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Development of a specific aggregation lure for *Apis cerana javana*

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and Development Corporation**

by Michael Lacey

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Foreword

The Asian hive bee *Apis cerana* is an exotic pest that is listed in the AUSVET PLAN. It has spread throughout Indonesia and Papua New Guinea and has infiltrated Australian islands of the Torres Strait. Very recently there have been successive incursions of *A. cerana* into Darwin and Brisbane.

If *A. cerana* eventually succeeds in establishing itself on the Australian mainland, it will have major consequences because it will:

- seriously damage the honey bee industry by competing aggressively with *A. mellifera* for floral sources;
- introduce the ectoparasitic mites *Varroa jacobsoni* and *V. underwoodi*;
- disrupt pollination worth over one billion dollars per annum to the Australian economy;
- threaten the survival of native bee populations.

The presence and spread of *A. cerana javana* in the Torres Strait is constantly being monitored by quarantine personnel. This necessitates ground searches that are both labour-intensive and expensive.

The development of a sensitive attractant for *A. cerana javana*, as demonstrated in the present report, provides a crucial step for improving the efficiency of protective monitoring measures. This report describes the identification and application of pheromone constituents for *A. cerana javana* that enable the development of a specific attractant for this pest. It demonstrated the successful field testing of natural and synthetic pheromone blends as aggregation lures for *A. cerana javana*.

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Disclaimer

The information, advice, data and calculations contained in this report are provided to assist in the development and assessment of specific aggregation lures to attract the Asian hive bee *Apis cerana javana*. CSIRO does not make any warranties and does not accept any liability for any loss or damage resulting from the use and/or reliance upon the information, advice, data and/or calculations provided in this report.

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Executive Summary

- Pilot trials were conducted to develop selective attractants for the Asian hive bee *Apis cerana javana* based on the natural scents (pheromones) of the pest.
- Successful bioassays were conducted in Papua New Guinea with *A. cerana javana* workers. These close-range experiments were accomplished under field conditions within small arenas.
- The PNG trials demonstrated that worker bees were very attracted to a five-component synthetic blend based on the natural queen substance that we identified for *A. cerana javana*.
- *A. cerana javana* workers in analogous close-range trials in Java responded with fanning behaviour rather than aggregation in reaction to the synthetic *A. cerana javana* queen pheromone blend.
- Natural queen pheromone extracts from *A. cerana javana* promoted aggregation of workers in similar close-range trials in Java.
- Intermediate-range trials designed to attract bees in flight to a choice of lures were conducted successfully under field conditions in Java.
- Synthetic queen pheromone blend of *A. cerana javana* was selected exclusively by worker bees from other pheromone blends and individual lures in the intermediate-range trials.
- The matrix of the dispensers protects the pheromone components from deterioration and controls their release rates.
- The successes of these trials demonstrate that the five-component synthetic blend and dispenser for *A. cerana javana* have significant potential for the development of a specific long-range attractant for this pest.

1. Introduction

The regional strain of the Asian hive bee *A. cerana javana* is an exotic pest that will have serious consequences for Australian rural industries if it eventually establishes itself on the mainland. There are frequent incursions of the Asian hive bee from Papua New Guinea on to the northern islands of the Torres Strait. Moreover, there was an intrusion of a colony into Darwin in August, 1998, and into Brisbane in August and December, 1999.

Current protective measures for monitoring the presence and spread of *A. cerana javana* are labour-intensive, expensive and difficult. The efficiency of monitoring procedures to target the pest will improve greatly through development of selective attractants based on pheromones, as described in this Report.

2. Objectives

The objects of the research were:

- to identify all the constituents of the queen substance of *A. cerana javana*, including those present at low concentrations;
- to prepare sufficient quantities of the synthetic pheromone constituents and other possible attractants for field trials of the blends as potential aggregation lures;
- to evaluate possible dispensers that will allow controlled aerial release of the various pheromone constituents over a prolonged period without deterioration;
- to conduct field trials of the lures in order to optimise their effectiveness in traps designed for protective monitoring of *A. cerana javana*.

3. Methodology

Compounds that were identified as semiochemical constituents of *A. cerana javana* and *A. mellifera*, or were possible candidates for mediating their behaviour, were synthesised and incorporated as standard solutions in sealed ampoules. Other compounds and blends were incorporated within controlled-release dispensers of medical rubber tubing (length 25 mm, OD 5 mm, ID 3 mm) and, after evaporation of the solvent, were sealed in pairs within glass tubing. All solutions and controlled-release dispensers were transported to Indonesia and PNG and maintained at ambient temperatures before the trials to ascertain whether they were sufficiently robust for practical applications as lures.

Because the laboratory facilities in the overseas locations were very limited, it was necessary to choose the qualitative and quantitative characteristics of the semiochemical blends in advance. One equivalent of the synthetic queen pheromone blend for *A. mellifera* was that determined previously from several measurements (Kaminski *et al.*, 1990; Plettner *et al.*, 1997) to represent the average content of the mandibular glands of a honeybee queen. One equivalent of the synthetic queen pheromone blend for *A. cerana javana* was assumed to be that with the same amount of 9-keto-2*E*-decanoic acid (9-ODA) as *A. mellifera*, even though the quantities of 9-ODA actually recorded (Lacey, 1998; Plettner *et al.*, 1997) were generally lower. The various combinations, solvents and concentrations of the synthetic components were as follows:

- (i) Solutions of *A. cerana javana* queen pheromone:
 - (a) blend of 5 components in methanol, synthesised 16/10/98, for trials in PNG. Unused portion stored at -20°C in the intervening period. 2 sealed ampoules for trials in Indonesia. 10 μL = 0.1 queen equivalents. **Solution A.**

