout PNG, including the offshore islands of New Britain, Boigu, Saibai and Dauan.

In March 2003, the same type of Asian honeybee was discovered more than 1000 km east of PNG in the Solomon Islands, on the Islands of Guadalcanal and Savo. Its rapid spread has also created new threats for Australia. Since 1995, 9 swarms of Asian honeybee, most originating from the New Guinea region, have been intercepted and destroyed on vessels at Australian seaports. A further 2 swarms from the region have penetrated Australia's quarantine barrier, the first at Darwin in June 1998 and the second at Cairns in May 2007 (Barry et al., 2010). The Darwin incursion was quashed, but an attempt to eradicate the Cairns incursion is still on-going. To date more than 300 colonies of the bee have been detected and destroyed in the Cairns region (Crook, 2011).

The discoveries of Asian honeybees on Guadalcanal and Savo Islands in the Solomon Islands coincided with the demise of most managed European honeybee colonies on both islands and the total cessation of honey production. At the time it was assumed that the colony losses were due to the effects of parasitic *Varroa* mites that the newly arrived bees were carrying. However, a CSIRO assessment of the incursions in 2004 showed that this was not the case, as the Asian honeybees were carrying a Java strain of *Varroa jacobsoni* and it could not colonize the few surviving European honeybee colonies, as it lacked the ability to reproduce on that bee's brood (Anderson, 2004). This was identical to the behaviour previously reported for this mite in European honeybee colonies in Java and New Guinea (Anderson, 1994). The CSIRO assessment concluded that the Asian honeybee had become well established on Guadalcanal and Savo Islands, could not be eradicated, and would eventually spread to other islands. The losses of managed European honeybees that coincided with the incursions were attributed to increased competition for floral resources from and, perpetual robbing by, Asian honeybees (Anderson, 2004).

Representatives of the Solomon Islands Government, the local honey industry and other stakeholders met in Honiara in 2004 and decided that the local beekeeping industry founded on European honeybees imported from Australia and New Zealand should be saved from the threat of Asian honeybees and efforts should be made to monitor the further spread of the Asian honeybees. Several areas were identified for immediate research including:

- Development of methods to reduce the negative impacts of Asian honeybees on managed European honeybees;
- Development of surveillance for Asian honeybees on islands still free of the bees;
- Surveys of Asian and European honeybees for pests and diseases.

This project was implemented in 2007 following a request to ACIAR for assistance from the Solomon Islands Government. The over-arching aims of the project were to (a) obtain more information on Asian honeybees in the Solomon Islands and (b) develop methods that would assist Solomon Island beekeepers reduce the negative impacts of Asian honeybees on managed European honeybees. Most project activity was directed at improving the foraging competiveness of managed honeybees in the presence of Asian honeybees. Extension and training was also carried out to address knowledge gaps and to support the uptake of new and improved beekeeping methods aimed at reducing the negative impacts of Asian honeybees. Other project activities involved implementing surveillance for Asian honeybees on islands still free of the bees and determining the pest and disease status of European and Asian honeybee populations.

Shortly after this project commenced, a survey carried out in New Guinea by the Australian project leader found that a previously harmless form of *Varroa* mite carried by Asian honeybees in PNG (the Java haplotype of *V. jacobsoni*) had developed a newfound ability to reproduce on European honeybee brood. This 'new' mite was destroying European honeybee colonies and causing hardship for beekeepers. At the same time, the same mite haplotype on Asian honeybees in neighbouring Papua and distant Java (where the haplotype originated) still lacked the ability to reproduce on European honeybee brood, although there were indications that it may be beginning to gain that ability in Papua (Anderson, 2008). This worrying new development added increased significance to studies in this project on *Varroa* mite behaviour in European honeybee colonies, as the mite type carried into the Solomon Islands by Asian honeybees was also the Java haplotype of *V. jacobsoni* (Anderson, 2004).

The project is aligned with the medium-term strategy of ACIAR for addressing biosecurity-related issues in Pacific Island countries and an ACIAR priority for the Solomon Islands of providing assistance with major pest problems affecting the smallholder honey industry. By delivering research outputs applicable across the Australasian-Pacific region, particularly to the New Guinea region, the project is also aligned with the organization's broader strategy of delivering research outputs that constitute public goods across regions and countries. The project builds on previous ACIAR projects on Asian bees and their mites in PNG, Indonesia and across the entire Asian region (PN 9028, AS2/1994/017, AS2/1994/018, and AS2/1999/060) in which valuable information has been obtained on the epidemiology, taxonomy, genetics, host-relationships and control of Asian honeybees and their parasitic mites.

4 Objectives

- Develop methods for reducing the negative impacts of Asian honeybees (*Apis cerana*) on managed European honeybees (*Apis mellifera*) in the Solomon Islands.
- Develop and implement surveillance for the early detection of Asian honeybees in the Solomon Islands.
- Determine the pest and disease status of Asian and European honeybees in the Solomon Islands.
- Obtain further information on *Varroa* mite reproduction.

5 Methodology

5.1 Suppressing Asian honeybee populations

The prime objective of this project was to develop methods that would reduce the negative impacts of Asian honeybees on managed European honeybees in the Solomon Islands. As there was no likelihood that the Asian honeybees could be domesticated, the approach taken here was to improve the foraging competiveness of the managed honeybees in the presence of Asian honeybees. Two options were available - permanent eradication or temporary suppression of the Asian honeybees. As Asian honeybees were already well established on Guadalcanal and Savo Islands and possibly on other Islands, temporary suppression became the only viable option. This is the first reported study in which attempts were made to suppress *A. cerana* populations in particular regions for the sole purpose of making those regions more conducive to beekeeping with *A. mellifera*.

The method used here to achieve suppression of Asian honeybees relied on the use of the broad-spectrum slow-acting insecticide fipronil to selectively destroy feral Asian honeybee colonies in defined areas. It was based on a method previously used in New Zealand and Australia to destroy European honeybee colonies (Taylor, 2003; Clark et al., 2006). It involves first attracting foraging honeybees from as many colonies as possible to ‘bait-stations’ with the lure of a food (sugar-based) reward. Then, when large numbers of bees have become familiar with regularly receiving the reward, fipronil is added to the reward. The bees transport the fipronil to their respective colonies before they themselves are poisoned and, as more and more lured bees enter colonies, the colonies become poisoned and are destroyed. Hence, the method has 2 main components. First, large numbers of foraging bees must be lured to bait-stations with the offer of a food reward. Studies in New Zealand suggest that more than 300 European honeybees simultaneously visiting a bait-station is adequate to cause the destruction of many colonies (Taylor, 2003), but the numbers of Asian honeybees that would need to be attracted to achieve a similar result is not known. Second, fipronil is added to the food reward at a concentration that allows individual lured bees to return to their respective colonies before being poisoned and dying. Hence, trials were conducted to (a) develop a means of attracting large numbers of foraging Asian honeybees to bait-stations and (b) finding a concentration of fipronil that, when added to a food reward, would effectively lead to the destruction of many feral Asian honeybee colonies.

5.1.1 Attracting foraging Asian honeybees to bait-stations

Trials were carried out on Savo Island to develop a means of attracting foraging Asian honeybees to bait-stations. This island is small, relatively isolated and had become totally free of European honeybees following the arrival of the Asian honeybee.

Foraging European honeybees can be lured to a ‘bait-station’ that has been provisioned with a feeder containing honey or sugar-syrup (50% or 60% sucrose in water) as a reward (Ribbands, 1953). The bait-station can be the feeder itself, or any structure that houses a feeder. Large numbers of foraging European honeybees can also be trained to visit bait-stations at a specific time of day, if the food reward is only made available on consecutive days at the required time (Wenner, 1961).

Hence, trials were carried out to develop an efficient feeder/bait-station for offering a food reward to foraging bees and a suitable food reward for attracting foraging bees. Subsequent trials were also conducted to determine the time of day when most foraging bees and other ‘non-target’ insects visited bait-stations and some environmental conditions that affected the numbers of foraging bees visiting bait-stations.

(a) Finding an efficient feeder/bait-station

This trial was conducted in the middle of the day in calm, clear weather and was repeated the following day in similar conditions at a new location. Sugar-syrup (60% sucrose dissolved in tank water) and squashed honeycomb (removed from a feral Asian honeybee colony and which contained honey and pollen) was offered to foraging Asian honeybees in 5 different feeders placed side by side in replica in both shaded and un-shaded locations (under a tree and in the open in direct sunlight respectively). The feeders were:

1) Commercially available yellow wasp trap suspended from a rope strung between 2 supports (usually trees) 1.5 meters above the ground. This trap had additional holes drilled in its lid so that arriving bees were free to arrive and depart the trap. The trap design is shown in Appendix 1B (traps of this kind were kindly supplied by Robert Ingram, DAFF, Canberra);
2) Commercially available cardboard flytrap suspended as in (1) 1.5 meters above the ground (traps of this kind were kindly supplied by Dr Mike Lacey, CSIRO Ecosystem Sciences, Canberra);
3) A 30cm diameter x 4cm deep metal enamel dish (hereafter referred to as a dish dispenser) placed 1.0 meter above the ground on top of 3 stacked empty bee hive boxes. Twigs were placed inside the dish to act as landing platforms for arriving bees (Appendix 1D). Dishes of this kind were purchased from a store in Honiara.
4) Standard Petri dish placed on a cardboard base and suspended 1.5 meter above the ground as in (1). Dishes of this kind were sourced from the CSIRO bee laboratory in Canberra.
5) One liter plastic drink bottle, modified to resemble the wasp trap described in (1), suspended 1 meter above the ground as in (1). Traps of this kind were included in the trial because they were cheaper than the commercially produced traps used in (1) and could be constructed in the Solomon Islands from recycled drink bottles.

