Australian Government

Rural Industries Research and Development Corporation

Honeybee Research Report 2004

Research completed and in progress for the Honeybee R & D Program

May 2004

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Foreword

On 1 July 1995, the former Honeybee Research and Development Council became a committee of the Rural Industries Research and Development Corporation.

This publication, Honeybee Research Report 2004, provides details of honeybee research from July 2003 until June 2004 and lists projects commencing in the 2004/2005 financial year. It follows the Honeybee Research and Development Council Research Report 1980-1995 and the RIRDC Reports 1995-1997, 1998-2003, which were a collection of final report and progress summaries of levy funded honeybee research until June 2003. All of these research summaries plus many of the full research reports have been included on the recently released version 2 of the Honeybee R&D CD. This incorporates a web based styled search system for easily finding all research on a particular issue. These are available from RIRDC as 'Honeybee Program-Research Reports' on CD, Version 2 (CD03/001) for $26.00 which includes postage and handling and GST costs.

This report provides information to help apiarists and others access research recommendations and research in progress, together with researcher contact details, in a simple, easy to read format.

This report, a new addition to RIRDC's diverse range of over 1000 research publications, forms part of our Honeybee R&D program, which aims to improve the productivity and profitability of the Australian beekeeping industry

Most of our publications are available for viewing, downloading or purchasing online through our website:

- downloads at www.rirdc.gov.au/fullreports/Index.htm
- purchases at www.rirdc.gov.au/eshop

Alternatively, there is a RIRDC order form included on the last page of this publication.

Simon Hearn
Managing Director
Rural Industries Research and Development Corporation

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Continuing Projects 2003-04

PRODUCTION - Bee Husbandry & Management

Project Title	Drone honey bees- semen production

RIRDC Project No.: DAN-205A
Start Date: 07/01/2002
Finish Date: 31/07/2005
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Objectives

- To provide data on the effects of drone age, season (time of year), and differences between unrelated drone breeding lines on the production and quality of drone semen.

Current Progress

Data collection spring drones (2003) and summer drones (2003-2004) has been completed. One breeding line of the four lines being examined has consistently reared small numbers of drones resulting in an insufficient number of drones from this line available for the summer 35 day old drone examinators. All other data required to be collected from the summer drones has been collected. Autumn drones have been reared with data collected from 14 day old drones. Data collection from the 21 and 35 day old drones is expected to be completed by mid May 2004.

PRODUCTION-Diseases & Pests

Project Title	A study of Gluconobacter – gluconic acid producing bacteria, symbionts of bees: development of biological control for chalkbrood

RIRDC Project No.: ANU-58A
Start Date: 01/01/2002
Finish Date: 30/05/2007
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Objectives

- To isolate and characterise bacteria from varied Australian bee hives that produce antifungal agents effective against the chalk brood disease. The results of this strategic basic research will provide specific information to carry out applied research in the future to develop a biological control of chalk brood disease.

Current Progress

This project is studying the symbiotic association of bacteria with Australian honeybees. Previous studies by Margaret Hilton isolated gram-positive bacteria from the hypopharyngeal gland and intestinal tract of adult bees. She showed some of these bacteria produce gluconic acid and other unidentified antifungal agents, some of which can inhibit the chalk brood fungus. In the present research samples were taken from different bee types and associated bee products (adult worker and nurse bees, larvae, stored honey and bee-bread supplies on the hives). A number of different gram negative bacterial species have been identified in these samples, such as *Klebsiella* and *E.coli*, using gram-specific media.. These bacterial strains are currently being characterised and some have been shown to strongly inhibit chalk brood on chalk brood specific media (Yeast-Glucose-Phosphate agar). Bacterial isolates that produce anti-fungal agents that inhibit chalk brood will be screened to determine if they have the potential to be used in control of the chalkboard disease.. Hilton's study has shown that there is selection for bacteria that can compete with fungi like chalk brood in the intestinal tract of honeybees. This may be beneficial to bees in controlling disease. Presently, the control for chalk brood is very limited and expensive.

PRODUCTION-Diseases & Pests

Project Title	Clarification of aspects of Varroa reproduction - first stage of a possible new control method

RIRDC Project No.: CSE-87A
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Objectives

- To determine the hormone profiles in the blood of pre and post-pupal stages of Apis mellifera and Apis cerana drone brood in Java, Indonesia, and in the blood of Varroa mites infesting those stages. This information will form a basis for developing a possible new method for controlling Varroa destructor on Apis mellifera.

Current Progress

Of the 30 or so genotypes of *Varroa* mites that have so far been identified in Asia on the Asian honey bee *Apis cerana* (the natural host of *Varroa* mites), only 2 can reproduce on the European honey bee *A mellifera*. These are the Korea and Japan genotypes of *Varroa destructor*. All other *Varroa* genotypes, including those *V. jacobsoni*, *V. rindereri* and *V. underwoodi*, will attempt to reproduce on *A. mellifera* if given the opportunity. However, these genotypes lack the ability to lay eggs on the *A. mellifera* brood at a critical stage of the infection cycle. Finding the factor(s) that prohibit egg-laying in these mites could lead to the development of a new method for controlling the Korea and Japan genotypes on *A. mellifera*.

To find the factor(s) responsible for *Varroa* egg-laying, a *Varroa* reproduction model is being developed using known insect reproduction models as a guide. Female *V. destructor* and *V. jacobsoni* mites have been collected from *A. mellifera* and *A cerana* broods in Java at specific time intervals after the broods have been capped. These mites are now being sectioned and examined by light microscopy to determine the time when their egg-laying was initiated.