- (b) blend of 5 components in methanol, freshly synthesised 3/3/99. 6 sealed ampoules. 10 μL = 0.1 queen equivalents. **Solution B.**
 - (c) Solution B diluted tenfold. 6 sealed ampoules. 10 μL = 0.01 queen equivalents. **Solution C.**
 - (d) Solution B diluted one hundredfold. 1 sealed ampoule. 10 μL = 0.001 queen equivalents. **Solution D.**
 - (e) blend of 5 components in methanol, freshly synthesised 3/3/99. 1 sealed ampoule. 10 μL = 1.0 queen equivalents. **Solution E.**
 - (f) blend of 5 components in dichloromethane, freshly synthesised 5/3/99. 1 sealed ampoule. 10 μL = 10.0 queen equivalents. **Solution F.**
 - (g) Methanol solvent. 6 sealed ampoules.
 - (h) Dichloromethane solvent. 6 sealed ampoules.
- (ii) Solutions of *A. mellifera* queen pheromone:
- (a) Commercial blend (Bee-Lure, Phero Tech Inc, BC, Canada) in methanol prepared 16/10/98 for trials in PNG. Unused portion stored at -20°C in the intervening period. 6 sealed ampoules for trials in Indonesia. 10 μL = 0.1 queen equivalents. **Solution G.**
 - (b) Commercial blend in methanol prepared 3/3/99. 6 sealed ampoules for trials in Indonesia. 10 μL = 0.1 queen equivalents. **Solution H.**
 - (c) Commercial blend in dichloromethane, prepared 5/3/99. 2 sealed ampoules. 10 μL = 10.0 queen equivalents. **Solution I.**
- (iii) Solutions of *A. cerana javana* Nasonov pheromone:
- (a) Blend of 1:1 linalool and citral in methanol. 6 sealed ampoules. 10 μL = 10 μg of each component. **Solution J.**
 - (b) Solution J diluted tenfold. 10 μL = 1.0 μg of each component. **Solution K.**
 - (c) Blend of 1:1 linalool and citral in dichloromethane. 1 sealed ampoule. 10 μL = 200 μg . **Solution L.**
- (iv) Solutions of 11-eicosen-1-ol in methanol. 4 sealed ampoules. 10 μL = 15 μg . **Solution M.**
- (v) Solutions of 2-octenyl acetate in methanol. 4 sealed ampoules. 10 μL = 10 μg . **Solution N.**
- (vi) Solution of 2-octenyl acetate in dichloromethane. 1 sealed ampoule. 10 μL = 200 μg . **Solution O.**
- (vii) Solutions of ethyl Z-9-octadecenoate in ethanol. 4 sealed ampoules. 10 μL = 20 μg . **Solution P.**
- (viii) Solution of ethyl Z-9-octadecenoate in ethanol. 1 sealed ampoule. 10 μL = 200 μg . **Solution Q.**
- Controlled release lures:
- (i) 1 mg aliquot of *A. cerana javana* queen substance blend, solution F (50 μL), absorbed on medical rubber tubing (2.5 cm length). The solvent was evaporated and the 6 dispensers sealed in pairs in glass tubes.
 - (ii) 1 mg aliquot of *A. mellifera* queen substance blend, solution I (50 μL), absorbed on medical rubber tubing (2.5 cm length). The solvent was evaporated and the dispensers (6) sealed in pairs in glass tubes.
 - (iii) 1 mg aliquot of *A. cerana javana* Nasonov blend, solution L (50 μL), absorbed on medical rubber tubing (2.5 cm length). The solvent was evaporated and the dispensers (6) sealed in pairs in glass tubes.

- (iv) 1 mg aliquot of 2-octenyl acetate, solution O (50 μ L), absorbed on medical rubber tubing (2.5 cm length). The solvent was evaporated and the dispensers (6) sealed in pairs in glass tubes.
- (v) 1 mg aliquot of ethyl Z-9-octadecenyl acetate, solution Q (0.5 mL), absorbed on medical rubber tubing (2.5 cm length). The solvent was evaporated and the dispensers (5) sealed in glass tubes.

Bioassay arenas for close-range trials of worker bees were constructed from covered plastic Petri dishes (15 cm x 2 cm), similar to those described by Kaminski *et al.* (1990). A hole (0.8 cm diam.) was drilled into the side wall immediately below the lid for introduction of lures and 4 holes of the same bore were drilled into the lid and protected with gauze to allow aeration of the confined bees. Test probes ('pseudo-queens') were made from Pasteur pipettes, cut and flame-sealed at both ends. A small cup was formed in the barrel end of the pseudo-queen to allow evaporation of sufficient volume of a test solution (aliquots of up to 10 μ L) in preparation for each bioassay.

A gazebo tent (2 m length x 1.5 m width x 2 m height) had been transported previously to Indonesia as a portable field cage and was retrieved at Parung Panjang for the present studies. An improved spoke assembly (diameter 1.2 m) for suspension from the roof of the field cage was constructed from brass and aluminium by the CSIRO Entomology workshop. This design overcame the difficulties of the previous assembly (Lacey, 1998) and enabled up to 6 different lures to be evaluated per intermediate-range trial.

4. Detailed Results

(a) Close-range trials in Papua New Guinea

Preliminary close-range trials with *Apis cerana javana* worker bees were carried out in October, 1998, by Dr Denis Anderson at the National Agricultural Research Institute, Aiyura, PNG. The blends for assay were synthetic *A. cerana javana* queen pheromone (**solution A**) and synthetic *A. mellifera* queen pheromone (**solution G**). The solutions were prepared 5 days before the trials and were sealed in ampoules for transport from Canberra to PNG. Solvent (methanol in ampoules) was included as a control. The ampoules were left at ambient temperatures for the first 3 days but were stored thereafter in a refrigerator.

The *A. cerana javana* workers were obtained from a single mature colony **H1** at Aiyura. The hive was almost certainly queenright because, although the queen was not spotted, the colony had brood. Groups of 15 workers were selected at random from colony **H1** as required. Each group was placed in a clean covered Petri dish arena immediately before the designated assay and the arenas were placed 25 cm apart. The bees were allowed to distribute themselves throughout the arena and its entrance hole was blocked by a clean pseudo-queen probe to prevent escape. The bees tended to clump in one area of the dish and settled completely within 3 minutes.

In **trial 1**, 0.06 queen equivalent of synthetic *A. cerana javana* pheromone (**solution A**, 6 μ L) was allowed to dry on the tip of a clean pseudo-queen **P1** and 6 μ L of methanol was dried on a control probe **P2**. After 30 seconds evaporation, the stoppers in the 2 arenas, each oriented towards the right of its arena, were replaced by the treated pseudo-queens and the number of bees within a 2.5 cm radius of **P1** and **P2** were recorded.

The 15 bees within the arena exposed to the tip of the pseudo-queen **P1** reacted within 3 seconds and the original clump began to disperse. Within 10 seconds all 15 bees had aggregated within 2.5 cm of the pseudo-queen. Antennation and licking of the treated zone and fanning by individual bees were also noted. The aggregation by the 15 bees persisted for 160 seconds whereupon some of the bees began to disperse. The number gradually diminished to 9 bees in the vicinity of pseudo-queen **P1** after 200 seconds and 0 after 280 seconds. Conversely, there was no aggregation of the bees around the

control pseudo-queen **P2** over the same observation period, demonstrating that the bees responding to pseudo-queen **P1** in the parallel arena were not merely attracted to solvent residues.

In **trial 2**, the experimental procedure was essentially the same as that in trial 1 except that 3 clean arenas each containing 15 freshly obtained *A. cerana javana* workers from colony **H1** were used. The bees quickly settled and tended to clump within 3 minutes of being introduced into the arenas. In the meantime, 0.06 queen equivalent of synthetic *A. cerana javana* queen pheromone (**solution A**, 6 μL) was applied to pseudo-queen **P3**, 0.06 queen equivalent of synthetic *A. mellifera* queen pheromone (**solution G**, 6 μL) to pseudo-queen **P4** and 6 μL of methanol (control) to pseudo-queen **P5**. After 30 seconds evaporation, the pseudo-queens were inserted into the 3 adjacent arenas.

The 15 bees within the arena exposed to pseudo-queen **P3** reacted quickly and the original clump began to disperse. Within 10 seconds all 15 bees had aggregated within 2.5 cm of **P3**. The aggregation by the 15 bees persisted for 80 seconds whereupon some of the bees began to disperse. The number diminished to 9 bees in the vicinity of **P3** after 120 seconds and 0 after 200 seconds, with only occasional visits thereafter. Conversely, there was no attraction of the bees to the pseudo-queens **P4** and **P5** over the same observation period.