The numbers of bees simultaneously visiting each feeder were counted once every 15 minutes over a 2-hour period.

(b) Determining a food reward for luring forager bees

Again, this trial was conducted in the middle of the day in calm, clear weather. In trial (a) above, sugar-syrup was found to be more attractive to foraging bees than honeycomb. Hence, a trial was conducted to determine whether melted wax honeycomb from an Asian honeybee colony, which would have contained honey pollen and possibly Asian honeybee pheromones, was more attractive to forager bees than sugar-syrup. The following three rewards were offered in replica dish dispensers at bait-stations in un-shaded locations. Each dispenser contained twigs that provided a landing platform for arriving bees.

1) Sugar-syrup (60% sucrose dissolved in tank water).
2) Raw honey removed from a feral Asian honeybee colony.
3) Cooled melted wax Asian honeybee comb (containing honey and pollen). The comb had been melted in silver foil over an open fire before being cooled (Appendix 1C).

The numbers of bees simultaneously visiting each feeder were counted once every 15 minutes over a 2-hour period.

(c) Assessing peak visitation times for forager bees at bait-stations and the impact of environmental conditions on bee visitations.

For this trial, a single bait-station, that contained a dish dispenser containing 60% sugar-syrup (which was replenished on a continuous basis) was placed in an un-shaded location and checked for the number of simultaneous visiting bees once every 60 minutes between 6.00am-6.00pm over a 2-week period.

These counts determined:

(a) The time of day of most bee visits (peak visitation time);

(b) The time of day of most non-target insect visits;

(c) Environmental conditions that impacted on bee visitations.

5.1.2 The use of fipronil to suppress Asian honeybee populations

The method developed here for suppressing Asian honeybee populations was based on the use of fipronil, as was used to successfully destroy European honeybees in Australia and New Zealand (Taylor, 2003; Clark, 2006). In those trials the insecticide was found to persist inside of poisoned colonies and could be transported from those colonies to healthy colonies nearby via robbing bees from the healthy colonies. The toxicity of the insecticide in poisoned colonies eventually broke down. This effect from using fipronil had to be considered in the current study.

The method involved initially attracting as many foraging Asian honeybees as possible to a bait-station at the same time each day, with the lure of a food reward. When large numbers of bees had been regularly attracted to the bait-station, the food reward was replaced for a 1-hour period with a fresh reward, but which contained 0.05% fipronil. The form of fipronil used was TERMIDOR® (residual termicide, active constituent 100g/l fipronil).

Trials were carried out to determine whether a low concentration of fipronil (0.05%) would kill feral Asian honeybee colonies and whether it could be used safely by beekeepers to reduce the negative impacts of Asian honeybees on their managed European honeybees. An initial trial with the insecticide was carried out on Savo Island. When this trial proved safe, a further trial was carried out on the larger and more accessible Guadalcanal Island.

Fipronil trial on Savo Island

The aims of this trial were to determine how efficient a low concentration of fipronil was at suppressing feral Asian honeybee colonies at increasing distances away from a bait-station and to assess the time taken for the Asian honeybee population to rebuild after being suppressed with fipronil.

The trial began by locating feral Asian honeybee colonies that could be monitored both before and after the application of fipronil. During October 2008 a total of 8 accessible feral colonies were located in a 0.5 km^2 area on the eastern part of the island. Most were located in the walls of houses and hence bee flight activity (details below) could be monitored at their entrances (Appendix 2B). The location of the colonies in relation to the bait-station is shown in Appendix 2C. The closest and most distant colony from the bait-station was approximately 30 and 400 meters respectively. Other feral colonies were also present in the area, but they were inaccessible (mostly in the canopy of coconut trees) and could not be monitored.

Flight activity was monitored by counting the numbers of bees departing and arriving at the entrances of each colony over 3 consecutive 60-second periods between 11.00am-12.00pm each day from 11-13 November 2008. On 14 November, fipronil was applied at the feeder between 11.00am-12.00pm (details below) and flight activity on that day was similarly measured at each colony between 10.00am-11.00am and again between 1.00pm-2.00pm (that is, immediately before and after the application of fipronil). Then, from 15-21 November (a 1 week period) between 11.00am-12.00pm the colonies were monitored for bee and bee flight activity.

The application of fipronil was as follows. Asian honeybee foragers within the area were lured to a single bait-station provisioned with 2 dish dispensers containing 60% sugar-syrup (Appendix 2A) on a continuous basis between 10.00am and 12.00pm each day from 7-13 November 2008. On 14 November the sugar-syrup was initially offered in the dish dispensers for 1 hour between 10.00am-11.00am, and then replaced between 11.00am and mid-day with sugar-syrup containing 0.05% fipronil. At the end of this 1-hour period unused fipronil-ladened sugar-syrup was buried in the ground and the dish dispensers cleaned using water and detergent.

The area where the bait-station had been located was revisited on 4 April, 20 May and 5 December 2009 (or at 141, 188 and 386 days post-fipronil treatment) to assess the numbers of feral Asian honeybee colonies and thus gauge the time it took for Asian honeybees to re-colonize the site.

Fipronil trial on Guadalcanal Island

This trial was carried out at Sisters of the Church, Teteni Kolivuti Training Centre, about 7km east of Honiara airport. The aims were to verify results of the Savo Island trial and to determine the time required before managed European honeybee colonies could be safely introduced to an area in which fipronil had been used to suppress Asian honeybees. There were no hived European honeybee colonies on Guadalcanal Island before this trial commenced. However, as the trial commenced, six 6-frame nucleus-hives containing European honeybee colonies were introduced from Malaita Island and kept at the DAL compound in Honiara until needed at the end of the trial.

As on Savo Island, the first task was to locate feral Asian honeybee colonies that were accessible within a 0.5 km^2 area around the Training Centre. During April 2009, 10 accessible colonies were located in walls of buildings attached to the Centre. The location of each of these colonies in relation to the bait-station is shown in Appendix 2H. The closest and most distant colony from the bait-station was approximately 35 and 310 meters respectively.

Foraging Asian honeybee within the area were lured to a single bait-station provisioned with 2 dish dispensers containing 60% sugar-syrup (Appendix 2A) on a continuous basis between 10.00am and 12.00pm each day from 1-8 May 2009. Bee flight activity at the entrances of each the 10 Asian honeybee colonies was monitored between 11.00am-12.00pm each day from 6-7 May 2009, as described previously. On 8 May sugar-syrup was initially offered in the dish dispensers for 1 hour between 10.00am-11.00am, and then replaced between 11.00am and mid-day with sugar-syrup containing 0.05% fipronil. At the end of this 1-hour period the unused poisoned sugar-syrup was safely disposed of, as described above. On this day bee flight activity was also monitored at the entrances of each of the 10 colonies between 10.00am-11.00am and again after application of fipronil between 1.00pm-2.00pm. Then, on each day during a 1-week period following application of the fipronil (from 9-15 May) bee and bee flight activity was monitored at each colony.

To assess the time required for fipronil to breakdown in Asian bee colonies before managed European colonies could be safely introduced, two 6-frame nucleus European honeybee colonies were introduced on 22 May 2009 (2 weeks after application of fipronil) to the site where the bait-station had been located. A further 2 nucleus colonies were introduced to this site on 19 June 2008 (6 weeks after application of fipronil). These nucleus colonies were then monitored for abnormal brood, abnormal bee behaviour and signs of bee population decline.

5.2 Implementing surveillance for Asian honeybees

To establish the presence and spread of the Asian honeybee in the Solomon Islands, project staff visited San Cristobal, Guadalcanal, Savo, Florida, Malaita, New Georgia, Kolombangara, Gizo, Ranongga, Vella Lavella and Choiseul Islands to liaise with local people and beekeepers. When surveillance seemed warranted, it was initiated with the help of regional DAL Officers and local beekeepers. The surveillance adopted at particular locations depended on local available resources, but involved a combination of some or all of the following:

- Erection of bait-stations provisioned with sugar-syrup to attract foraging bees;
- Visual checks for foraging Asian honeybees on flowering plants;
- Placement of 'bait hives' (empty European honeybee hives) for attracting newly arrived Asian honeybee swarms and;
- Following-up on reports from the public of bee swarms and wild bee colonies.

To assist with surveillance efforts, trials were also carried out on Savo Island to determine whether chemical lures would improve the attractiveness of sugar-syrup presented to foraging Asian honeybees. Solutions of the chemical lures were dissolved in controlled-release dispensers of medical rubber (4 cm length, 0.5 mm OD, 0.3 cm ID). In order to provide a reward as well as a potentially attractive odor, the lures were placed in pairs on small globules of honey in an array on top of 3 stacked wooden hive boxes (Appendix 1E). The box was located 4 m away from two dish dispensers, one containing sugar-syrup and the other honey. The chemical lures were:

- Acetic acid (5 mg). Acetic acid is well known as an attractive odor for flying insects, particularly those searching for carbohydrates;
- Mixture of acetic acid (5 mg) and isobutanol (5 mg). Isobutanol is prominent in the odor of molasses;
- Mixture of citral (10 mg) and geraniol (10 mg). These are well known floral odors;
- Queen aggregation pheromone of *A. cerana javana* (20 queen equivalents), identified previously;
- Nasanov aggregation pheromone of *A. mellifera* (20 worker equivalents) (it has been claimed that the Nasanov pheromones of A*. mellifera* and *A. cerana javana* are the same);
- Mixture of queen aggregation pheromone and Nasanov aggregation pheromone.
- 1-Eicosanol (1 mg) (it has been reported that this substance is very attractive to *A. cerana indica*);
- 1-Eicosanol (5 mg).