PRODUCTION-Diseases & Pests

Project Title	Using temperature manipulation to control small hive beetle

RIRDC Project No.: DAN-215A
Start Date: 01/04/2003
Finish Date: 31/12/2004
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Objectives

- To develop hive disinfestation procedures involving chilling/freezing of hives/supers to control small hive beetle.

Current Progress

A colony of Small Hive Beetle (SHB) was established using adult beetles collected at Richmond (NSW). Under the insectary conditions (29°C) eggs hatch within 2 days of oviposition. There are four larval 'instars'. The larval period is comprised of a feeding period (6 d) and a non-feeding pre-pupal period (4-10 d). Full-size larvae pupate over the next 13 days if transferred into moist, sandy soil. Emergence of adult beetles begins 13 days later. To test whether exposure of stored comb to low temperatures could be used to disinfest comb, eggs, full-size larvae and adult beetles were transferred to containers inside a freezer (-10°C) or a refrigerator (4°C). After various times the insects were retrieved into normal growing conditions. Recovery assessed 24 h later indicated that 30 min in the freezer killed eggs and adults but 1 h was required to kill larvae. Egg hatch was unaffected after 2 h in the refrigerator but was reduced thereafter. Survival of larvae in the refrigerator was unaffected for at least 48 h but mortality increased to 92 % after 96 h exposure. Larval recovery remained low up to 7 days exposure. No larvae recovered after 8 days.

PRODUCTION-Diseases & Pests

Project Title	Insecticidal control of small hive beetle

RIRDC Project No.: DAN-216A
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Finish Date: 31/12/2004
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Objectives

- To identify the most appropriate insecticides and insecticide application methods to control small hive beetle in hives.

Current Progress

A treated-surface, self-dosing test (bioassay) was developed. One mL of insecticide in is applied to 9 cm diameter filter papers. Ten adult beetles are exposed for up to 48 h to these treated surfaces inside petri dishes. Full dose-response bioassays have been conducted using tau-fluvalinate, coumaphos, flumethrin, diazinon, methomyl and fipronil. LC50s (the concentration lethal to 50% of the population) suggest that fipronil and diazinon are superior to the other insecticides.

A bioassay to measure the susceptibility of SHB larvae to insecticidal soil treatments was developed. Aqueous pesticide solutions are sprayed onto soil contained in 9 cm diameter plastic pipe. Full size larvae are added to the pots. The number of adult beetles emerging relative to untreated controls is used to determine the relative toxicity of the insecticides and to measure their residual effectiveness. Insecticides selected for testing are permethrin, imidacloprid, thiacloprid, chlorpyrifos, profenophos, diflubenzuron and bifenthrin. These were chosen because formulations are already registered as soil treatments for other purposes (usually termite control) or are known to be very residual in soil (diflubenzuron) or are covered by a Pesticide Order for the control of SHB larvae (permethrin). In trials to date permethrin and imidacloprid performed best.

PRODUCTION-Diseases & Pests

Project Title	Transmission of American foulbrood (AFB) disease of honeybees through replacement of queen bees

RIRDC Project No.: DAQ-293A
Start Date: 01/11/2002
Finish Date: 30/03/2004
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Objectives

- To assess the risk of transmitting American foulbrood (AFB) disease to apiaries through use of contaminated queen bees.

Current Progress

Field work commenced initially in September 2002 and continued until March 2003 when it was postponed due to irregular AFB detections in control hives.

Fieldwork recommenced in September 2003 with splitting and requeening of nucleus colonies. Problems with poor conditions and a lack of locally available mated queens have delayed the project. Artificial infection of the hives will take place in spring 2004. The full project is expected to be completed by March 2006.

PRODUCTION-Diseases & Pests

Project Title:	The sensitivity of Paenibacillus larvae isolates (AFB) to oxytetracycline

RIRDC Project No.: DAN 219A
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Objectives

- To determine whether Australian isolates of *P. l. larvae* have acquired resistance to OTC and whether isolates cultured from imported honey are resistant to OTC.

Background

American foulbrood (AFB), caused by *Paenibacillus larvae* subsp. *larvae*, is a major bacterial honey bee disease which causes significant economic loss to the beekeeping industry in Australia and around the world. Oxytetracycline hydrochloride (OTC) has been used overseas to treat AFB for 4 decades. However, in recent years OTC resistant strains have emerged in the USA, Canada and Argentina.

Research

Seventy nine *P. l. larvae* isolates were obtained by culture of honey samples or larval smears recently submitted by beekeepers in Australia, and from imported honey collected from supermarket shelves and honey samples from individual Argentina beekeepers provided by a honey packing plant. *P. l. larvae* isolates from the late 1980s were also tested to determine whether any resistance to OTC has developed in *P. l. larvae* in Australia of the past 15/16 years. The minimum inhibitory concentration (MIC) of OTC was determined for all isolates using the agar dilution method. (The MIC of OTC is the minimum concentration of OTC required to inhibit the growth of *P. l. larvae*)

Outcomes

All *P. l. larvae* isolates from Australian sources including all the isolates from the late 1980s but excluding one isolate from honey originating in Victoria had MICs for OTC of the order of 0.04 µg/ml. The Victorian isolate had an MIC of 0.6 µg/ml. Of the 20 isolates cultured from blended (imported/local) honey or imported honey 6 had MICs of the same order as the bulk of the Australian isolates. The remaining 14 isolates had an MIC of the order of 0.6 µg/ml.

Implications

This study has demonstrated that *P. l. larvae* isolated from Australian sources are very sensitive to OTC and that no resistance to OTC appears to have developed over the past 15/16 years. Most isolates from imported honey had higher MICs for OTC than Australian isolates but the difference was so minor that they would all still be considered to be very sensitive to OTC. This work also indicates that imported honey from Argentina has not been a significant source of OTC-resistant *P. l. larvae*.