In **trial 3**, the experimental procedure was essentially a replicate of that in trial 2 except that the positions of all the pseudo-queens were rotated 45 degrees to those in trial 2. 0.06 queen equivalent of synthetic *A. cerana javana* queen pheromone (**solution A**, 6 μL) was applied to pseudo-queen **P6**, 0.06 queen equivalent of synthetic *A. mellifera* queen pheromone (**solution G**, 6 μL) to pseudo-queen **P7** and 6 μL of methanol (control) to pseudo-queen **P8**. After 30 seconds evaporation, the pseudo-queens were inserted into the 3 clean arenas each containing workers freshly obtained from colony **H1**.

The results for **trial 3** essentially duplicated those for **trial 2**. The 15 bees within the arena exposed to pseudo-queen **P6** reacted quickly and within 10 seconds all 15 bees had aggregated within 2.5 cm of **P6**. The attraction to the 15 bees persisted for 80 seconds whereupon some of the bees began to disperse. The number diminished to 5 bees in the vicinity of **P6** after 100 seconds and 0 after 150 seconds, with only occasional visits thereafter. There was no attraction of the bees in the adjacent arenas to the pseudo-queens **P7** and **P8** over the same observation period.

(b) Close-range trials using synthetic blends at Parung Panjang

The initial **trial 4** was designed to replicate the successful close-range trials in PNG. Workers of *A. cerana javana* for **trial 4** at the National Beekeeping Centre were obtained from a queenright hive **H2**. 15 bees were placed in each of 2 arenas 25 cm apart on a table outside under a canvas shade cloth. However, unlike those in PNG, the bees became noticeably more agitated when confined in the arenas. Although they were somewhat settled after a further 20 minutes when the assays began, some of the bees were still moving and there was minimal clumping. 0.1 queen equivalent of synthetic *A. cerana javana* queen pheromone (**solution A**, 10 μL) was applied to pseudo-queen probe **P9** and 0.01 queen equivalent (**solution A**, 1 μL) to pseudo-queen probe **P10**. After 30 seconds evaporation, the stoppers in the two arenas were replaced by the treated pseudo-queens. Within 5 seconds, the bees in each arena responded to **P9** and **P10** by dispersing. There was no significant migration towards the lures and the bees gradually became agitated over 1-2 minutes with frenetic movement around the arenas.

These initial results for **trial 4** in Parung Panjang were surprisingly different to those encountered in **trials 1-3** in PNG. Some possible complications were:

- the hive **H1** in PNG might have been a queenless colony, for which the workers could be somewhat more receptive to artificial queen substance odours;
- the ambient temperature, pressure and humidity are quite different for the two locations and might mediate the type of response;

- **solution A** might have changed in composition during storage since **trials 1-3**;
- the bees in Parung Panjang were more agitated than those in PNG and therefore would be less receptive to artificial queen pheromone odours.

Workers bees for **trial 5** were therefore obtained from a queenless hive **H3**. 30 bees were placed in each of 3 petri dish arenas under the shade cloth and were assayed in sequence. The bees in the first arena settled after 20 minutes and formed a small clump of 10-15 bees. 0.05 queen equivalent of *A. cerana javana* queen pheromone blend (**solution A**, 5 μL) was evaporated on pseudo-queen **P11** which was then inserted into the arena. The bees responded by dispersing the clump within 5 seconds and began movement towards **P11**. The majority (20) of the bees had moved to the vicinity of the pseudo-queen within 20 seconds. The group began to disperse after 70 seconds and by 120 seconds all had moved away from **P11**. Thus, this result was similar to those obtained in PNG although the response appeared to be more muted.

0.05 queen equivalent of freshly synthesised pheromone blend (**solution B**, 5 μL) was evaporated on pseudo-queen **P12** which was then inserted into the second arena containing reasonably becalmed bees from hive **H3**. All the bees responded within 3 seconds by moving towards **P12** but they began to get agitated within 40 seconds and dispersed rapidly, moving around the arena.

0.05 queen equivalent of queen pheromone blend (**solution A**, 5 μL) was evaporated on pseudo-queen **P13** which was then inserted into the third arena containing bees from hive **H3**, though the workers had not settled even after 1 hour. The bees displayed no apparent reaction to **P13** and continued to move frenetically within the arena.

In order to match the lower ambient temperature of Aiyura in the PNG Highlands, **trial 6** was conducted in an air-conditioned room (temperature 20°C). Workers were obtained from queenless hive **H4** and 15 were placed in an arena, which was cooled in a refrigerator (5°C) until the bees were pacified. 0.02 queen equivalent (**solution A**, 2 μL) was applied to pseudo-queen **P14** which was inserted into the arena just as the bees began to revive. The workers began to move towards **P14** and then commenced rapid fanning within 20 seconds.

A similar behavioural response was found in **trial 7** for bees from hive **H4** in 2 additional arenas that had been pre-cooled to reduce the agitation. 0.1 queen equivalent (**solution A**, 10 μL) was applied to pseudo-queen **P15** which was inserted into an arena as the bees revived. The bees aggregated at about 5 cm distance from the pseudo-queen and then began to fan rapidly. Similarly, 0.001 queen equivalent (**solution D**, 10 μL) was applied to pseudo-queen **P16** which was inserted into an arena as the bees revived. One of the bees approached **P16** while the remainder fanned rapidly.

The fanning behaviour was reminiscent of that recorded for *A. cerana javana* in response to natural queen pheromone during the previous bioassays at Parung Panjang (Lacey, 1998). Nevertheless, the expected behavioural response in the present close-range experiments was that of attraction and aggregation and it was unclear at this stage what were the factors that mediated the different responses.

(c) Close-range trials of other semiochemicals at Parung Panjang

Ethyl Z-9-octadecenoate is a volatile chemical constituent of *Varroa jacobsoni* (Sasagawa *et al.*, 1999) and therefore it was considered that it might induce a behavioural response from *A. cerana javana*. For **trial 8**, 100 μg of ethyl Z-9-octadecenoate (**solution P**, 50 μL) was evaporated on a pseudo-queen probe **P17** and inserted into an arena containing 15 settled workers from queenless hive **H5**. There was a reaction to the vapour within 5 seconds and this quickly resulted in mutual grooming behaviour by the workers throughout the arena. The hygienic grooming response may be part of the reason why *V. jacobsoni* (reproducing strain) has never established itself in *A. cerana javana* colonies.

A second arena of 15 settled *A. cerana javana* workers from queenless hive **H5** was tested in **trial 9** with their synthetic Nasonov pheromone blend. 20 µg (**solution H**, 10 µL) was evaporated on a pseudo-queen probe **P18** and inserted into the arena. The initial clump of workers dispersed within 5 seconds and the bees became very irritated and aroused. There was clearly a behavioural response to **P18** but no attraction to the Nasonov blend. It was possible that the design of this bioassay was inappropriate for testing the attractiveness of Nasonov pheromone.

(d) Close-range trials with *A. mellifera* at Bogor

It has been established in the literature that *A. mellifera* workers were attracted to synthetic queen pheromone blends in close-range trials (Kaminski *et al.*, 1990). Because *A. cerana javana* workers in Java were not responding in the same manner, it was decided to evaluate the analogous responses of *A. mellifera*. These trials were conducted in the kampung of Pasir Gaok near Bogor where there were several hives of *A. mellifera* available.