The numbers of bees visiting the lures were counted over a 20-minute period. However, initially, there were very few bee visits to, and no bee landings on, the dispensers over the monitoring period. It was evident that the bees were not being diverted from the favored location of the dish dispensers nearby. Hence, the dishes were removed from their location and placed close to the box containing the array of chemical lures. There were immediate responses by the bees and bee landings on the alternative dispensers were recorded over 20 minutes. Landings were disregarded when they merely involved landing on the globules of honey.

Because *A. cerana* foragers were observed to be highly attracted to the flowers of coconut palms and bananas, the odors of each of these flowers were also extracted by dissolution in dichloromethane. In each case, the solution was filtered and evaporated in a dish dispenser to regain the odorous essence. Sugar syrup (200 mL) and twigs (that provided a landing platform for bees) were added to each dish. The relative attractiveness of the two flower-laced dishes to foraging honeybees was then assessed with that of a dish containing only sugar-syrup.

5.3 Establishing the disease status of Solomon Island honeybees

Samples of dead, diseased and healthy honeybees and parasitic bee mites were collected from Asian and/or European honeybee colonies on islands that were colonized by only *Apis mellifera* (Malaita, Gizo, Ranongga, Vella Lavella and Choiseul), only *Apis cerana* (Guadalcanal and Savo) and both *Apis cerana* and *Apis mellifera* (San Cristobal, New Georgia and Kolombangara). The mites and apparently healthy honeybees were collected directly into 70% alcohol, whereas dead and diseased bees were frozen at -20^{o}C immediately after being collected. All collected samples were transported under AQIS permit (No. 200110142) to CSIRO Entomology laboratories in Canberra and stored at -20^{o}C until being tested.

In the laboratory extracts were obtained from dead, diseased and apparently healthy honeybees and tested for the presence of known and unknown bacterial, fungal, protozoan and viral pathogens and, in the case of healthy bees, tracheal mites, using methods described by Anderson (1990). The identity of *Varroa* mites was determined from their mtDNA COI gene sequence (Anderson and Fuchs, 1998) and from morphological measurements (Anderson and Trueman, 2000).

Laboratory tests were also carried out in Spain to determine the identity of *Nosema* spores found in Asian and European honeybee colonies in the Solomon Islands. Abdomens of 10-15 bees from each sample were macerated in 5 ml of distilled water with a mortar and pestle and, after centrifugation, DNA was extracted as described by Higes et al., (2008) and tested for *N. apis* and *N. ceranae* by PCR, using primers and methods described by (Martín-Hernández et al., 2007).

5.4 Extension and training activities

Project staff visited beekeepers and training centres throughout the Solomon Islands to liaise with beekeepers and conduct workshops on Asian honeybees and their control. The islands visited included San Cristobal, Guadalcanal, Savo, Malaita, New Georgia, Kolombangara, Gizo, Ranongga, Vella Lavella and Choiseul. These visits also allowed staff to assess local honey yields, beekeeper access to hive and honey processing equipment and the state of current bee stock.

Work was also carried out on Malaita Island to develop a European honeybee box hive that would help prevent robbing by Asian honeybees. Malaita Island is currently free of Asian honeybees, but most of the hive material used in the Solomon Islands is constructed there. During this work, training was also provided to local beekeepers in queen rearing and colony propagation, which would enable beekeepers to produce and sell excess colonies and queens to beekeepers on those islands where Asian honeybees have reduced the European honeybee population.

5.5 Obtaining information on *Varroa* mite reproduction

As mentioned earlier, soon after this project commenced a survey of bees and bee mites in New Guinea and Indonesia found that a previously harmless form of *Varroa* mite carried by Asian honeybees in PNG (the Java haplotype of *V. jacobsoni*) had developed a newfound ability to reproduce on European honeybee brood (Anderson, 2008). This new development added increased significance to observations in this project on *Varroa* mite behaviour in European honeybee colonies, as the mite type carried into the Solomon Islands by Asian honeybees was also the Java haplotype of *V. jacobsoni* (Anderson, 2004).

In this project detailed information was obtained on the reproductive behaviour of *Varroa* mites on Asian and European honeybees. Generally, when a European honeybee colony was sampled, the owner beekeeper was first questioned on the health, honey yields and movements of the colony and of other colonies in the apiary. The hive was then opened and the adult bee population on combs visually examined for the presence of external mites. Brood frames were removed and visually inspected for signs of mite infestation. The caps of about 300 worker or drone brood cells were then removed using a pair of fine forceps, the brood removed, and the bottoms of cells inspected for signs of mite infestation (mite excreta and the presence of mite nymphs). The reproductive status of an invading mother mite was noted (that is, whether she was reproducing or not reproducing). Mites were placed in vials containing 70% ethanol and later identified at the laboratory in Canberra as described previously. The reproductive behaviour of female mites in capped Asian honeybee worker and drone cells was likewise examined.

To obtain more information on the reproductive system of *Varroa* mites, a 3D model of a female *Varroa destructor* mite was partially constructed from ultra thin sections embedded in wax. However, as this work progressed it was found that the organization and structure of the mite's internals organs could be more easily viewed using a simpler fluorescent staining technique. This technique involved placing mite samples on microscopic slides, covering them with Dako's Fluorescent Mounting Media, leaving overnight to solidify then examining with a fluorescent microscope in the green channel (wavelength 490nm). The success obtained with this technique obviated the need for further construction of 3D models from ultra thin sections.

6 Achievements against activities and outputs/milestones

Objective 1: To develop and implement a method for suppressing feral Apis cerana *populations on Guadalcanal and Savo Islands.*

No.	Activity	Outputs/ Milestones	Completion Date	Comments
1.1	Identify areas where *Apis cerana* populations will be suppressed, erect bait-stations of different designs that contain sugar-syrup without insecticide. Monitor the numbers and kinds of insect visitors.	Information on the effectiveness of different bait-station designs for attracting *Apis cerana* but not non-target insects. Beekeepers and collaborators trained in the use of bait-stations.	30 June 2008	Savo Island was considered to be the most appropriate site to conduct an initial trial to suppress Asian honeybees, as it was small, relatively isolated and totally free of European honeybees. Preliminary trials had to be conducted first to find the best means of attracting foraging Asian honeybees to bait-stations. A simple but effective bait-station was developed that attracted foraging bees and it was used in subsequent trials using fipronil to suppress Asian honeybees, as well as for surveillance purposes. A local farmer (Mr James Tom) and DAL staff assisted in setting up and monitoring the Savo Island trials.
1.2	Erect bait-stations shown to be most efficient at attracting *Apis cerana*, remove hived *Apis mellifera* colonies from test areas, add fipronil to bait-stations and monitor the numbers of *A. cerana* visitors. Remove fipronil from bait-stations and monitor numbers and kinds of insect visitors. Re-introduce *A. mellifera* to treated areas.	Information on the effectiveness of fipronil for suppressing *A. cerana* populations. Successful methods communicated to farmers and collaborators.	30 June 2010	Fipronil proved successful in suppressing Asian honeybee populations in a defined area for between 6-12 months. Its use caused no observable detrimental effects to humans, domestic animals, non-target insects or wildlife. Mr James Tom assisted in setting up and monitoring the fipronil trials on Savo Island. After the Savo Island trial proved safe a further trial using fipronil was carried out on the larger and more accessible Guadalcanal Island. The Guadalcanal trial verified the results obtained on Savo Island and also showed that hived European colonies could be safely introduced rather quickly to an area in which the feral Asian honeybee population had been suppressed with fipronil. Project and- non-project staff of DAL assisted with the Guadalcanal trial.

PC = partner country, A = Australia

Objective 2: To develop and implement a surveillance system for the early detection of Apis cerana *in the Solomon Islands.*

No.	Activity	Outputs/ Milestones	Completion Date	Comments
2.1	Introduce an *Apis cerana* surveillance system on to Malaita and Kolombangara Islands.	A system in place for monitoring the spread of *A. cerana* in the Solomon Islands. Beekeepers and collaborators trained in bee surveillance	30 June 2008	Surveillance for the Asian honeybee was implemented on Malaita, Gizo, Ranongga, Vella Lavella and Choiseul Islands. It was not necessary to implement surveillance on Kolombangara Island, as Asian honeybees had already colonized that Island. Regional DAL officers and local beekeepers assisted with this work.
2.2	Assess and improve the effectiveness of *Apis cerana* pheromone blends for attracting *A. cerana* (on Guadalcanal and Savo Islands). Upgrade and monitor surveillance systems on Malaita and Kolombangara Islands.	An effective pheromone for use in attracting *A. cerana* to bait-stations. Collaborators trained on how to make the pheromone	30 June 2010	Sugar-syrup proved to be the most effective lure for attracting foraging Asian honeybees to bait-stations. The addition of known insect attractants, Asian and European honeybee pheromone blends and flower extracts to the sugar-syrup did not improve its attractiveness to foraging bees. Mr James Tom assisted in this work.

PC = partner country, A = Australia

Objective 3: To determine the pest and disease status of Apis cerana *and* Apis mellifera *in the Solomon Islands.*

No.	Activity	Outputs/ Milestones	Completion Date	Comments
3.1	Collect samples of dead and diseased bees from *Apis cerana* and *Apis mellifera* colonies in the Solomon Islands and transport them under permit to CSIRO laboratories in Canberra. Test samples for pests and diseases.	An official health status for Solomon Island honeybees. Beekeepers and collaborators trained in the recognition of symptoms of known bee diseases.	30 June 2010	European honeybees throughout the Solomon islands were found to be relatively pathogen free, compared to honeybees in other regions. The Asian honeybee almost certainly introduced the serious microsporidian pathogen *Nosema ceranae* into the Solomon Islands and it now infects European honeybees on islands that have been invaded by Asian honeybees. This pathogen may have played a role in the demise of European losses that coincided with the arrival of Asian honeybees. Importantly, the *Varroa* mite introduced by Asian honeybees into the Solomon Islands was found to be harmless to European honeybee colonies.