PRODUCTION-Diseases & Pests

Project Title:	Evaluating alternative antibiotics for control of European Foulbrood disease

RIRDC Project No.: DAV-198A
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Objectives

- To determine the potential of antibiotics, other than oxytetracycline (OTC), to control the bacterial honey bee brood disease, European Foulbrood (*Melissococcus pluton*) (EFB).
- To identify suitable efficacious antibiotics that quickly degrade and leave no residues in honey extracted from treated hives.

Background

European Foulbrood disease of honeybees is an endemic disease in the commercial apiaries of South-eastern Australia. The current treatment method is to apply the antibiotic oxytetracycline. This antibiotic can result in long lasting antibiotic residues of honey produced and stored in treated hives. Detection of oxytetracycline contamination in Australian honey by our export partners would have serious consequences for the Australian apiary industry.

Research

Antibiotics were selected from all antibiotics registered for use by the Australian Pesticides and Veterinary Medicines Authority. From this listing, antibiotic types with rapid breakdown rates, which were likely to be effective against *Melissococcus pluton*, were identified. The selected antibiotics were tested for their effectiveness in stopping *M. pluton* growth in laboratory culture, and that they were not detrimental to the growth of *Apis melifera* larvae. The comparative rate of degradation, of the candidate antibiotics, in honey was assessed using a bio-assay on antibiotic spiked honey samples.

Outcomes

This project has identified two suitable candidate antibiotics; ampicillin and amoxicillin. They fulfil the selection criteria set in the project objectives, as they are both highly effective at controlling *M. pluton in-vitro* and they are rapidly degraded in honey

Implications

These antibiotics may be a suitable alternative to oxytetracycline for the control of European Foulbrood.

PRODUCTION-Nutrition

Project Title	Predicting the productivity of honeybees from the nutritional value of pollen

RIRDC Project No.: ANU–57A
Start Date: 01/12/2001
Finish Date: 31/12/2004
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Objectives

- To devise a rapid method for explaining the nutritional status and productivity of a colony of bees from the nutritional value of pollen they eat.

Current Progress

We have developed a model to predict the crude protein content of fresh pollen. This model contains 58 samples from 43 plant species and provides accurate predictions of the protein content ($r^2 = 0.99$). We are currently working on models for amino acids, particularly the ten essential amino acids. Our pilot study with pollen stored for several years and analysed in different laboratories produced reasonable models for predicting amino acid composition ($r^2 = 0.6 - 0.8$). We envisage much better models from our analyses of fresh pollen.

We now have a system for conducting feeding studies on small groups of bees (50-100 newly-emerged workers) confined to cages measuring 14 x 12 x 6.5 cm. Bees fed high protein pollen weigh more and develop larger hypopharyngeal glands than do bees fed pollen with less protein. These glands produce protein-rich brood food. Researchers elsewhere have shown that the size of the hypopharyngeal gland depends on the quality of the pollen eaten by the newly emerged worker. In other experiments we have succeeded in getting small groups of bees to raise eggs to the sealed stage. Again, the number of eggs raised depends on the protein content of the pollen fed to the workers.

PRODUCTION-Nutrition

Project Title	Production of a publication on honeybee nutrition in Australia 'Fat bees/skinny bees'

RIRDC Project No.: DAN-186A
Start Date: 01/01/2000
Finish Date: 30/10/2003
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Objectives

- To produce an extension publication on honey bee nutrition, incorporating research findings from past RIRDC projects, literature searches and anecdotal examples of applications in the Australian context in a format that will be readily understood and adopted by beekeepers.

Current Progress

Interviews with the following beekeepers have been completed:

WA: John Davies, Peter Detchon, Harry East, Colin Fleay, Ron Jasper, Rod Pavy, Bob Power, Steve Richards.
SA: Leigh Duffield, John Fuss, Geoff Smith, Graham Wagenfeller.
TAS: Ken Jones, Bill Oosting, Col Parker, Ian Stephens, Julian Wolfhagen.
VIC: Kevin & Glen Emmins, Ken Gell, Ian Oakley, Ray Phillips, Craig Scott.
NSW: Rosemary Doherty, Warren Jones, Dayl Knight, Keith McIlvride, Greg Mulder, John and Keiren Sunderland, Warren Taylor

Interviews with the remaining beekeepers are being finalised these include:
NSW: Fred Taylor, Monte Klinger, Mike Nelson, Dave Fisher, Trevor Billett, Wayne Fuller.
QLD: Rod Palmer, Ken Olley, Don Keith, David Stephens, Will Tiswell.
Quarantine Station: Bruce White.

A full set of information for most of Australia has been assembled in this process and a guide to current practices will be finalised in a book by the end of 2004.

PRODUCTION-Nutrition

Project Title	Nutrition field trial to maximise colony population

RIRDC Project No.: DAN-214A
Start Date: 01/04/2003
Finish Date: 30/10/2005
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Objectives

- To provide evidence of the effectiveness of supplementary feeding honeybee colonies to achieve population increase.

Current Progress

Essentially the 2003 trial failed to provide a strategy for apiarists to artificially increase populations of bees over the winter period. Even so, a significant result was achieved in providing evidence of the reasons why this was not accomplished, and possible future research directions.

Two commercial apiaries were utilised in the trial. At times there were significant differences between the apiaries independent of the treatments indicating strong climatic and floral reward variations between apiary locations. The strongest response in this area was observed between one apiary having access to a flowering canola crop after almond pollination, when the other apiary did not have access to canola blossom. In this case the frames of bees per hive were not significantly different between apiaries, although the area of brood was twice as large in the colonies that had access to canola pollen and nectar followed by pear pollen and nectar than the colonies that did not.