0.1 queen equivalent of *A. mellifera* commercial blend (**solution G**, 10 µL) was evaporated on pseudo-queen **P19** which was then inserted into an arena containing quiescent bees from a naturally queenless hive **H6** (**trial 10**). 3 of the workers approached **P19** within the first 10 seconds but did not stay in the vicinity of the probe. The majority (10) responded to the odour from **P19** by fanning. The assay was repeated with 3 more arenas of 15 bees from both queenless and queenright hives but, while the workers responded by fanning, none of the pseudo-queens promoted any significant attraction. This response was analogous to that found with *A. cerana javana* in **trial 6** but contrasted with that recorded for *A. mellifera* by Kaminski *et al.* (1990) and observed for *A. cerana javana* in PNG (**trials 1-3**).

Because there was a slight possibility that the dilute methanol solution of the queen pheromone blend (**solution G**) might have allowed some derivatisation of the carboxylic acids, a further assay (**trial 11**) was carried out using a dichloromethane solution of the pheromone blend. 1.0 queen equivalent (**solution I**, 1 µL) was evaporated on a pseudo-queen probe **P20** and inserted into an arena of 15 settled *A. mellifera* bees from a queenless hive **H7**. After 10 seconds exposure, 12 of the 15 workers had commenced fanning but there was no concerted movement towards **P20**. Nevertheless, there was not the ensuing agitation that was typical of the bioassays with *A. cerana javana*. The fanning reaction lasted for 10 minutes after which some agitation became evident. Thus, the behavioural responses to synthetic pheromone blends by *A. mellifera* and *A. cerana javana* were unexpected but were not a consequence of deterioration of the test solutions.

Other semiochemicals were also tested with the *A. mellifera* colonies. Z-11-Eicosenol is one of the major components of *A. mellifera* venom (Pickett *et al.*, 1982) and *A. cerana* venom (Schmidt *et al.*, 1997) and it is thought that it may serve to attract foragers to marked floral sources. For **trial 12**, 15 µg of 11-eicosenol (**solution M**, 10 µL) was evaporated on a pseudo-queen probe **P21** and inserted into an arena containing 20 settled workers from the queenless hive **H6**. There was no significant attraction to the probe. Most bees remained at a remote distance from **P21** and there was some fanning by 3 bees. There was occasional touching of **P21** over 5 minutes but this was random. Thus, 11-eicosenol does not appear to be a short-range attractant. Similarly in **trial 13**, 20 µg of ethyl Z-9-octadecenoate (**solution P**, 10 µL) was evaporated on pseudo-queen **P22** and inserted into an arena containing 15 settled workers from hive **H6**. There was no hygienic grooming behaviour evident over 5 minutes observation time. Thus the response of *A. mellifera* to the semiochemical (**trial 13**) is different from that of *A. cerana javana* workers (**trial 8**).

(e) Close-range trials of natural queen blends at Parung Panjang

Analogous close-range trials were conducted with natural queen extracts of *A. cerana javana* in order to ascertain some of the factors that mediated attraction to synthetic blends in the arenas. Two mature queens of *A. cerana javana* were decapitated and their heads extracted with a small volume of solvent;

one in 100 μL dichloromethane (**Q1**) and the other in 100 μL of methanol (**Q2**). Assays with the natural extracts were carried out in an air-conditioned room at 20°C. In **trial 14**, 0.1 queen equivalent (**solution Q1**, 10 μL) was evaporated on a pseudo-queen probe **P23** and inserted into an arena containing 15 workers from a queenless hive **H7**. There was almost immediately an attraction to **P23**. 3 bees licked the probe within 15 seconds and 12 within 45 seconds. There was much antennation and all the bees aggregated around the probe for 5 minutes before they began to disperse.

In **trial 15**, the same pseudo-queen **P23** was transferred to a fresh arena containing 15 bees obtained directly from a queenright colony **H8**. There was some attraction and antennation by 5 of the bees within the first 60 seconds but they dispersed within 100 seconds. This result indicated that the bees from queenright colonies at Parung Panjang needed an extended time to acclimatise to the absence of the queen and colony odours before their responses were assayed. This delay was not a prerequisite for successful close-range assays of synthetic blends in the PNG Highlands.

In **trial 16**, 0.1 queen equivalent (**solution Q2**, 10 μL) was evaporated on a pseudo-queen probe **P24** and inserted into an arena containing 15 workers from the queenless hive **H7**. The bees were agitated at first with only occasional licking of the probe. After 3 minutes all the bees had aggregated around the probe but after 5.5 minutes they began to disperse. Thus, there appeared to be a delayed response to **Q2** compared with **Q1**, possibly because the bees had not settled before the assay.

The experiment was repeated in **trial 17**. 0.1 queen equivalent (**solution Q2**, 10 μL) was evaporated on a pseudo-queen probe **P25** and inserted into an arena containing 15 freshly obtained workers from the queenless hive **H7**. The bees were moving frenetically in the arena before insertion of the probe. There was no attraction to **P25** over the observation period of 10 minutes, demonstrating that the bees need to be quiescent before a close-range trial.

A more consistent response from different colonies now appeared possible. In **trial 18**, 15 workers were obtained from a queenless hive **H9** and left to stand for 1.5 hours until they were quiescent. 0.1 queen equivalent (**solution Q1**, 10 μL) was evaporated on a pseudo-queen probe **P26** and inserted into the arena. The bees were attracted to the pseudo-queen within 10 seconds and all 15 aggregated within 2 minutes. The aggregation gradually diminished in 4-5 minutes whereupon the bees dispersed and became agitated.

The natural extracts could be diluted without significant loss of efficacy. Thus, 10 μL of **Q1** was diluted with 40 μL of dichloromethane to yield **solution Q3**. In **trial 19**, 0.02 queen equivalent (**solution Q3**, 10 μL) was evaporated on a pseudo-queen probe **P27** and inserted into an arena containing 15 workers (queenless hive **H9**) that were quiescent. The bees were attracted to the pseudo-queen within 5-10 seconds and all 15 aggregated within 2-3 minutes. Similarly in **trial 20**, 0.01 queen equivalent (**solution Q3**, 5 μL) was evaporated on a pseudo-queen probe **P28** and inserted into an arena containing 15 workers (queenless hive **H10**) that had been left until settled. The lure became attractive within 2 minutes and aggregation by 8 of the 15 was sustained for 5 minutes. There was some antennation of **P28** and noticeable fanning by the workers.

The natural pheromone solutions were left at ambient temperatures for a prolonged period during the course of these assays. Thus, it was important to assess their durability because this was also relevant to the stability of the synthetic blends. A freshly obtained mature queen *A. cerana javana* was decapitated and its head extracted with 500 μL of dichloromethane (**solution Q4**). After 15 minutes extraction, 400 μL of **Q4** were preserved at -180°C in the dry shipper (**solution Q5**) while 100 μL of **Q4** were left in a specimen tube at room temperature and in natural light for 2 days (**solution Q6**).

In **trial 21**, 0.02 queen equivalent (**solution Q5**, 10 μL) was evaporated on a pseudo-queen probe **P29** and inserted into an arena containing 15 settled workers obtained from queenless hive **H11**. The bees were attracted to **P29** within 5 seconds and aggregated around the probe. The attraction began to diminish after 3 minutes.