PC = partner country, A = Australia

Objective 4: To obtain information on the Varroa *mite reproduction system*.

No.	Activity	Outputs/ Milestones	Completion Date	Comments
4.1	Construct 3D models of the male and female *Varroa* mite reproductive system from ultra-thin sections presently scanned onto computer CD's.	Information on the organization and structure of the *Varroa* mite reproductive system.	30 June 2010	A simple fluorescent staining technique was found to be better for viewing the organization and structure of the *Varroa* mite's internals organs than the laborious construction of 3D models from ultrathin sections. More attention was given to examining the reproductive behavior of *Varroa* mites in Asian and European honeybee colonies in the Solomon Islands than was intended before the project began. This was necessary after the discovery in PNG in 2008 that the same *Varroa* mite genotype carried by Asian honeybees in the Solomon Islands had developed a newfound ability to reproduce on European honeybee brood.

PC = partner country, A = Australia

7 Key results and discussion

7.1 Suppressing Asian honeybee populations

7.1.1 Attracting foraging Asian honeybees to bait-stations

Finding an efficient feeder/bait-station

No foraging Asian honeybees were observed visiting feeders that were located in shaded situations during the 2-hour observation period of 2 independent trials. In contrast, foraging bees visited 4 of the 5 feeders placed in un-shaded situations in these trials (Appendix 1A, Table 1). The feeder that did not attract foragers in the un-shaded situations was a commercially available cardboard flytrap.

It generally took an hour or more for foraging bees to be first attracted to the feeders. Fresh honeycomb (from an Asian honeybee colony) appeared to attract foragers slightly faster than sugar-syrup (a mean of ~80 minutes versus 86 minutes respectively in trial 1 and ~67 minutes versus 71 minutes in trial 2), although these differences were not significant. However, the rate of recruitment of other foragers once the reward was discovered was greatest at those feeders that contained sugar-syrup. For example, the total numbers of foraging bees that visited feeders with sugar-syrup during the 2-hour observation period was 23 and 20 in trials 1 and 2 respectively, while 9 and 8 foraging bees visited feeders containing honeycomb in these respective trials.

The feeder referred to as a 'dish-dispenser' attracted the most foraging bees and it also attracted bees faster than the other feeders, regardless of the type of food reward offered. This dispenser was a 30cm diameter x 4cm deep metal enamel dish in which twigs were placed to act as landing platforms for bees (Appendix 1D).

In summary, the most efficient bait-station for attracting foraging Asian honeybees was a single dish dispenser containing 60% sugar-syrup as a reward placed on the top of empty bee boxes in direct sunlight. The dish is sold at local shops throughout the Solomon Islands. This bait-station was used in further trials to determine whether the food reward offered in it could be made more attractive to foraging bees.

Determining a food reward for luring forager bees

It was overwhelmingly clear that sugar-syrup was far more attractive to foraging Asian honeybees than raw honey or cooled melted honeycomb, the latter two obtained from an Asian honeybee colony (Appendix 1D). For surveillance purposes, further trials were conducted to determine whether sugar-syrup could be made more attractive to foraging Asian honeybees by the addition of pheromones and flower extracts, and the results of those trials are reported in Section 7.3.

Assessing peak visitation times for forager bees at a bait-station and the impact of environmental conditions on bee visitations.

Foraging Asian honeybees were observed visiting the bait-station between 6.00am and 6.00pm. However, fewer bees visited the station early in the day, between 6.00am-9.00am, and late in the day, between 4.00pm-6.00pm, than at other times of the day. The numbers of bees visiting the station steadily increased from about 9.00am, reached a maximum between 12.00pm-1.00pm, and declined thereafter.

Most non-target insects, such as butterflies, flies and stingless bees, visited the bait-station early in the day, between 6.00am-9.00am. Their visitations declined rapidly after 9.00am as visitations by Asian honeybee increased. Flies tolerated the increase in

Asian honeybee visitations more than other non-target insects, but even their visits ceased as the Asian honeybee visits peaked.

The presence of flowering plants in the local environment and adverse weather had a notable effect on the numbers of foraging Asian honeybees visiting the bait-station. Usually the number of visitations fell in overcast conditions and usually totally ceased when it rained. Visitations also fell when nearby Malaysian apple (*Syzygium malaccense*) was in flower close to the feeding station (Appendix 1F).

In summary, more foraging Asian honeybees visited the bait-station in the middle of the day than at other times of the day. During this peak visitation time there were virtually no non-target insects visiting the station. Bees were also less attracted to the bait-station during adverse weather conditions and when there was a competing natural food source (flowering plants) in the local environment.

7.1.2 Suppressing Asian honeybees using Fipronil

Fipronil trial on Savo Island

Flight activity at the entrances of each of the 8 Asian honeybee colonies, both before and after application of the fipronil, is shown in Appendix 2D, Table 2 and more graphically in Appendix 2E. A notable drop in flight activity two days before the application of fipronil was due to rain and windy weather conditions. On the morning prior to the application of fipronil more than 500 foraging bees were simultaneous visiting the bait-station. It took about 20 minutes following the application of fipronil for bees visiting the bait-station to show abnormal symptoms. Some began to rest on nearby vegetation and groom themselves. Thirty minutes after fipronil application some bees had become flightless and were spinning in circles on the ground. Forty-five minutes after application of the fipronil bee visitations to the bait-station had completely ceased.

In the hour following application of fipronil, flight activity was significantly reduced at the entrances of each of the 8 colonies (Appendix 2D; 2E). Over the next 7 days, 6 of the 8 colonies died, 1 had absconded and 1 continued to survive. The sole surviving colony was the most distant colony from the bait-station and its entrance was pointed in the opposite direction to the bait-station. Nonetheless, flight activity at the entrance of this colony fell continuously over the 7-day observation period. Some of the effects that fipronil caused to one colony, which was located 40 metres from the bait-station and which died within 2 days of the application of the fipronil, are shown in Appendix 2F and Appendix 2G.

It took more than 4 months for feral Asian honeybee colonies to begin to re-colonize the trial site following application of fipronil. Five months after application of fipronil, 2 new colonies were found in the walls of houses at the site. A month later, a further colony was found. Twelve months after application of fipronil a total of 6 new colonies were found at the site.

In summary, the application of 0.05% fipronil for a 1-hour period between 11.00am-midday at the bait-station effectively suppressed feral Asian honeybee colonies in a 0.5 km^2 area for more than 6 months and possibly up to 9-12 months. The results indicated that wide suppression of Asian honeybee colonies could be achieved by using more than one bait-station, each placed 0.5 km apart.

Fipronil trial on Guadalcanal Island.

On the morning prior to the application of fipronil more than 2,000 foraging bees were simultaneous visiting the bait-station. Like at Savo Island, it took about 20 minutes following the application of fipronil for bees visiting the bait-station to show signs of poisoning and, by 45 minutes following application of fipronil, bee visitations to the station had totally ceased.

The general pattern of flight activity at the entrance of the 10 colonies before and after application of the fipronil was also very similar to that found at Savo Island (Appendix 2I, Table 3). In the hour following application of fipronil, flight activity was significantly reduced at the entrances of each colony. During the next 7 days, 9 of the 10 colonies died. One of the colonies that died had attempted to escape the effects of the fipronil by building new comb in the same cavity, a short distance from the old combs.

Hived European honeybee colonies, that were introduced 2 and 6 weeks after the fipronil treatment to the site where the bait-station had been placed, showed no visible signs of being affected by fipronil when inspected at fortnightly intervals up to 6 months after their introduction. This indicated that the fipronil had lost its toxicity in the poisoned Asian honeybee colonies or that the European honeybee colonies were not robbing from the poisoned hives. Observations indicated that the poisoned Asian honeybee colonies were being destroyed rather quickly after application of the fipronil, mostly by wax moth larvae that did not appear to be affected by the fipronil, and this may have quickly dispersed fipronil residues remaining in the colonies.

In summary, the Guadalcanal trial verified the results obtained during the Savo Island trial and showed that managed European honeybee colonies can be introduced into a fipronil-treated area as little as 2-weeks after application of fipronil. However, as a precautionary measure, it is recommended a withholding period of 4-6 weeks between the application of fipronil in a designated area and the introduction of managed colonies into that area.

The Guadalcanal Island trial did not allow for an assessment of the 'safe distance' that managed European honeybee colonies would have to be moved from an area due to receive fipronil treatment. However, this distance would be equivalent to the distance that European forager bees fly away from their hive to collect pollen or nectar, plus the distance where fipronil loses its effectiveness from a bait-station. In the Savo Island and Guadalcanal trials fipronil appeared to be effective up to a distance of 0.5km from a feeding station. Further, as a rule of thumb, the foraging distance of European worker bees extends for 3 km, but, in rare instances, may be twice or three times this distance (Winston, 1987). In the Solomon Islands this foraging distance would probably be about 4-5 km at most, due to the typical vegetation of dense plantations or jungle. Hence, for the Solomon Islands it is recommended that managed European honeybee colonies be moved about 5.5 km away from the closest bait-station prior to fipronil treatment.

7.2 Implementing surveillance for Asian honeybees

DNA fingerprinting confirmed that the Asian honeybee now present in the Solomon Islands is the Java 'haplotype' of *A. cerana*. This is the same bee that has become invasive in nearby PNG and which is now present in northern Australia, following its artificial introduction into the Indonesian province of Papua from Java during the 1970's (Anderson, 1994).

Over the course of this project the Asian honeybee continued to spread through the Solomon Islands. It is now well established on the Islands of San Cristobal (Makira Province), Guadalcanal (Guadalcanal Province), Savo, Florida (Central Province), New Georgia and Kolombangara (Western Province). Surveillance for the bee was implemented on Malaita, Gizo, Ranongga, Vella Lavella and Choiseul Islands, all still free of the bee. During early 2010 project staff were also informed that a single Asian honeybee swarm had been detected and destroyed in late 2009 on the south coast of Gizo Island. Subsequent surveillance has not detected any more Asian honeybees on the island.