The allocated 5 litres of syrup and 500 gram patties per application was, in many cases, excessive for the colony's ability to remove the supplements. These treatment volumes were applied irrespective of the size of the colony which was found to be wanting. As a result of measuring left over syrup and protein patties, it is recommended that 50 grams of pattie and 500 ml of syrup per frame of bees per month be considered a maximum level for the mild winter conditions. Pollen supplements and sugar syrup should be provided to a colony on a volume or weight basis per size of population formula.

There is strong evidence that any benefit from the various supplements provided to the colonies was overridden by an adult bee disease *Nosema apis*. This disease is known to reduce the longevity of adult bees and thus suppress population increase when nectar and pollen conditions should provide the stimulus for a colony to expand its population. Also, the trial provided evidence that the provision of supplements to a colony during the winter period may have increased Nosema levels in adult bees.
If colonies are required to be a certain size population in late winter or early spring, then management strategies must be implemented during the autumn period prior to winter, giving sufficient time to expand the population of the colonies to the required size prior to winter with little or no management activity on the hives during the winter period, except for the possibility of sugar supplementation in the event of imminent starvation although this may take the form of dry sugar rather than syrup.

PRODUCTION-Nutrition

Project Title	An Australian survey of pollens for their fatty acid composition

RIRDC Project No.:	DAW 100A
Start Date:	01/05/2001
Finish Date:	31/07/2005
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Objectives

- To analyse the fatty acid composition of pollen from at least 120 of Australia's major honey and pollen producing species by 2004 (20 species from each State).

Current Progress

The progress of this project is largely unchanged since the previous report. Thirty pollen samples were only received in the last period. Some 80 odd samples need microscope analysis to determine the genus to verify to a degree the stated species collected by beekeepers. Analysis of data will commence in July-August.

PRODUCTION-Nutrition

Project Title	The effect of high and low fat pollens on honey bee longevity

RIRDC Project No.: DAW 105A
Start Date: 01/07/2003
Finish Date: 30/11/2004
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Objectives

- To ascertain the effect of oleic and linoleic acids on honey bee longevity and their effect on body fat composition.

Current Progress

Three 42 day replicates of testing oleic and linoleic acids have been completed. However, the 2nd replicate was found to have contaminated sugar syrup half way through the experiment (which was allowed to continue). Tests have shown contaminates to be fungal and therefore the 2nd Replicate needs to be repeated. It is possible that the 1st replicate might need repeating too as the same sugar syrup was used but possibly not contaminated then. The second half of the experiment will be delayed by a few months. Bees take 21 days to hatch for each and the feeding experiments are of 42 days duration, so therefore each experiment is taking 63 days and a number of days to measure comb and clean up cages before the next experiment.

Results so far indicate that honey bees will tolerate pollen with higher concentrations of linoleic acid than oleic acid. Honey bees did raise brood in cages with an additional 2% linoleic acid added to redgum *(Corymbia calophylla)* pollen.

Only the protein and fatty acid analyses has been completed for replicate 1. Results of lipid and mineral have yet to be completed. Samples from replicate 2 are being held in a freezer. Samples from replicate 3 await analyses.

PRODUCTION-Nutrition

Project Title	Review of honeybee nutrition research and practices

RIRDC Project No.: JLB-2A
Start Date: 16/04/2003
Finish Date: 16/04/2004
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Objectives

- Develop a review of all nutrition research, which has been undertaken for bees and if possible relate this to practical use by beekeepers.
- Assess how the current and past research RIRDC has funded fits into past research.
- Develop an integrated framework for bee nutrition research in Australia and identify priority areas.
- Present the results of the review at the Honeybee R&D Meeting to be held May 5 and 6 at ANU in Canberra. Develop the structure of the nutrition session if necessary. This may mean presenting the review as the opening session and then the way ahead as a summing up after presentations from current projects.
- After the Meeting provide a written report which includes the review in a form suitable for publication in a scientific journal, recommendations for future RIRDC funded research and mechanisms for developing adoption strategies for the industry.

Current Progress

A thorough review of the literature has been completed on the nutrition of honeybees. The nutritional status of a colony has a marked effect on the growth and development of individual bees, their lifespan, foraging capacity, brood rearing, sex differentiation and resistance to diseases. The sources of nutrients for honeybees and contributions from nectar, honeydew and pollen are reviewed. The composition, digestibility and nutritional value of pollens vary widely between plant species and also within plant species grown in different seasons and locations. Attractiveness of pollen and the amount consumed has a greater impact on the nutritional status of honeybees than pollen protein content. However, there are examples where specific amino acid, fatty acid, mineral or vitamin content of a pollen source may limit honeybee performance. An attempt was made within the review to quantify the requirements of honeybees for energy (expressed as glucose requirement), protein and essential amino acids, essential fatty acids (linoleic, linolenic and sterols), macro and micro minerals, water soluble and fat soluble vitamins. These nutrient requirement specifications and an understanding of factors that attract honeybees may help in the development of more effective pollen substitutes. Research is needed to develop an accurate and practical method for predicting when pollen supply is about to become limiting and will affect colony vitality.

RESOURCES

Project Title	Floral resource database for Tasmania

RIRDC Project No.: DAT-42A
Start Date: 25/03/2004
Finish Date: 01/08/2005
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Objectives

- Survey and document the floral resources on which the Tasmanian Beekeeping Industry depends. Relate the value of sites and floral resources (in beekeeping terms) to tenure, vegetation type and bioregions in order to help estimate the social, environmental and economic value of the Tasmanian Beekeeping Industry.