In a parallel experiment (**trial 22**), 0.02 queen equivalent (**solution Q6**, 10 μL) was evaporated on a pseudo-queen probe **P30** and inserted into an arena containing 15 settled workers from the same queenless hive **H11**. There was a fanning response from 10 of the workers but no significant attraction to the vicinity of the probe. However, when 0.02 queen equivalent of the preserved extract (**solution Q5**, 10 μL) was added and evaporated on the same probe (pseudo-queen **P31**), there was a rapid response by the workers within 10 seconds followed by aggregation. The results indicated that **Q6** had deteriorated in composition at room temperature.

Nevertheless, there was an alternative explanation for the contrasting responses to **Q5** and **Q6**. Since the more voluminous extract **Q5** contained the queen head, the solution **Q6** might have been more dilute than **Q5** because it was separated before equilibration. This was supported by the observation that the similarly diluted extract **Q3** was still active after 4 days use. Thus in **trial 23**, 0.02 queen equivalent (**solution Q3**, at ambient temperature in daylight for 2 days, 10 μL) was evaporated on a pseudo-queen probe **P32** and inserted into an arena containing 15 settled workers from the queenless hive **H11**. There was attraction to **P32** within 5 seconds resulting in aggregation and some antennation by workers. The bees began to disperse after 3 minutes.

The observation that the attraction of the bees in the arena to the natural and synthetic blends was often short-lived appears to be partly a consequence of the limited headspace and restricted mobility in the arena. This was evidenced by the result that when the pseudo-queen **P31** was left on the laboratory bench, it attracted 5 *A. cerana javana* escapees in the room. The bees aggregated around the probe and fanned, with the attraction lasting for more than 10 minutes, somewhat longer than generally observed within the arenas.

(f) Close-range trials at Sukabumi

The natural queen pheromone solutions were cooled and transported to Sukabumi within the dry shipper. The *A. cerana javana* colonies at the beekeeping centre (Gunung Arca) near Sukabumi proved to be significantly more responsive to the natural pheromone lures than the colonies at Parung Panjang. For example in **trial 24**, 0.02 queen equivalent (**solution Q5**, 10 μL) was evaporated on a pseudo-queen probe **P33** and inserted into an arena containing 20 settled bees from a queenright colony **H12**. Within 10 seconds a clump on the remote side of the arena had dispersed and within 1 minute, all the bees had aggregated around the probe. The aggregation continued for over 30 minutes with occasional licking and antennation and the bees only began to disperse after 45 minutes.

An even more striking example of the difference in responses of the bees at the two locations was evident in the contrasting responses to **solution Q6** in **trial 25**. 0.02 queen equivalent (**solution Q6**, 10 μL) was evaporated on a pseudo-queen probe **P34** and inserted into an arena containing 15 settled bees from the queenright colony **H12**. The bees began to aggregate around the probe within 3 minutes and the aggregation continued for more than 15 minutes. This was strikingly different from the response at Parung Panjang and confirmed the conclusion that **Q6** had not deteriorated markedly when left at room temperature for 2 days.

For **trial 26**, 1.0 queen equivalent of synthetic *A. cerana javana* queen pheromone (**solution F**, 1 μL) was evaporated on a pseudo-queen probe **P35** and inserted into an arena containing 15 quiescent workers from hive **H12**. 2 bees approached **P35** and licked it within the first minute but there was no general movement by the remainder towards the probe. By 5 minutes all the bees were fanning vigorously and there was some subsequent clustering but not at the probe. The pseudo-queen **P35** was therefore removed and replaced by a fresh one (**P36**) to which 0.02 queen equivalent (**solution Q6**, 10 μL) had been applied. All the bees aggregated around the probe within 30 seconds and remained in the vicinity for 5 minutes.

The results of **trial 26** demonstrated that the workers preferred the natural blend in these close-range bioassays but indicated that prior treatment by the synthetic pheromone blend did not necessarily preclude attraction. Despite the evidence that the bees at Sukabumi were more sensitive to queen pheromone than those at Parung Panjang, they did not aggregate around synthetic pheromone lures in the manner observed previously in PNG.

(g) Intermediate-range trials at Sukabumi

The field cage for intermediate-range trials was transported from Parung Panjang and erected in a grass clearing near the summit of Gunung Arca. The various lures were suspended by nylon fishing line from the arms of the spoke assembly, which could be rotated freely so that the initial positions of the lures within the cage were random. The lures were wrapped in a small square (4 cm x 4 cm) of gauze before attachment to the assembly. Each of the lures was suspended 30 cm above ground level. Mature queens were separated from the hives of *A. cerana javana* about 1 hour before each trial.

For **trial 27**, the different controlled-release lures were attached to the arms of the spoke assembly. 2-Octenyl acetate was included because it is a major component of the Nasonov glands of *A. cerana javana* (Lacey, 1997) and may be attractive in its own right. Its homologue 2-decenyl acetate is a component of *A. dorsata* and *A. florea* and appears to be an olfactory marker (Veith *et al.*, 1978).

The dispensers were placed in the following orientation:

Spoke 1	<i>A. cerana javana</i> queen pheromone blend, 10 queen equivalents
Spoke 2	Ethyl Z-9-octadecenoate, 1 mg
Spoke 3	2-Octenyl acetate, 1 mg
Spoke 4	<i>A. cerana javana</i> Nasonov pheromone blend, 1 mg
Spoke 5	<i>A. mellifera</i> queen pheromone blend, 10 queen equivalents
Spoke 6	Control dispenser

Workers from queenless hive **H13** were released on the grass beneath the spoke assembly and the tent was resealed. There was a slight cross-breeze and the sky was cloudy. Within 1 minute, bees began to land on the **spoke 1** dispenser, forming a clump around the dispenser. There were no landings on any of the other lures. The clump increased in volume over 15 minutes, after which time it decreased in size. Only a few bees remained on the lure after 20 minutes. The hive box **H13** and resident queen were therefore replaced in the field cage to retrieve the dispersed colony (Lacey, 1997).

The experiment was repeated 1 hour later with a fresh queenless colony **H14** but using the same lures and same orientation on the spoke assembly (**trial 28**). The workers from **H14** were released both beneath the assembly and on the ground elsewhere in the tent. Bees began to inspect the **spoke 1** dispenser within 30 seconds and landed after 50 seconds. The clump slowly grew in volume over 20 minutes and then began to decline. The colony was retrieved with the hive box **H14** and resident queen.

Because there was the possibility that the bees landing on the *A. cerana javana* queen pheromone dispenser in **trial 27** might have left a footprint pheromone and therefore have prejudiced the outcome of the replicate **trial 28**, all the lures were replaced with fresh dispensers and gauze enclosures for **trial 29**. Bees were obtained from a queenless colony **H15** and were scattered both on the grass near the spoke assembly and into the air within the tent. There was no noticeable breeze at the beginning of the trial. While clumps of bees formed on the roof of the tent in the first 5 minutes, there were no landings on any of the lures during the first 10 minutes, even though a few bees inspected the *A. cerana javana* queen pheromone dispenser on **spoke 1**. After 10 minutes, however, bees began landing on **spoke 1** in increasing numbers such that after 20 minutes, the clump had tipped the balance of the spoke assembly so that it almost reached the ground. The bees began to disperse over the next 10 minutes but even after 30 minutes, there was still aggregation around the dispenser on **spoke 1**.

demonstrating a sustained attraction. The trial was discontinued at this point and the bees were retrieved with hive box **H15** and its resident queen.