In experiments on Savo Island to determine whether chemical lures could improve the attractiveness of sugar-syrup to foraging Asian honeybees during surveillance, the numbers of bees that visited the different lures over a 20-minute period were as follows.

- 1-eicosanol (1 mg) – 9 landings
- Control (medical rubber) - 7 landings
- 1-eicosanol (5 mg) - 6 landings
- Queen aggregation pheromone - 5 landings
- Acetic acid/isobutanol - 2 landings
- Acetic acid - 2 landings
- Citral/geraniol - 2 landings
- Queen and Nasanov pheromones - 1 landing
- Nasanov pheromone - nil landings

While the number of landings on the control diminishes the significance of these results, the control had been placed equidistant from the two 1-eicosanol dispensers and may have benefited from their vicinal odor plumes. The responses to the 1-eicosanol dispensers were prominent in both experiments and increased with lower concentration. This suggested that the applied concentrations of 1-eicosanol were rather high and that levels of 0,1 mg or less may have been more attractive to *A. cerana.* However, this did not prove to be the case in subsequent experiments.

The acetic acid and isobutanol combinations appeared to be particularly attractive to foragers in the first experiment but this attraction was not sustained in the second experiment. The queen aggregation pheromone showed some attraction to foragers even though it was primarily designed to entice swarms and scout bees. The Nasanov aggregation pheromone displayed no attraction in either experiment, which is consistent with our previous findings that the Nasanov pheromone for *A. cerana javana* is completely different to that for *A. mellifera* (Lacey, 1999).

Sugar-syrup by itself proved to be the more attractive to foraging Asian honeybees that coconut and banana-flavored sugar-syrups. This may have been a consequence of the presence of residual footprint pheromone from its previous exposure to the foragers. For this reason, the original dish of sugar-syrup was removed and the relative attractiveness of the flower-laced dishes compared. The coconut-flavored syrup was clearly favored over the banana-flavored syrup by a factor of 30 to 4. Thus, the essence of the coconut flower may have a role to play in the attraction of foragers of *A. cerana javana*, but it was clear that the primary attractant was the sugar-syrup.

7.3 Establishing disease status of Solomon Island honeybees

Asian honeybees in Solomon Islands were found to carry the microsporidian pathogen *Nosema ceranae*, Kashmir bee virus and a Java strain of the parasitic mite *Varroa jacobsoni*. They do not host any other species of parasitic mite, including *V. destructor*, *V. underwoodi*, *Acarapis woodi* (the tracheal mite) or *Tropilaelaps* spp.

European honeybees were found to be relatively disease-free, compared to honeybees in other regions. They carry *N. ceranae*, and evidence suggests they have recently acquired this parasite from the Asian honeybee. For this reason, trade in live European honeybees (e.g. queen bees) and used beekeeping equipment should not be permitted from islands with Asian honeybees to islands without Asian honeybees. European honeybees in the Solomon Islands also carry sacbrood and chronic bee paralysis viruses, but are free of *Ascosphaera apis* (the cause of chalkbrood disease), *Melissococcus plutonius* (the cause of European foulbrood disease), *Paenibacillus larvae* (the cause of American foulbrood disease) and the parasitic mites *V. destructor*, *A. woodi* and *Tropilaelaps* spp. The European honeybee colonies in the Solomon Islands are also invaded by low numbers of adult female *V. jacobsoni*, which spread from the Asian honeybee colonies. These mites do not harm the colonies, as they are unable to reproduce on the European honeybee brood. This is discussed further in Section 7.6.

The cane toad (*Bufo marinus*) was also found to be a serious pest of managed European honeybees throughout the Solomon Islands.

7.4 Extension and training activities

Extension and training activities during the project confirmed that beekeeping with the European honeybee is a highly regarded activity in the Solomon Islands and there is much potential for expansion. Beekeeping is small scale with only a few beekeepers owning more than 50 hives; most own 25 hives or less. The European honeybees are stationary and produce honey yields of about 25kg/hive/year. Most of the honey is very dark and has a high moisture-content (19-21%). The majority of honey is collected during the drier season from April to October. Hive boxes and frames are made locally from local timber and are accessible to beekeepers with funds. However, many beekeepers continue to lack access to boxes and frames and their current equipment is in need of repair (Appendix 3A). Most beekeepers also experience difficulties in accessing other equipment that needs to be imported, such as foundation (Appendix 3B), hive tools, smokers, veils and honey processing equipment. Such equipment is often shared between many beekeepers. Hive boxes, lids and bases do not last much longer than 5 years, due to the hot humid conditions and limited use of wood preservatives.

Locally produced honey attracts a premium price and demand for it far outstrips supply. Honey is purchased for between SBD12 and SBD20 (Solomon Island dollars, Aus$1 currently equals approx. SBD7) and it is mostly sold locally, although some is exported or sold to foreign fishing boats, which attracts a much higher price.

The local European honeybee genetic stock showed acute signs of inbreeding at many locations and this negatively impacts on honey yields (Appendix 3C). There is an urgent need to import new improved stock. The difficulty in accessing new bee stock and beekeeping equipment has made it difficult to recruit new beekeepers into the industry.

Project staff worked with local beekeepers in developing and partially implementing a new beehive that will help European honeybees cope with Asian honeybees. This hive (shown in Appendix 3D) has a reduced entrance, which allows the European bees to better defend their colonies from robbing Asian honeybees. Increased ventilation near the top of the hive also improves airflow through the hive, thus countering the reduction in airflow caused by reducing the entrance. The added ventilation should also assist honey ripening and temperature regulation within the hive. A trial use of this new hive on Kolombangara Island allowed beekeepers to continue beekeeping in the presence of the Asian honeybee without the need to resort to suppress the Asian honeybee population by poisoning.

7.5 Obtaining information on *Varroa* mite reproduction

Varroa mites were not found in European honeybee colonies on islands that were free of Asian honeybees.

Varroa mites were found in all Asian honeybee colonies inspected. These were identified by DNA fingerprinting as the Java type of *V. jacobsoni*. These mites were only reproducing on the Asian honeybee drone brood, not on the worker brood.

On islands inhabited by both European and Asian honeybees, the managed European honeybee colonies were invaded by low numbers of adult female *V. jacobsoni*, which had spread from the Asian honeybee colonies and which were identified as the Java type of *V. jacobsoni*. However, these mites did not cause serious harm to the managed colonies, as they lacked the ability to produce eggs and offspring on either the worker or drone brood. Nevertheless, this situation will need to be continually monitored in the future because, on two separate occasions, an individual female mite was observed with accompanying offspring in a European honeybee drone cell, suggesting that the mite in the Solomon Islands may be in the very early stages of developing an ability to fully colonize the European honeybee, as it recently did in PNG.

8 Impacts

8.1 Scientific impacts – now and in 5 years

New information from this project on *Varroa* mites in Papua New Guinea (PNG) and the Solomon Islands has helped better understand the current and past invasion biology of these important parasites and shown how the mites may impact in the region in 5-10 years' time.

The *Varroa* mites now present in PNG and the Solomon Islands are the result of a 1970s introduction of the Asian honeybee into Papua (formerly known as Irian Jaya) from Java by humans. This bee is the native host of the Java type of *Varroa jacobsoni* and it carried mites of that type with it into Papua. Those mites gradually spread in the region as the Asian honeybee developed into an invasive pest. The mites and bee were first discovered in PNG in 1987 (Delfinado-Baker and Aggarwal, 1987). In 2003 they were both detected in the eastern regions of the Solomon Islands (Anderson, 2004).

For 20 years following their introduction into New Guinea the *Varroa* mites did not harm local European honeybees, as this particular strain lacked the ability to reproduce on the brood of those bees (Anderson, 1994). The same was the case following the introduction of the same strain into the Solomon Islands (Anderson, 2004). However, during the course of this project (in 2008) some mites in PNG were found to have developed an ability to reproduce on European honeybee brood (Anderson, 2008). In doing so, they were able to colonize the European honeybee colonies. This host-switch, which is now only the third recorded for *Varroa* mites on Asian honeybees, was confined to PNG. Mites in Papua and Java (the mites' place of origin) had not switched host, although there were signs that small numbers of individual female mites in Papua were in the process of colonizing European honeybees, as they were found with accompanying offspring in capped drone cells, but not worker cells, of European honeybee colonies (Anderson, 2008). In PNG, the mites that switched host were present in large numbers in European honeybee colonies, were reproducing on both the drone and worker broods, were killing large numbers of colonies and causing hardship for beekeepers (Anderson, 2008).

During the course of this project the mites in the Solomon Islands showed similar behaviour to that reported by Anderson (2004). That is, they could not colonize the local European honeybee as they lacked the ability to reproduce on that bee's brood. Nevertheless, on two separate occasions, an individual female mite was observed with accompanying offspring in a European honeybee drone cell, suggesting that the mite in the Solomon Islands may be in the very early stages of developing an ability to fully colonize the European honeybee. Therefore, the mite situation in the Solomon Islands will need to be monitored over the next 5-10 years in case the mite develops a full-blown ability to colonize European honeybee colonies, as it recently did in PNG.

8.2 Capacity impacts – now and in 5 years

The following activities have built capacity in the Solomon Islands that will be sustained well beyond the life of this project.