Current Progress

The project has been going about six months and the following have been completed:

- Preliminary fieldwork on Leatherwood relicts in an attempt to better understand factors affecting distribution.
- Census forms designed and distributed. Census returns and visits now cover nearly all Tasmanian operators with greater than 200 hives.
- Access database has been designed and data entry has begun.
- Definition of variables affecting honey production and hive condition has been largely completed.
- Leatherwood ecology in published works has been investigated and two papers have been written.
- Preliminary Modeling and GIS work for Leatherwood distribution have been done.

The next stages will involve:

- Collate species from survey and run models based on available biological data in GTSpot.
- Complete data entry and spatial analysis of hive site feeding catchments.
- Plan and conduct fieldwork for 04-05 beekeeping season: September-February. Aim is to investigate and refine initial species models as necessary as well as to check any anomalous species names.
- Run workshops on important species models (Feb-Mar 2005).
- Analysis of entered site data for outcomes listed in project briefing.
- Write and submit final report.

RESOURCES

Project Title	The effect of logging on nectar production in NSW forests

RIRDC Project No.: SFN-2A
Start Date: 30/08/2002
Finish Date: 30/10/2005
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Objectives

- To quantify the impact of logging on nectar production in two eucalypt species (Spotted Gum and Grey Ironbark) by measuring nectar production in different tree sizes in forest under different stages of regeneration from logging.
- A better understanding of nectar production in logged forests, when widely communicated, will allow an integration of apiculture with forest management and thus promote sustainability and accessibility.

Current Progress

Nectar in spotted gum was measured in the winter of 2003 in Currumbene State Forest, near Nowra. Poor flowering this year (one of the worst on record) prevented adherence to the original experimental design. Instead, the opportunity was taken to refine field techniques and collect preliminary data. Standing crops of nectar volume and sugar concentration were measured from 39 trees. Standing crops rapidly declined to almost zero after 9:30 am, probably due to the combination of nectar-feeding birds and insects depleting nectar from the small patches of flowering trees available in 2003. Taking only pre-9:30 standing crops, we found that tree diameter did not influence sugar production per flower. However, larger trees have many more flowers than smaller trees, resulting in greater overall sugar standing crops. These values need scaling up to the stand/site level using the abundance of flowering trees.

In late summer 2004 nectar measurements began on Grey Ironbark (n=39). Again, only low flowering levels were experienced. For this species, both nectar production (bagged flowers) and standing crops (unbagged) were measured. Standing crops were almost zero, even at dawn, indicating pollinators depleted nectar as fast as it was produced. Bagged flowers produced much more nectar and like spotted gum, production per flower was not related tree size. However, large trees produced many more flowers than smaller trees, resulting in greater overall sugar standing crops.

RESOURCES

Project Title	Eucalypt regrowth thinning trails to optimise leatherwood honey production
RIRDC Project No.:	FTA-1A
Start Date:	21/01/1999
Finish Date:	30/06/2003
Researcher:	Ms Frieda Heese
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Objectives	• To demonstrate that non-commercial thinning of eucalypt regrowth will enhance leatherwood regrowth at no extra cost. • To establish a set of prescriptions for the timing and intensity of eucalypt regrowth thinning. • To communicate main findings to the beekeeping and forestry industries.
Current Progress	The project is basically completed, however, a detailed report was not provided at the time of printing.

RESOURCES

Project Title	Natural Resource Database for the South Australian Apiary Industry

RIRDC Project No.: DEH-1A
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Objectives

- To identify the major floral resources used by South Australian apiarists and document the locations used by beekeepers when exploiting different floral resources and to compile these data in a suitable electronic form.

Background

There has been no detailed survey that has identified the key floral resources used by the beekeeping industry in South Australia. Such information is important for regional planning particularly with respect to protecting floral assets and maintaining access for beekeepers to these assets.

Research

The primary method of collecting data was by a questionnaire that was distributed to 216 beekeepers that had registered at least 40 hives in South Australia. Each beekeeper was asked to answer a series of questions about their beekeeping operations. These included providing details of the locations that they used for their bees, the times of the year that these sites were used, the numbers of hives placed at each site and the main floral resources that were being used at each site. The initial response to the questionnaire was poor (<30%) and additional data were collected by interviewing individual beekeepers willing to be included. Beekeepers were also asked to identify potential threats or changes to the floral resources that they used.

Outcomes

Based on the responses of 103 beekeepers, at least 118 plant species were identified as providing important floral resources to beekeepers in South Australia. 69 of these were native species (mainly various eucalypts), 27 were introduced plants (many were agricultural weeds) and 22 were crop plants. The most important and widespread resources were Salvation Jane (*Echium plantagineum*) and South Australian Blue Gum (*Eucalyptus leucoxylon*) being recorded as important resources at 26 and 25% of the 750 or so locations listed by South Australian beekeepers. Other widespread key plants included lucerne (14%), *Eucalyptus diversifolia* (14%), and *Eucalyptus camaldulensis* (14%). Of the plants listed as being important to beekeepers, most of the crop and introduced plant species were reliable produces from one year to the next. Many of the native plants, particularly various eucalypts did not flower or provide reliable resources every year. The major factors listed as reducing resource availability for the industry were dieback of eucalypts, grazing of understorey, more frequent dry conditions in recent years, reduction of agricultural weeds associated with a shift from grazing to cropping. Shifts in the types of crops and to more intensive agriculture were also issues. Vegetation clearance was an issue but not in the last ten years. Examination of individual beekeepers' production records supported some of these changes in resource use and some native species such as Pink or Hill Gum *Eucalyptus fasciculosa* which were significant components of honey production from the 1960s-1980s were now minor contributors, suggesting widespread decline for some native plant species.