Since it was possible that proximity effects of adjacent lures could have compromised the choices of the workers, the lures on the spoke assembly were reoriented to obviate this possibility:

Spoke 1	<i>A. cerana javana</i> Nasonov pheromone blend, 1 mg
Spoke 2	Ethyl Z-9-octadecenoate, 1 mg
Spoke 3	2-Octenyl acetate, 1 mg
Spoke 4	<i>A. cerana javana</i> queen pheromone blend, 10 queen equivalents
Spoke 5	Control dispenser
Spoke 6	<i>A. mellifera</i> queen pheromone blend, 10 queen equivalents

The wind was allowed to die down to a gentle breeze for **trial 30**. Workers from a queenless colony **H16** were dispersed within the tent, mostly in the air but some on the ground. Bees began to inspect and land exclusively on the **spoke 4** dispenser after 10 minutes and by 15 minutes, sufficient bees had aggregated to tip the balance so that the clump reached the ground. The attraction was sustained for 45 minutes after which the clump began to decrease in size. The colony was retrieved with hive box **H16** and its resident queen.

After use in the assays, the *A. cerana javana* queen pheromone dispensers from **trials 28, 29 and 30** were stored in stoppered glass specimen tubes to prevent further vapour loss. The 3 dispensers were combined with a fresh dispenser in a single gauze cover for **trial 31** in an attempt to ascertain whether multiple dispensers were more effective as a single-source lure than one dispenser. After a rainstorm and wind had died down, worker bees from a queenless colony **H17** were dispersed in the tent. Many of the bees clustered on the roof of the tent but it took 15 minutes before they began to land on the *A. cerana javana* queen pheromone lure. Aggregation took place rapidly over the next 10 minutes but it was not sustained. The clump surrounding the lure began to decrease in size after 25 minutes. The colony was retrieved with hive box **H17** and its resident queen.

Although the results of **trial 31** suggested that multiple lures were not superior to single lures, the bees may have been less receptive than in previous intermediate-range trials because they may have been:

- sensitive to the change in humidity;
- irritated during the initial 10 minutes by occasional outside intervention to disrupt the roof clusters;
- disturbed by non-resident bees remaining in the tent from the preceding **trial 30**.

To avoid these possible complications, the remaining trials were conducted under similar atmospheric conditions; the workers were left undisturbed after their release; and the field cage was emptied completely of residual bees before commencing the subsequent trial.

In order to assess the synthetic pheromone solutions that had been used in most of the short-range assays but whose durability had been questioned, the *A. cerana javana* queen pheromone blend (**solution B**, 1 mL, 10 queen equivalents) was absorbed onto 2 dispensers and the solvent (methanol) allowed to evaporate. The gauze-covered lure was substituted on **spoke 4** for **trial 31** and bees from queenless colony **H18** were scattered in the tent. After 3 minutes bees began to land exclusively on the lure on **spoke 4** and continued to do so to the extent that the clump reach the ground after 40 minutes. Aggregation persisted for at least 50 minutes whereupon the trial was discontinued. The colony was retrieved with hive box **H18** and its resident queen. All remaining bees were dispersed from the tent enclosure. Clearly the methanolic **solution B** had not deteriorated to the extent indicated by the earlier short-range experiments at Parung Panjang.

In the final intermediate-range trial (**trial 32**), an attempt was made to assess the relative potencies of natural and synthetic queen pheromone blends. The residual amounts of **Q5** and **Q6** solutions preserved in the dry shipper were combined (approximately 0.5 queen equivalent) and absorbed onto a

3 mm length of medical rubber. It was assumed that the release rate of pheromone blend from this lure would be of the same order as that emanating from the synthetic pheromone dispenser (10 queen equivalents on 25 mm length). The natural lure was suspended from **spoke 4** and bees from queenless colony **H19** were scattered in the tent. There were occasional visits to the vicinity of the lure by one or two bees but no landings over the observation time of 20 minutes.

To ensure that colony **H19** was actually receptive to the queen pheromone odour, a fresh dispenser of the synthetic *A. cerana javana* blend was substituted on **spoke 4** for the natural lure. The bees were attracted almost immediately to **spoke 4** and the adhering clump of workers gradually tipped the balance of the spoke assembly such that it reached the ground. Aggregation continued for 20 minutes after which the bees began to leave the clump. After 30 minutes the trial was terminated and the colony was recovered with its hive box **H19** and resident queen. Apparently the synthetic lure was superior to the natural lure in these intermediate-range assays, even though the converse was the case for many of the short-range assays.

(h) Close-range trials with *A. mellifera* in Canberra

Experiments were conducted on return to Canberra to elucidate the reasons why *A. mellifera* responded with fanning behaviour rather than the expected aggregation (Kaminski *et al.*, 1990) in the close-range **trials 10** and **11** in Bogor. Some of the possible differences were that the bees in the study by Kaminski *et al.* were pacified by brief treatment with gaseous carbon dioxide and that the workers remained in the arenas before their trials for a prolonged period (3-5 h) with access to 50% aqueous sugar syrup. *A. mellifera* workers from a mature colony at Ginninderra, ACT, were deprived of their resident queen for 1 h. The bees were narcotised in the laboratory with carbon dioxide and divided into groups within arenas before they revived. The arenas were left on a laboratory bench, 30 cm apart, for 0.5-1 h at 20°C, which were sufficient conditions to permit some clump formations.

0.1 queen equivalent of freshly prepared *A. mellifera* queen pheromone blend (10 µL) was evaporated on pseudo-queen probe **P37** and inserted into an arena of 20 bees that had been left for 0.5 h to acclimatise (**trial 33**). The bees commenced fanning within 10 seconds and after 2 minutes 3 of the workers approached **P37**. By 4 minutes there was aggregation around the pseudo-queen by the majority (15) and occasional licking of the pheromone by individuals. After 6 minutes the bees began to disperse. In a similar trial, 0.1 queen equivalent of freshly prepared *A. mellifera* queen pheromone blend (10 µL) was evaporated on pseudo-queen probe **P38** and inserted into an arena of 15 bees that had been left for 1 h to acclimatise (**trial 34**). Fanning commenced within 10 seconds and within 1 minute all the workers were fanning vigorously. After 5 minutes there was antennation of **P38** by 6 of the bees and aggregation around the pseudo-queen by the majority (10). The bees dispersed after 7 minutes.

A further trial (**trial 35**) was carried out using a sample of *A. mellifera* queen pheromone blend from an ampoule that had been transported to Indonesia and left at room temperature after its return to Canberra. 0.1 queen equivalent of *A. mellifera* queen pheromone blend (**solution G**, 10 µL) was evaporated on pseudo-queen probe **P39** and inserted into an arena of 15 quiescent bees that had been left for 1 h. The workers reacted to the vapour from **P39** within 10 seconds. Some of the bees (8) began to fan after 1 minute. There was a general movement towards the probe after 4 minutes by 11 of the bees and 5 of the bees antennated and crawled over **P39**. The bees dispersed after 6 minutes.