- Solomon Islands Government apiary officers were trained in different aspects of bee management (particularly how to deal with Asian honeybees), disease and pest recognition, queen production and colony propagation. Two officers (Ms Salome Ete and Mr Boginald) were also sponsored by the project to attend the 2009 Queensland Beekeepers' Association Annual Conference from 2-4 July at Cairns. The theme of the Conference was "Asian honeybees". After the Conference both officers spent 2 days visiting queen bee production yards in southeast Queensland to learn queen-rearing techniques. Throughout the project Ms Salome Ete also assisted with experimental trials during which she became proficient with the use of the fipronil-based method for suppressing Asian honeybee populations. At the beginning and completion of the project meetings were held with Department of Agriculture and Livestock, to discuss beekeeping and its sustainability in the presence of Asian honeybees.

- At various stages during the project, beekeeper field days were conducted, which covered beekeepers on most of the large islands. At these field days beekeepers were trained in different aspects of beekeeping and in recognizing Asian honeybees and their effects on managed European honeybees. Beekeepers on Malaita Island were trained in the construction and use of a newly designed hive box that will reduce the effects of Asian honeybees on managed honeybees.

8.3 Community impacts – now and in 5 years

8.3.1 Economic impacts

The last official report on beekeeping in the Solomon Islands was released by the Central Bank of the Solomon Islands in 2004 (published in May 2005), one year after the first sighting of the Asian honeybee. That report estimated that there were approximately 2,000 European beehives producing about 50 tons of honey throughout the Solomon Islands. That level of production could not meet the high domestic demand and, as such, no honey was exported in 2004. The retail price of honey in Honiara was $36.00/kg bringing the total value of the honey crop to about $1.8 million. Those statistics probably reflect the current state of beekeeping in the Solomon Islands. With the future of beekeeping now looking bright, due to the development of control methods for Asian honeybees in this project, benefits in the form of increased honey yields will soon begin to be realised and should continue to be realised well into the future. However, increased on-going extension activities will be needed to ensure these benefits are realised. The beneficiaries will be smallholder beekeepers and rural settlements whose livelihoods depend on income generated from the sale of honey.

The continued spread of the Asian honeybee through the Solomon Islands will have an economic impact on beekeeping, the environment and public health, but the associated costs are not known.

Australia has benefitted from the tactical component of the research in the Solomon Islands. The Australian project leader is a member of the Consultative Committee for the current Asian honeybee incursion at Cairns. As a result, project findings have been regularly conveyed to the incursion-response team that is trying to eradicate the bee. The eradication of the Asian honeybee at Cairns will produce significant economic benefits for crop growers (that depend on honeybees for pollination), public health, and the environment. The public health benefits alone are conservatively estimated to range from $84,114 to $88,637 per 100,000 people, while the cost estimates for the public nuisance aspects are estimated to range from $4,580 to $33,660 per 100,000 people (Goswami and Antony, 2010; Ryan, 2010).

8.3.2 Social impacts

Beekeeping with European honeybee fits in well with the social structure of the Solomon Islands, as men and women of all ages can undertake it. A rural women's group on Malaita Island uses small-scale honey production as a way to earn money to cover the costs of school fees, kerosene and soap. Beehives can also be located on small tracts of land that may be unsuitable for other agricultural activities. Income generated through the activities of this project will flow through to whole families and village groups, having a particularly positive impact on women and children.

Environmental impacts

Beekeeping is widely recognised as an environmentally friendly activity. In the Solomon Islands the European honeybee has been used for honey production and also as a highly efficient pollinator of a wide variety of fruit and vegetable crops.

The spread of the Asian honeybee through the Solomon Islands will have a severe impact on the environment and the extent of this impact warrants further investigation.

8.4 Communication and dissemination activities

- Project staff visited the Islands of San Cristobal (Makira Province), Guadalcanal (Guadalcanal Province), Savo, Florida (Central Province), Malaita (Malaita Province), New Georgia, Kolombangara, Gizo, Ranongga, Vella Lavella (Western Province), and Choiseul (Choiseul Province) and gave practical demonstrations to beekeepers and local Government staff on queen rearing, colony propagation and hive management techniques that reduce the impact of Asian honeybees on European honeybees.
- Project staff held annual meetings with Solomon Islands Department of Agriculture and Livestock management and apiary officers to discuss project activity and findings.
- Project findings were presented at:
  - Apimonida, Melbourne Vic, 9-14 Sept 2007.
  - Australian Almond Industry Conference, Rowland Flat SA, 31 Oct 2008.
  - American Beekeeping Federation Conference, Reno USA, 15 Jan 2009;
  - Western Australia Beekeepers Conference, Perth WA, 5 Jun 2009;
  - Plant Health Australia Workshop on *Varroa* mite, Melbourne Vic, 10 Jun 2009;
  - CSIRO Biosecurity Workshop, Canberra ACT, 16 June 2009;
  - Victorian Apiarist Association Annual Conference, Bendigo Vic, 23 Jun 2009.
  - Queensland Beekeepers Association (QBA) Annual Conference, Cairns, Qld, 3 Jul 2009
  - NSW Apiarists Association Annual Conference, Sydney NSW, 10 Jul 2009;
  - Australian Honey Bee Industry Council meeting, Sydney NSW, 13 Jul 2009;
  - Plant Health Australia Workshop on *Varroa* mite, Melbourne Vic, 19 Aug 2009;
  - PERSA Biosecurity Conference, Adelaide SA, 30 Apr 2010;
  - Tasmanian Beekeepers Association Annual Conference, Launceston Tas, 28 May 2010;
  - QBA and AHBIC Conference, Ipswich Qld, 18 Jun 2010.
- Project findings were disseminated to Australian quarantine officials through informal meetings or through the Consultative Committee overseeing the Asian honeybee incursion at Cairns.
- In the Solomon Islands the project received coverage on local television news and was discussed in Parliament.
- Overviews of the project were published in the *Australasian Beekeeper* (Annand, 2008) and *The Land* newspaper, 28 May 2009.
- The Australian project leader presented project findings to beekeepers and Government staff in PNG at:
  - Provincial DAL Office Goroka, 28 May 2009
  - National DAL Office Konidobu, Port Moresby, 13 June 2008
  - Provincial DAL Office Goroka, 16 Apr 2010.

9 Conclusions and recommendations

9.1 Conclusions

The Asian honeybee is now well established in many parts of the Solomon Islands. Its means of introduction are not known. However, the bee's sequential invasion, first of the eastern Islands of San Cristobel, Guadalcanal and Savo and, later, of the western Islands of New Georgia and Kolombangara, clearly indicate that it did not 'island-hop' into the Solomon Islands from Papua New Guinea (PNG). The presence of the bee on Islands with commercial forest logging activities, and its conspicuous absence from islands without such activities, suggests that logging activities have aided its spread in the Solomon Islands.

Since arriving in the Solomon Islands, the Asian honeybee has seriously impacted on beekeeping with the European honeybee. The Asian honeybee was first discovered in the Solomon Islands in 2003, near the end of a period of civil unrest. Its discovery coincided with reports of large losses of European honeybee colonies and declines in honey production. Some of those colony losses may have resulted from the civil unrest, but severe losses were also reported from Training Centres that were largely shielded from the unrest. An assessment of the incursion by CSIRO in 2004 concluded that the colony losses were not due to the *Varroa* mite that the Asian honeybee was carrying (as the mite lacked the ability to reproduce on European honeybee brood), but rather, to the effects of robbing and increased competition for floral resources from the Asian honeybees. The discovery of *Nosema ceranae* in the Solomon Islands during this project suggests that this pathogen may have also played a role in the colony losses. *N. ceranae* was first discovered on *A. cerana* in China (Fries et al., 1996) and is thought to have recently switched host from that bee to the European honeybee. In recent times the pathogen has been reported from European honeybees in many countries, where it has been linked to severe colony losses (Higes et al., 2008). It has also been implicated in colony collapse disorder in the United States (Cox-Foster et al., 2007; Bromenshenk et al., 2010). In the Solomon Islands *N. ceranae* was only found in European honeybees that had come in contact with Asian honeybees. It was not found in European honeybees that were present on islands free of Asian honeybees (Malaita Island), but it was found in Asian honeybees that were present on islands free of European honeybees (Savo Island). These observations suggest that *N. ceranae* was introduced into the Solomon Islands by the Asian honeybee. Hence, quarantine procedures will now be needed to prevent *N. ceranae* from spreading to European honeybees on islands that are currently free of Asian honeybees (see Section 9.2).

The Asian honeybee has developed into more than simply a pest of European honeybees in the Solomon Islands. It is now also a nuisance pest of human communities. The bee rapidly reproduces through frequent swarming and establishes colonies in cavities in a variety of situations, particularly favouring the wall-cavities of buildings. During this project 9 colonies of the bee were found in the wall-cavities of a single building on Savo Island. The increase in bee densities in cities and towns has also impacted on human health by way of increased incidences of bee stings.

The development of methods during this project for controlling the Asian honeybee should now enable the continuation/re-establishment of beekeeping in areas of the Solomon Islands that have been affected by the bee. The first of these methods, suppression of Asian honeybee populations using fipronil, is cheap, effective and does not appear to negatively impact on humans, domestic animals or wildlife. However, beekeepers will need to be trained on how and when to use the method, as there was insufficient time to do so during this project. Many beekeepers, particularly those on Malaita Island were nevertheless introduced to a second control method, the use of a modified hive box to reduce robbing of managed European honeybee colonies by the Asian honeybee. It may even be possible for many beekeepers to use this new hive as a stand-alone method for controlling the Asian honeybee, and not have to resort to suppressing the Asian honeybee population by poisoning. Nevertheless, beekeepers are still likely to suffer reduced honey yields because of competition from the Asian honeybee for floral resources.

There is an urgent need to improve and expand extension services for beekeepers in the Solomon Islands and to improve beekeepers' access to hive and honey processing equipment. The European honeybee stock used by beekeepers throughout the Solomon Islands is also showing signs of in-breeding and needs to be improved urgently.