Implications

More detailed assessments and monitoring of the health and vigour of many of the key floral resources used by beekeepers in South Australia, particularly Pink Gum *Eucalyptus fasciculosa*, is warranted to better track changes in resource availability and to better quantify temporal aspects of flowering intensity.
Since approximately half of South Australia's registered beekeepers did not contribute to this survey some caution is required before assuming that the above surveys have captured all of the key resources used by South Australian apiarists.

OFF FARM ISSUES

Project Title	High power ultrasound for candied liquid honey liquefaction and controlled creamed honey crystallisation

RIRDC Project No.:	UQ-101A
Start Date:	01/10/2002
Finish Date:	30/11/2005
Researcher:	Dr. Bruce R D'Arcy
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Objectives

- To reduce the amount of expensive heating and loss in quality during liquefaction of candied honey by developing an alternate, cost-effective ultrasound based method for the partial or complete liquefaction of candied honey by 2005, with a view to ultrasound having direct application for beekeeper control of honey crystallisation, or for liquefying candied honey prior to decanting in a honey packing plant.
- To better control the texture of creamed honey spread by developing an ultrasound based method that enhances the nucleation rate and produces uniform crystal growth in a creamed honey system by 2005, with a view to it being used by beekeepers and honey processors for producing consistent and high quality creamed honey.

Current Progress

A significant review of the literature related to the use of high power ultrasound in food science, with particular emphasis on foods containing sugar, has been completed. A study of the cavitation process that occurs in sugars solutions during ultrasound treatment is nearing completion. A Malvern Mastersizer is being used to measure the size of the bubbles produced during such ultrasound cavitation. This involves the use of laser diffraction, which is used to determine particle size. Some technical difficulties have arisen due to the recording of large bubble diameters. Use of a larger rectangular glass container that contains the sugar solution within the Malvern Mastersizer is being pursued to overcome this problem. Further, an appropriate mathematical model will be fitted to the bubble size distribution to increase the accuracy of the measurement. In addition, studies have commenced on optimising the ultrasound conditions for treatment of candied yapunyah honey. Initial experiments have found that setting the power levels for a set period of time does not impart the expected energy into the honey for some ultrasound power levels. This is due to honey being quite viscous, with it taking some time for the energy to be transferred from the ultrasonic probe to the honey. Thus, experiments are proceeding, whereby the amount of energy transferred to the honey is now set, while the time period of the treatment is not (although it is recorded).

OFF FARM ISSUES

Project Title	Antioxidants as Health and Nutritional Components of Australian Floral Honeys

RIRDC Project No.: UQ-102A
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Objectives

- To extract antioxidant flavonoids and other polyphenols from straightline samples of three species-specific floral types of Australia honey, namely yapunyah, leatherwood and Salvation Jane honeys;
- To identify and quantify antioxidant flavonoids and other polyphenols in extracts from straightline samples of three species-specific floral types of Australia honey, namely yapunyah, leatherwood and Salvation Jane honeys.

Background

Polyphenols in foods are thought to play important roles in human health such as cancer preventative, and anti-inflammatory, radical scavenging and antioxidative activities. The most important classes of antioxidant polyphenols are the flavonoids and phenolic acids. It is these substances in tea, wine, fruits and vegetables that are most responsible for the antioxidant characteristics, and thus the healthy image of these foods. However, little data exists on these components in Australian floral honeys, hence the need for this study.

Research

The research activities involved two stages: (1) A method for the extraction of antioxidant flavonoids and phenolic acids from honey using Amberlite XAD-2 resin was optimised, and recovery studies undertaken. (2) Identification and quantification of honey flavonoids and other polyphenols was done using high performance liquid chromatography (HPLC) with diode array detection (DAD) at 290 nm (phenolic acids) and 340 nm (flavonoids), and liquid chromatography (LC) - mass spectrometry (MS), including the use of the sensitive, selective ion response (SIR) mode. The HPLC methodology needed to be developed so that separation of flavonoids and phenolic acids was maximised, enabling accurate quantification. However, even with the use of LC-MS, many polyphenols could not be identified, although they were quantified against a standard polyphenol to give some indication of their relative concentrations.

Outcomes

During the optimisation of the Amberlite XAD-2 extraction method, it was found that, contrary to previous studies, the phenolic acids, gallic acid, chlorogenic acid and ellagic acid were not retained on the resin during the extraction under the acidic conditions ideal for such retention. In addition, the recoveries of other phenolic acids such as caffeic acid, *p*-coumaric acid and ferulic acid, reported by Yao (2002) to be in yapunyah and other honey types, varied between 15.5 and 62%. However, this does not mean that other phenolic acids detected in this study were not recovered in higher yields. In contrast, the extraction efficiencies for flavonoid standards such as quercetin, hesperetin and chrysin were much better, with the latter two having recoveries of >83%, in agreement with previous literature studies.

On analysis of extracts from five samples of yapunyah honey using the SIR mode of LC-MS analysis, it was found that the phenolic acids, gallic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, and ellagic acid were detected in negligible levels (<13.2 g/ 100 g), which suggests that the previous

reporting of these phenolic acids in yapunyah honey in much higher concentrations by Yao (2002) was in serious error. This was probably because the study of Yao (2002) did not involve LC-MS analysis, only HPLC-DAD analysis. However, a number of other phenolic acids were detected in the yapunyah honey samples during this study, but could not be identified, even with the use of LC-MS. Flavonoids identified and quantified in yapunyah honey were tricetin, pinobanksin, quercetin, luteolin, quercetin 3-methyl ether, and 8-methoxy kaempferol.

The study of ten leatherwood honey samples quantified the phenolic acid, caffeic acid, and the flavonoids, tricetin, pinobanksin, luteolin, pinocembrin, and chrysin, as well as many unidentified phenolic acids. Leatherwood honey did not contain many flavonoids, but was rich in many phenolic acids. This is a very interesting result when considered in the light of the high levels of volatiles previously found by D'Arcy et al. (2001) in an earlier RIRDC project.