(i) Natural queen extracts of *A. cerana javana*

Since the quality and quantity of the synthetic queen pheromone blends for *A. cerana javana* had been based solely on the chemical analyses of just 2 queens (Lacey, 1998), additional samples of the natural pheromone were collected for transport in the dry shipper at -180°C. The heads of 3 mature queens from Parung Panjang and 3 from Sukabumi were each dissected into dichloromethane (0.3 mL) solvent for extraction and subsequent analysis in Australia.

The quantities of queen pheromone constituents in micrograms were:

Pheromone constituent	Q1	Q2	Q3	Q4	Q5	Q6
9-keto-2 <i>E</i> -decanoic acid	238.6	129.2	54.1	122.0	104.4	101.3
(<i>R,S</i>)-9-hydroxy-2 <i>E</i> -decanoic acid	51.7	34.4	18.3	46.1	36.3	8.6
4-hydroxy-3-methoxyphenylethanone	18.3	8.6	10.0	15.8	23.7	5.6
methyl 4-hydroxybenzoate	7.1	6.9	4.8	38.4	31.6	2.6
Total yield	315.7	179.1	87.2	222.3	196.0	118.1

(j) Long-range field trials with *A. cerana javana*

As a contribution to the response to the accidental incursion of *A. cerana javana* into Brisbane in August, 1999, a batch of 50 pheromone lures was prepared urgently and despatched to Jack Shield (QDPI), head of the Asian Honey Bee Local Disease Centre. Individual lures comprised two dispensers of medical rubber tubing (length 35 mm, OD 5 mm, ID 3 mm), each containing 2.72 mg 9-keto-2*E*-decanoic acid, 0.50 mg (*R,S*)-9-hydroxy-2*E*-decanoic acid, 0.36 mg 4-hydroxy-3-methoxyphenylethanone and 0.30 mg methyl 4-hydroxybenzoate. Laboratory studies indicated an approximate first-order release rate of 50 µg pheromone blend per day at 22°C with the lure exposed to a wind velocity of 1.8 km/hr. Thus, the lures were expected to remain attractive to *A. cerana javana* in the field for up to 3 months. Each lure was completely sealed in a glass envelope to preserve its integrity before application. It was recommended to personnel that the lure be wrapped in a portion of nylon flyscreen netting before opening (by breaking the glass envelope) in order to facilitate handling and to minimise exposure of the pheromone contents to photodegradation. There were no reports of capture of *A. cerana javana* on the mainland during the subsequent period of intensive monitoring operations using modified Lucitraps and it was concluded that the colony had failed to re-establish itself.

In November, 1999, a limited trial with the previously prepared *A. cerana javana* pheromone lures was conducted on Dauan Island in the Torres Strait by the Northwatch Response and Surveillance Technical Officer, Mr Mark Trinca. Three modified Lucitraps were set up in similar situations in neighbouring mango trees. Traps A and B were each baited with the synthetic *A. cerana javana* pheromone while trap C served as a control. In December, the honorary Northwatch officer on Dauan, Michael Asela, reported that the pheromone traps were catching bees. After the traps had been in place for 30 days, the adhesive sheets within the traps were collected and forwarded to the Northwatch entomologist in Cairns, Mr Paul Dangerfield. All the trapped bees were identified by Mr Dangerfield as *A. cerana javana*. The pheromone traps A and B caught 12 and 47 bees respectively while the control trap C caught none.

In order for quarantine officers and QDPI personnel to conduct further long-range trials with the pheromone, an additional 160 aggregation lures were prepared in January-February, 2000. These were despatched to AQIS (Dr David Banks) and QDPI (Mr Jack Shield) and will enable more extensive field trials and testing of swarm traps for monitoring purposes.

5. Discussion of Results

The strong attraction by *A. cerana javana* workers to the synthetic *A. cerana javana* pheromone blend in the close-range **trials 1-3** and the lack of attraction to the synthetic *A. mellifera* pheromone blend in **trials 2-3** was significant and confirmed our conclusions that the queen substances of *A. cerana javana* and *A. mellifera* are distinctly different. While Plettner *et al.* (1997) have reported that *A. cerana* workers in Sri Lanka respond to the synthetic queen pheromone blend for *A. mellifera* in analogous short-range bioassays, this behavioural difference may reflect the genetic diversity of *A. cerana* throughout its endemic range.

The conclusions from close-range **trials 1-3** were:

- *A. cerana javana* workers from a queenright hive are consistently attracted to the 5 component synthetic pheromone blend represented by **solution A**;
- the short-range bioassay using glass pseudo-queens as lures is applicable to *A. cerana javana*;
- the quantity of **solution A** applied to the pseudo-queen is within the appropriate range to ensure aggregation by *A. cerana javana*;
- the attraction persists for 1-3 minutes but diminishes gradually thereafter, possibly reflecting saturation of the headspace within the arena;
- *A. cerana javana* workers are not attracted to the synthetic *A. mellifera* queen blend represented by **solution G**.

The inferences from the results of close-range **trial 5** were:

- the composition of synthetic **solution A** had not changed significantly in the intervening period between the trials in PNG and Parung Panjang;
- workers from queenless hives in Parung Panjang are more responsive in close range bioassays than those from queenright hives;
- the workers in the arenas will not respond to pseudo-queen lures unless they are settled beforehand;
- the responses of the bees at Parung Panjang were more muted and less predictable than those in PNG, possibly because of climatic differences and because the former were more agitated than the latter.

The conclusions from the results of intermediate-range **trials 27 and 28** were:

- Synthetic *A. cerana javana* queen pheromone blend (five component) is selected exclusively by worker bees from the other synthetic blends and individual semiochemicals;
- Synthetic *A. cerana javana* queen pheromone blend can attract bees in flight;
- *A. cerana javana* workers are not attracted to the synthetic queen pheromone lure of *A. mellifera*;
- the matrix of the dispensers protects the pheromone components from deterioration, even though the pheromone lures were left at ambient temperatures for 2 weeks before testing;
- the synthetic blend is effective as a lure in the intermediate-range assays (bees in flight) while, apart from PNG, it was less effective than the natural blend in the short-range assays (restricted movement);
- the chosen matrix is effective in controlling the release rates of vaporisation of the pheromone blend.

The results of close-range **trials 33-35** indicated that narcotising *A. mellifera* workers with carbon dioxide at least 0.5 h before the close-range trials ensured that they were receptive to synthetic queen pheromone lures. The initial fanning response was analogous to that observed in **trials 10 and 11** with *A. mellifera* in Bogor but it was not possible to use carbon dioxide in the latter field trials to induce the subsequent attraction. Preliminary narcosis with carbon dioxide may have been the means of becalming *A. cerana javana* workers to ensure an aggregation response to synthetic pheromone blends in close-range **trials 4-26**. Nevertheless, the results with *A. cerana javana* workers from PNG (**trials 1-3**) showed that this was not an essential prerequisite and may have reflected the fact that the test hives in PNG were much more mature than those sampled in Parung Panjang and Bogor.