European honeybees in the Solomon Islands are relatively disease-free, compared with those in other regions. The maintenance of current quarantine measures for importing honeybee stock and the implementation of new measures for moving honeybees between islands (recommended in the next Section) will help maintain this health status.

It is particularly fortunate that the strain of *Varroa* mite that was introduced into the Solomon Islands by the Asian honeybee is harmless to the European honeybee (as it lacks the ability to reproduce on the brood of that bee). However, this situation could rapidly change as it recently did with the same mite strain in neighbouring PNG. There were signs during this project that the mite may be in the early stages of developing an ability to colonize European honeybees in the Solomon Islands. This makes it imperative that further incursions of the Asian honeybee be suppressed, both in the Solomon Islands and in Australia.

9.2 Recommendations

- The Solomon Island Government and local and foreign aid agencies continue to support beekeeping in the Solomon Islands as a viable economic and socially beneficial activity.
- Extension and training services provided to Solomon Island beekeepers be increased and expanded, with the primary aim of increasing honey yields. Part of this activity should include facilitating improved beekeeper access to hive and honey processing equipment and improved genetic stock (queen bees).
- New European honeybee breeding stock in the form of queen bees be imported into the Solomon Islands to improve current stock. A lack of local expertise to perform artificial insemination, and the costs associated with the use of this technology, makes it impractical to import bee semen to improve stock at this time. To maintain the Solomon Islands' current good honeybee health status imported stock should be sourced from a region where honeybees are known to be relatively free of diseases and parasites, such as Western Australia.
- Training in the use of the fipronil-based method for suppression of Asian honeybee populations and in the construction and use of the improved hive box for reducing Asian honeybee robbing should be core components of future training programs for Solomon Island beekeepers. Wider use of the improved hive box design for controlling Asian honeybees should be encouraged and training on its use should be supplemented with a 'fact-sheet' describing its construction and advantages. The fipronil-based method for suppressing Asian honeybees should only be used when Asian honeybee populations reach high densities locally and become a serious nuisance, and the method should only be used with Government Apiary Officer consent and supervision.

- Surveillance of the Asian honeybee be maintained in the Solomon Islands, so that beekeepers on islands still free of the bee can receive training and assistance in controlling the bee as soon as it is detected.
- Trade in live European honeybees (nucleus bee colonies and queen bees) and used beekeeping equipment be prohibited from islands with Asian honeybees to islands without Asian honeybees. This is primarily to prevent the spread of *Nosema ceranae*.
- ACIAR initiate and support new research on the *Varroa* mite in PNG and the Solomon Islands. The mite genotype that is now present on Asian honeybees in the Solomon Islands (the Java type of *Varroa jacobsoni*) is currently harmless to European honeybees as it is unable to reproduce on the brood of that bee. However, this situation will need to be continually monitored in the future in case the mite suddenly develops an ability to reproduce on European honeybee brood, as it recently did in PNG. Discussions and meetings with PNG beekeepers during this project indicated that the new form of *Varroa* mite infesting their bee hives is causing losses and hardship and, even though the beekeepers have received assistance from Australia in combating the initial outbreak of the mite, research is urgently needed to develop ways of controlling and living with the mite in the long term. The presence of the mite in PNG is also a new and serious biosecurity threat to Australian beekeepers and industries that depend on honeybees for pollination.

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11 Appendixes

Appendix 1: Attracting foraging Asian honeybees (*Apis cerana*).

A. Finding an efficient feeder/bait-station.

Table 1. Shows details of two independent trials carried out on Savo Island in which foraging Asian honeybees visited different feeders that were placed in un-shaded situations and provisioned with sugar-syrup (SS) or honeycomb (HC) as bait (see text for detail). NV = no visits.

Trial 1

Feeder Type[a]	Bait	Time (mins) to First Visit	Total Number of Visits
1	SS	90	2
1 (Replica)	SS	90	3
1	HC	75	1
1 (Replica)	HC	90	1
2	SS	NV	0
2 (Replica)	SS	NV	0
2	HC	NV	0
2 (Replica)	HC	NV	0
3	SS	60	4
3 (Replica)	SS	60	5
3	HC	45	2
3 (Replica)	HC	60	1
4	SS	105	2
4 (Replica)	SS	90	1
4	HC	105	1
4 (Replica)	HC	90	1
5	SS	105	3
5 (Replica)	SS	90	3
5	HC	75	1
5 (Replica)	HC	105	1

TRIAL 2

Feeder Type[a]	Bait	Time (mins) to First Visit	Total Number of Visits
1	SS	90	2
1 (Replica)	SS	90	2
1	HC	75	1
1 (Replica)	HC	60	1
2	SS	NV	0
2 (Replica)	SS	NV	0
2	HC	NV	0
2 (Replica)	HC	NV	0
3	SS	45	4
3 (Replica)	SS	45	3
3	HC	45	1
3 (Replica)	HC	60	1
4	SS	75	2
4 (Replica)	SS	60	3
4	HC	75	1
4 (Replica)	HC	60	1
5	SS	90	2
5 (Replica)	SS	75	2
5	HC	90	1
5 (Replica)	HC	75	1

[a] The feeders are those listed in Section 5.1.1

4) Different feeders were tested for their ability to attract foraging Asian honeybees. The feeder on the far right (with yellow base) is a commercially available wasp trap suspended from rope strung between 2 trees. The feeder of the far left is a dish dispenser placed on top of a stack of European honeybee hive boxes (*Photo courtesy Denis Anderson*).

C. Wax comb from an Asian honeybee colony was melted, cooled and tested for its ability to attract foraging Asian honeybees (*Photo courtesy Denis Anderson*)**.**

D. Sugar-syrup (in right dish dispenser) was more attractive to foraging Asian honeybees than honey (in left dish dispenser) or melted honeycombs. Short twigs in the dispensers were to provide landing platforms for the visiting bees (*Photo courtesy Denis Anderson*).

E. Bee pheromones, chemicals and floral odours were tested for their ability to attract foraging Asian honeybees (*Photo courtesy Denis Anderson*).

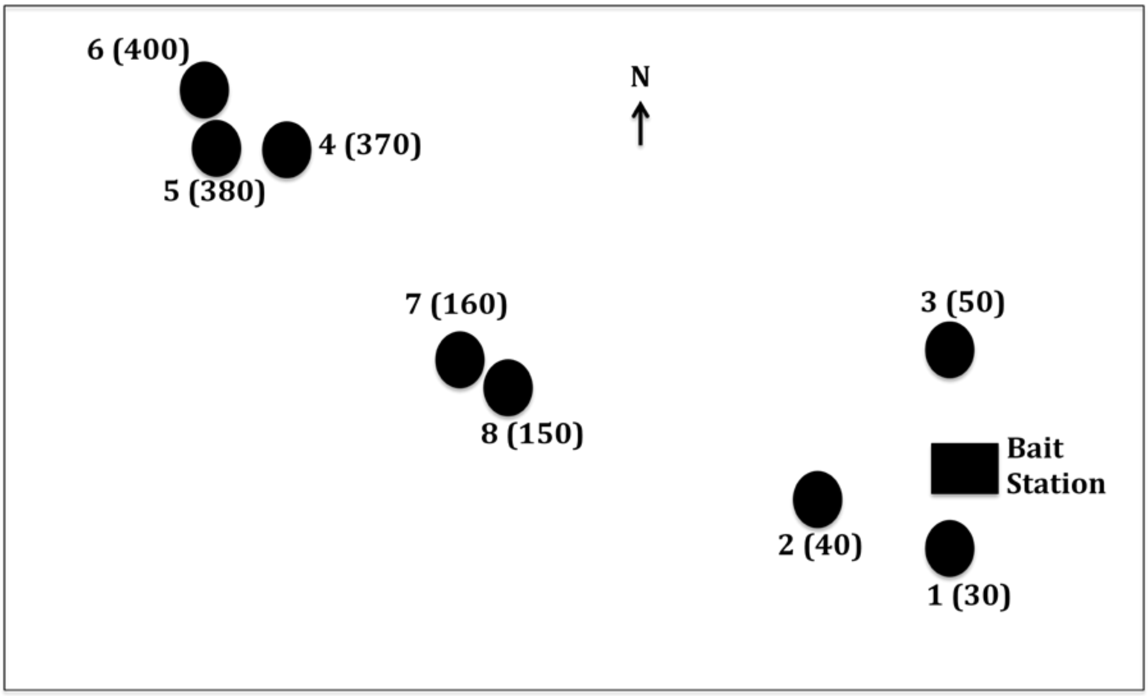
F. Fewer foraging Asian honeybees visited bait-stations when Malaysian apple trees (*Syzygium malaccense*) were in flower (*Photo courtesy Denis Anderson*).

Appendix 2: Suppressing Asian honeybees with fipronil.

5) The bait-station used to attract foraging Asian honeybees during fipronil trials on Savo and Guadalcanal Islands (*Photo courtesy Denis Anderson*).

B. Bee flight activity was monitored at the entrance of Asian honeybee nests on Savo Island prior to and following the application of fipronil at the bait-station (*Photo courtesy Denis Anderson*).

C. The relative positions of 8 feral Asian honeybee colonies (numbered circles) monitored for their response to fipronil on Savo Island, and their approximate distances (metres in brackets) from the bait-station (rectangle).