For the seven Salvation Jane honey samples, the flavonoids, pinobanksin, luteolin, kaempferol and pinocembrin, and the phenolic acids, 4-hydroxyphenyllactic acid and α-cyano-4-hydroxycinnamic acid were quantified. In addition, there were a significant number of other flavonoids and phenolic acids quantified, whose identity could not be determined even with the use of LC-MS.

Implications

Knowledge of the health and nutritional values of three floral types of Australian honey has been developed by determining the identity and levels of antioxidant flavonoids and other polyphenols in straightline samples. This scientific data will enable the further marketing of honey as a healthy and nutritious food to the Australian food industry and consumers, in addition to its use as a sweetener.

OFF FARM ISSUES

Project Title	Determination of pollen content of canola (*Brassica napus*) honey

RIRDC Project No.: DAN-218A
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Objectives

- To determine the pollen content, by weight, in canola honey.

Background

Genetically modified (GM) varieties of canola (*Brassica napus*) have been developed and introduced into various countries including Canada and Australia although GM canola has not yet been released commercially in Australia. In the event that it is commercially produced in Australia beekeepers would place bees on these crops for pollination purposes and would be likely to produce honey in the process. Pollen represents the most likely source of transgene DNA and novel proteins in bee products. It is also commonly present in the most widely-consumed product, honey.

In Australia mandatory labelling of GM foods, where introduced DNA or protein is present in the final food, came into effect on 8th December 2001. Food or ingredients labelled GM either contain new genetic material or protein as a result of modification. A 1% threshold, where labelling is not required, exists for the unintended presence of GM material in non-GM foods. This is a more stringent requirement than if honey containing up to 1% of the GM material itself was permitted, since transgene DNA or novel protein will comprise only a fraction of the weight of a GM pollen grain.

Honey, which contains more than 1% of a genetically modified component must be labelled as being genetically modified. This may have an impact on the sales potential for such honey and also influence the export potential of honey from this crop.

Research

Thirty two canola honey samples from New South Wales, Victoria, South Australia, Western Australia and two GM canola honey samples sourced from Canada were used in this study. There is no evidence to indicate that the pollen content of non-GM canola pollen is any different from GM canola hence non-GM canola honey was also used in this study. The percentage of pollen by weight was determined using the number of pollen grains detected in 10 ml of canola honey and the average calculated weight of a canola pollen grain.

Outcomes

The percentage of canola pollen by weight in Australian canola honey ranged from 0.15% to 0.443% with a mean of 0.1951% $\pm$ 0012. The two GM canola honey samples sourced from Canada contained 0.192% and 0.236% by weight.

Implications

This study has determined that the canola pollen content of canola honey is significantly less than 1% by weight of honey from which it originates. As such, honey produced from GM canola crops will not need to be labelled as a GM food.

GENETIC IMPROVEMENT

Project Title	Development of two genetic markers for hygienic behaviour of honeybees

RIRDC Project No.: US-123A
Start Date: 01/06/2003
Finish Date: 31/03/2007
Researcher: Dr Ben Oldroyd
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Objectives

- To identify two genes related to hygienic behaviour at the level of their sequence
- To produce a diagnostic test so that individuals carrying the allele that confers hygienic behaviour can be identified without field testing
- To develop general procedures for the identification of economically important behavioural genes of the honeybee and protocols for their exploitation by industry

Current Progress

We have identified two genetic markers, which are strongly linked to hygienic behaviour, using colonies classified as hygienic and non-hygienic from field studies. By sequencing the surrounding regions of one of the markers, we have developed a single-nucleotide polymorphic (SNP) marker, which would allow for the diagnosis of potential hygienic stock without the need of field testing. However, results from initial tests have shown that the SNP marker has narrower potential in identifying hygienic stock than we had hoped.

From the sequencing of the original genetic marker, we have also pinpointed the location of the marker in the honeybee genome. Based on the marker's position in the genome as well as protein homology, we have generated a list of potential candidate genes for hygienic behaviour. From this we hope to identify a gene related to hygienic behaviour and understand how this behaviour is regulated in individual bees.

New Projects –2004/2005

The following projects have been approved by RIRDC for commencement in the 2004/2005 year:

Title	Researcher	Phone
Securing long-term floral resources for the honeybee industry (HBE04-05)	Dr. David Paton	(08) 8303 4742
Plantations as a resource for native fauna and honeybees (MUL04-17)	Mr. Doug Somerville	(02) 4828 6619
An investigation into the therapeutic properties of honey (HBE04-08)	Dr. Dee Carter	(02) 9351 5383
Biological control of chalkbrood by anti-fungal bacterial symbionts of bees (Extension of ANU-58A) (HBE03-01)	Dr. Murali Nayudu	(02) 6125 3643
Investigating attractants and pheromones for small hive beetle management (HBE04-01)	Dr. Mofakhar Hossain	(03) 5833 5229
Bacteriophage control of European foulbrood (HBE04-02)	Mr. Stephen Doughty	(03) 9210 9222
Literature review of nosema apis (HBE04-10)	Dr. Michael Hornitzky	(02) 4640 6311