While the compounds 9-keto-2*E*-decenoic acid, (*R,S*)-9-hydroxy-2*E*-decenoic acid and methyl 4-hydroxybenzoate have been recently identified as mandibular gland constituents of *A. cerana* queens derived from Malaysia (Plettner *et al.*, 1997), the constituent 4-hydroxy-3-methoxyphenylethanone has never been reported previously to be present in *A. cerana* or in any other *Apis* species. As has already been demonstrated for *A. mellifera* (Winston and Slessor, 1998), it is crucial that all components of a

bee pheromone be identified in order to mimic the full range of behavioural responses to a synthetic blend.

The successful close-range **trials 1-3** and intermediate-range **trials 27-32** demonstrate that sufficient components of the natural pheromone blend for *A. cerana javana* have been identified to ensure that the synthetic mixture described in this report can mimic the natural blend as a very sensitive and selective attractant for the pest. The preliminary result of the long-range trial in the Torres Strait supports these conclusions and demonstrates that the matrix of the dispenser prolongs the evaporation of the pheromone and preserves its constituents from deterioration.

As was evident from the preliminary trial on the Torres Strait, the pheromone lure needs to be contained within an appropriate design of swarm box (artificial nest cavity) for prolonged monitoring purposes. Dr Denis Anderson (personal communication) has reported that traps made from hollow coconut palm logs were the most attractive to *A. cerana javana* swarms in Irian Jaya, while Mr Jack Shield (personal communication) is exploring various designs arising from the successful Lucitrap. Visual attractiveness will enhance the efficacy of the pheromone trap for protective monitoring of *A. cerana javana* in Australia.

6. Implications

- The five-component synthetic blend based on the natural queen substance of *A. cerana javana* attracts conspecific worker bees both in close-range assays (maximum distance from lure 15 cm) and in intermediate-range assays (maximum distance to lure 200 cm).
- Worker bees of *A. cerana javana* are not attracted in short-range or intermediate-range assays to the known synthetic queen pheromone blend for *A. mellifera*.
- The matrix of the controlled-release dispensers is effective for preserving and mediating the release rates of the various pheromone components for *A. cerana javana*.
- The five-component synthetic blend for *A. cerana javana* has significant potential use as a specific long-range attractant for scout bees and swarms of this pest.
- The ectoparasitic mite *V. jacobsoni* encompasses two distinct species of which one, now known as *V. destructor* (Korean haplotype), is the principal *Varroa* parasite of *A. mellifera* worldwide, though not as yet in Australia (Anderson and Trueman, 2000). *V. destructor* is reproductively isolated from *V. jacobsoni* and the latter is not a successful parasite of *A. mellifera*. The original host of *V. jacobsoni* is *A. cerana javana* while the original host of *V. destructor* is probably the Korean haplotype of *A. cerana* (Anderson and Trueman, 2000). Thus, an incursion into Australia of the Korean haplotype of *A. cerana* could present an even more damaging scenario than an incursion of *A. cerana javana*. The pheromones of the Korean haplotype are as yet unknown but a sensitive and selective pheromone attractant for the former could be developed and would offer a considerable advantage for protective monitoring. It is already known that the Nasonov pheromones of *A. cerana indica* (Naik *et al.*, 1988) and *A. cerana japonica* (Matsuyama *et al.*, 1996) are significantly different and therefore it is highly probable that pheromones for the Korean and Javanese haplotypes of *A. cerana* are distinctive as well.

7. Recommendations

- Since the successful intermediate-range trials were limited to a maximum flight range of 2 m, it is recommended that further long-range trials be conducted in the Torres Strait with the five-component synthetic blend for *A. cerana javana* to improve its potential for attracting scout bees and swarms.
- It is recommended that the pheromone dispensers be evaluated within swarm boxes of varying designs (artificial nest cavities) to assess both their efficacy and durability.

- It is recommended that the Consultant accompany Dr Denis Anderson to Irian Jaya to test pheromone lures and swarm boxes in a region of high endemic population of *A. cerana javana*.
- It is further recommended that field trials be carried out in Irian Jaya to gauge the optimum loading of the synthetic pheromone blend in the controlled release dispensers and the optimum density for sustained attraction by the lures.
- It is recommended that the natural queen pheromone blend of the Korean haplotype of *A. cerana* be identified as a necessary step in order to develop an analogous specific attractant for protective monitoring of this more serious strain of *A. cerana*.

8. Communication Strategy

Contacts have been established with relevant personnel in AQIS, QDPI and NAQS to ensure adoption of the research results. Communication of the research outcomes, where appropriate and with agreement by RIRDC and CSIRO, will be through publication in the scientific literature and media releases.

9. Bibliography

- Anderson, D.L. and Trueman, J.W.H. (2000).** *Varroa jacobsoni* (Acari: Varroidae) is more than one species. *Experimental and Applied Acarology*, **24**: 1-25.
- Kaminski, L.A., Slessor, K.N., Winston, M.L., Hay, N.W. and Borden, J.H. (1990).** Honeybee response to queen mandibular pheromone in laboratory bioassays. *Journal of Chemical Ecology*, **16**: 841-850.
- Lacey, M.J. (1998).** Identification and application of the aggregation pheromone of *Apis cerana*. Final report, RIRDC Project No. CSE-74A.
- Matsuyama, S., Suzuki, T. and Sasagawa, H. (1996).** Proceedings, 3rd Apicultural Association Conference, Hanoi, Oct. 6-10, S1-13.
- Naik, D.G., Gadre, R.V., Kapadi, A.H., Singh, M.K., Suryanarayana, M.C. and Kshirsagar, K.K. (1988).** Nasonov gland pheromone of the Indian honey bee *Apis cerana indica*. *Journal of Apicultural Research*, **27**: 205-206.
- Pickett, J.A., Williams, I.H. and Martin, A.P. (1982).** (Z)-11-Eicosen-1-ol, an important new pheromonal component from the sting of the honeybee, *Apis mellifera* L. (Hymenoptera, Apidae). *Journal of Chemical Ecology*, **8**: 163-175.
- Plettner, E., Otis, G.W., Wimalaratne, P.D.C., Winston, M.L., Slessor, K.N., Pankiw, T. and Punchihewa, P.W.K. (1997).** Species and caste-determined mandibular gland signals in honeybees (*Apis*). *Journal of Chemical Ecology* **23**: 363-377.
- Sasagawa, H., Matsuyama, S. and Peng, C.Y.S.** Recognition of a parasite: hygienic allo-grooming behavior induced by parasitic *Varroa* mites in the Japanese honey bee, *Apis cerana japonica* RAD. Paper presented at 13th Congress of the IUSI, Adelaide, December 29, 1998-January 3, 1999.
- Schmidt, J.O., Morgan, E.D., Oldham, N.J., Do Nascimento, R.R. and Dani, F.R. (1997).** (Z)-11-Eicosen-1-ol, a major component of *Apis cerana* venom. *Journal of Chemical Ecology* **23**: 1929-1939.
- Veith, J., Weiss, J. and Koeniger, N. (1978).** A new alarm pheromone (2-decen-1-yl acetate) isolated from the stings of *Apis dorsata* and *Apis florea* (Hymenoptera: Apidae). *Experientia*, **34**: 423.
- Winston, M.L. and Slessor, K.N. (1998).** Honey bee primer pheromones and colony organization: gaps in our knowledge. *Apidologie*, **29**: 81-95.