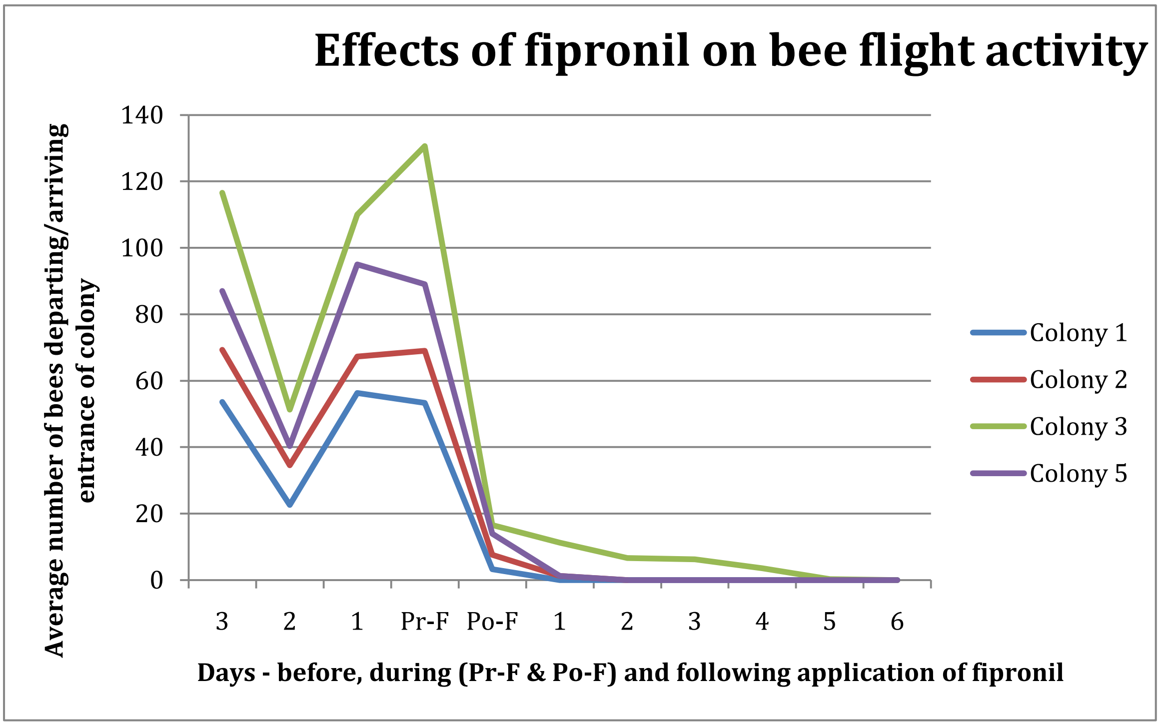
D. Effects of fiproil on Asian honeybee flight activity at nest entrances on Savo Island.

Table 2. Average numbers of bees departing from and arriving at the entrances of 8 feral Asian honeybee nests during three 1-minute periods in the days prior to, during and following the application of fipronil at the bait-station (see text for detail).

Nest No.	Day prior to application of fipronil			Day fipronil was applied		Day after application of fipronil						
	3	2(1)	1	Pre	Post	1	2	3	4	5	6	7
1	53.6	22.6	56.3	53.3	3.3	D(2)						
2	69.3	34.6	67.3	69.0	7.6	1.3	D					
3	116.6	51.3	110.0	130.6	16.6	11.3	6.6	6.3	3.6	0.3	D	
4	>150	115.0	>150	>150	116.0	A(3)						
5	87.0	40.3	95.0	89.0	14.0	1.3	D					
6	>200	161.3	>200	>200	139.0	102	111.3	105.3	93.6	88.3	61.6	52.6
7	>150	107.3	>150	>150	134.0	61.6	24.6	5.6	1.0	D		
8	>200	159.0	>200	>200	113.6	1.0	D					

(1) Overcast weather conditions; (2) D = Colony was dead; (3) A = Colony had absconded from its nest.

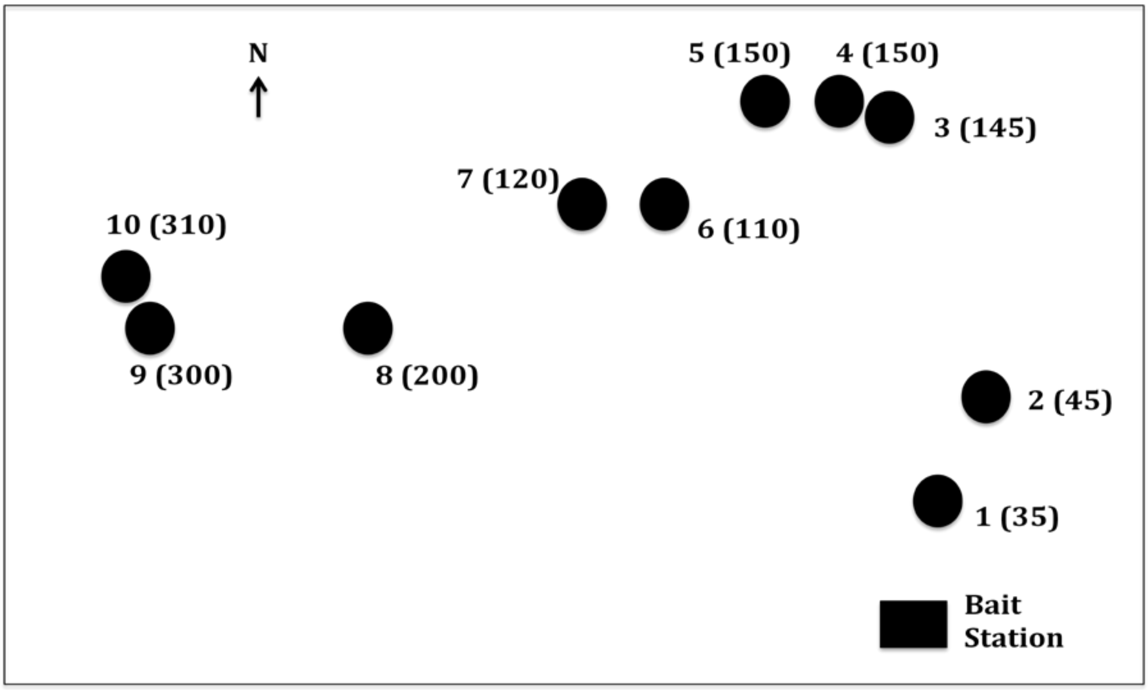
E. Plot of the effects of fipronil on Asian honeybee flight activity at nest entrances of 4 of the 8 Asian honeybee colonies monitored on Savo Island. Note that fipronil was added to sugar-syrup at the bait-station for a 1-hour period between Pr-F and PoF time intervals on the horizontal axis (Pr-F represent the hour prior to the application of fipronil and Po-F the hour following it). The decreased activity on day 2 prior to application of fipronil was due adverse weather conditions.



F. Only a few adult bees remain to tend brood in this Asian honeybee nest on Savo Island 24 hours after application of fipronil at the bait-station (*Photo courtesy Denis Anderson*).

G. Dead bees accumulate outside an Asian honeybee nest on Savo Island 24 hours after application of fipronil at the bait-station (*Photo courtesy Denis Anderson*).

H. The relative positions of 10 feral Asian honeybee colonies (numbered circles) monitored for their response to fipronil on Guadalcanal Island, and their approximate distances (meters, in brackets) from the bait-station (rectangle).



I. Effects of fiproil on Asian honeybee flight at nest entrances on Guadalcanal Island.

Table 3. Average numbers of bees departing from and arriving at the entrances of 10 feral Asian honeybee nests on Guadalcanal Island during three 1-minute periods in the days prior to, during and following the application of fipronil (see text for detail).

Nest No.	Day prior to application of fipronil		Day fipronil applied		Day after application of fipronil						
	2	1	Pre	Post	1	2	3	4	5	6	7
1	61.3	68.0	68.0	6.3	D([1]						
2	81.6	86.0	75.6	9.3	5.3	5	3..6	3.3	2.6	2	1.6
3	141.0	143.0	144.6	27.0	1.6	D					
4	>150	>150	>150	116.0	77.0	61.3	42.3	18	5.3	D	
5	18.0	19.3	18.0	3.0	D						
6	11.0	9.6	9.3	1.3	D						
7	>150	>150	>150	86.6	53.3	39.0	23.6	5.0	D		
8	>150	>150	>150	90.3	7.0	D					
9	54.0	47.3	45.6	26.6	16.0	7.0	5.3	3.3	1	D	
10	9.3	8	8.3	0	D						

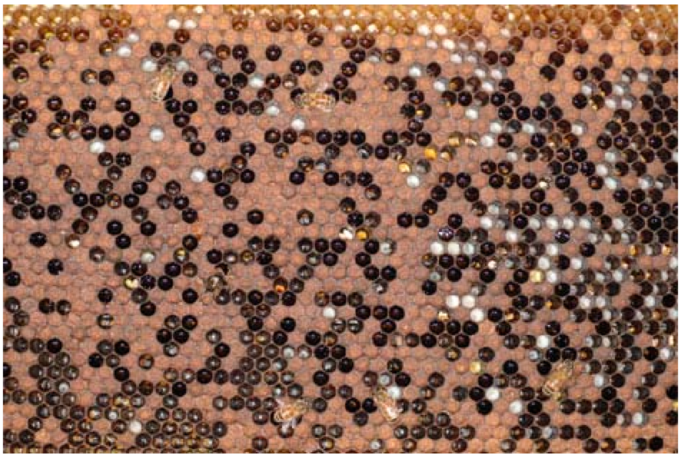
(1) D = Colony was dead.

Appendix 3: Extension activities in the Solomon Islands

A. A European honeybee hive on Kolombangara Island in need of repair (*Photo courtesy Denis Anderson*).

B. Many Solomon Island beekeepers lack access to hive comb foundation, and this has a detrimental impact on hive management and honey production (*Photo courtesy Denis Anderson*).

C. Spotty brood patterns in most European honeybee colonies in the Solomon Islands are a clear sign of inbreeding (*Photo courtesy Denis Anderson*).



D. The European honeybee hive developed during this project to reduce robbing by Asian honeybees. Note the narrowed entrance and added ventilation near the top of the hive to compensate for reduced airflow through the narrowed entrance (*Photos courtesy Nick Annand)*.