PUBLICATIONS AND ORDER FORM

Honeybee R&D Book List

Title	Pub No	Price	Order Amount
HUSBANDRY			
Introduction and Early Performance of Queen Bees	03/049	$16	
Successful introduction and performance of queen bees in a commercial apiary	SR 126	FREE	
DISEASES AND PESTS			
Evaluating alternative antibiotics for control of European Foulbrood disease	04/095	$16	
A beekeepers' guide to understanding control measures for European Foulbrood	04/091	$21	
Fatty Acids-An Alternative Control Strategy for Honeybee diseases	03/028	$16	
Study of the Small Hive Beetle in the USA	03/050	$21	
Breeding hygienic disease resistant bees	02/048	$16	
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Nosema Disease in Honeybees-Genetic Variation & Control	01/046	$16	
Hot Wax Dipping of Beehive Components	01/051	$16	
Honeybee Disease Barrier Management Systems	01/052	$16	
Literature Review of Chalkbrood-a Fungal Disease	01/150	$16	
European Foulbrood	99/020	$16	
Treating American Foulbrood	98/144	$16	
Control of European Foulbrood with OTC and apiary management	SR 135	FREE	
NUTRITION			
Pollen Analysis of Eucalypts in Western Australia	01/053	$16	
Nutritional Value of Bee Collected Pollens	01/047	$21	
Strategic Planning & Action Meeting for Honeybee Nutrition	98/128	$16	
RESOURCES			
Floral Resources used by the South Australian Apiary Industry	04/089	$21	
Beekeepers Use of Honey & Pollen Flora Resources in Victoria	01/050	$21	
Natural Resource Database for the QLD Apiary Industry	99/043	$16	
Impact of Commercial Honeybees on Flora & Fauna	99/015	$16	
Floral Resource Database for the NSW Apiary Industry	99/174	$21	
Beekeeping & Access to Public Land	97/026	$16	
POLLINATION			
The Use of Honeybees as a Transfer Vector for Control of Core Rot in Apples	02/046	$16	
Valuing Honeybee Pollination	03/077	$16	
CD - Pollination Manual 2000	CD00/001	$26	
OFF-FARM ISSUES			
Techniques for the detection of adulterated honey	02/047	$16	
A Quality Survey of Australian Honeys	01/044	$16	
Australian Liquid Honey	99/145	$21	
Bulk Honey Containers	SR 10	Free	
INDUSTRY			
Commercial Beekeeping in Australia	03/037	$21	
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Non-RIRDC Publications and Videos

The following publications and videos have been jointly funded by RIRDC but are not available from RIRDC. Ordering details as indicated.

Beekeeping in the NSW State Forest Districts

by NSW Agriculture, $5 each, phone (02) 4823 0616 to order

A series of reports which include information on beekeeping activities and honey and pollen flora of importance to beekeeping within each state forest district of New South Wales. Each report is approximately 20-26 pages.

Current reports in the series are:

- Queanbeyan/Badja State Forest Management Area – Apiary Management Potential (1995)
- Central Murray Valley Forestry Area – Apiary Management Survey (1995)
- Forbes Forestry District – Apiary Management Survey Results (1996)
- Beekeeping in the Bulahdelah State Forests (1997)
- Beekeeping in the Kempsey State Forests (1997)
- Beekeeping in the Narrandera State Forests (1997)
- Beekeeping in the Taree State Forests (1997)
- Beekeeping in the Tumut-Tumbarumba State Forests (1997)
- Beekeeping in the Wauchope State Forests (1997)
- Beekeeping in the Glen Innes State Forests (1997)
- Beekeeping in the Mildura Forestry Management Area (1997)
- Beekeeping in the Inverell State Forests (1997)
- Eden-Bombala Forestry District - Study of Beekeeping Usage and Importance (1997)
- Beekeeping in the Dubbo State Forests (1998)
- Beekeeping in the Urbenville State Forests (1998)
- Beekeeping in the Morisset State Forests (1998)
- Beekeeping in the Bathurst/Oberon State Forests (1998)
- Beekeeping in the Grafton State Forests (1998)
- Beekeeping in the Urunga State Forests (1998)
- Beekeeping in the Casino State Forests (1998)
- Beekeeping in the Gloucester/Walcha State Forests (1998)
- Beekeeping in the Dorrigo State Forests (1998)

Chalkbrood Disease of Bees

by NSW Agriculture, $25 (includes postage), phone (02) 6391 3433 or 1800 028 374 to order

Enables beekeepers to identify the symptoms of Chalkbrood, outlines measures to take to reduce the impact of this disease and outlines the epidemiology of this disease and how to correctly examine hives to detect Chalkbrood. 10 minutes

Bee Parasites Exotic to Australia

by NSW Agriculture, $30 (incudes postage), phone 02) 6391 3433 or 1800 028 374 to order

Enables beekeepers to identify external exotic parasites (varroa, trachael mites and tropilaelaps) and exotic bees (Asian, giant and dwarf honeybees) and be able to contact the right authorities should they see them in Australia. Includes biology of the parasites, how to inspect hives, how they spread and control measures should they enter Australia. Also covers how to legally import honeybees with approval from AQIS. 20 minutes

Endemic Bee Diseases (VDO5) 1992

by NSW Agriculture, $30 (includes postage), phone (02) 6391 3433 or 1800 028 374 to order

Enables beekeepers to identify endemic bee diseases (American Foulbrood, European Foulbrood, Sac Brood, Wax Moths, Braula Coeca (Tasmania only)) and other brood disorders. Enables beekeepers to identify the symptoms of the disease and pests, outlines measures to take to reduce the impact of this disease and outlines the epidemiology of the diseases and pests. How to correctly examine hives to detect problems. 49 minutes

Package Bee Production in Australia

by NSW Agriculture, $30 (includes postage), phone (02) 6391 3433 or 1800 028 374 to order

Enables beekeepers to follow a step-by-step guide on how to produce, handle and care for package bees, how to prepare package bees for shipment to overseas destinations. Inspection and certification requirements to overseas countries who buy package bees and Queen bees from Australia. 27 minutes