



Honeybee Research Report 2001

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

June 2001

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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 2001, provides details of honeybee research from July 2000 until June 2001 and lists projects commencing in the 2001/2002 financial year. It follows the Honeybee Research and Development Council Research Report 1980-1995 and the RIRDC Reports 1995-1997, 1998, 1999 and 2000, which were a collection of final report and progress summaries of levy funded honeybee research until June 2000.

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 600 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.rirc.gov.au/reports/Index.htm
- purchases at www.rirc.gov.au/eshop

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

Peter Core

Managing Director

Rural Industries Research and Development Corporation

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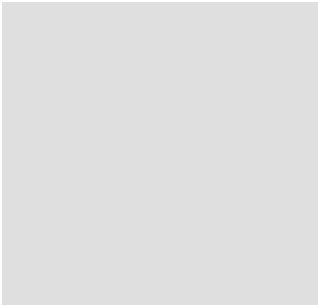
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RESEARCH IN PROGRESS REPORT 2001

Project Title	Improving queen bee production
RIRDC Project No.:	CSE-85A
Start Date:	01/07/99
Finish Date:	31/07/02
Researcher:	Dr. Denis Anderson
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Objectives	To improve the profitability of commercial queen producers by improving queen performance. This will be achieved by: <ol style="list-style-type: none">1. Determining whether nutritional supplements significantly improve queen and drone quality;2. Determining the factors which are responsible for causing disappearing disorder.
Current Progress	<p>Effects of nutrient supplements on queen cell-building colonies</p> <p>This sub-project aims to determine whether the feeding of nutrient supplements during queen production improves the quality of adult queen bees. During the 1999/00 season, the following supplements were each fed to 5 queen cell-building colonies of equal strength: (a) no supplement (control), (b) irradiated pollen, (c) irradiated pollen with added vitamins, (d) soya-flour based supplement, (e) soya-flour based supplement with added vitamins and (f) vitamins. These test-supplements were those recommended by queen producers and participants at the 'Honey Bee Nutrition Workshop' held in Sydney in May 1998. Sister-queens were subsequently raised in the cell-builders, open mated, and tested for: (1) weight, (2) number of ovarioles, (3) number of spermatozoa in spermathecae, (4) nosema levels and (5) body protein.</p> <p>No significant differences were found in the quality of adult queen bees raised from cell-builders fed the different diets. It was concluded that this unexpected result was due to the excellent forage conditions that prevailed in the local environment where the trials were conducted. It is planned to repeat these trials in the future when local forage conditions are poor. At the present time (during the latter part of the 2000/01 season), the effects of feeding nutrient supplements to queen banks is being assessed.</p> <p>Disappearing Disorder</p> <p>This sub-project aims to determine the cause of disappearing disorder which affects bee colonies at certain times of the year in North-East NSW and South-East Queensland.</p> <p>During the 1999/2000 season, the disorder was mild and tests conducted on the few samples that were collected showed no evidence for the presence of pathogenic micro-organisms or excessive levels of toxic</p>



elements in the nectar and pollen being consumed by the affected larvae. During the 2000/01 season, the disorder was moderately severe at Cunningham's gap in SE Queensland, but almost absent in the Gympie area to the north-east. Large numbers of samples of affected larvae were collected from an apiary at Cunningham's Gap. Tests on these larvae again showed no evidence that the disorder resulted from the presence of pathogenic micro-organisms or toxic elements. Further tests are currently being conducted on the samples.

RESEARCH IN PROGRESS REPORT 2001

Project Title	Introduction and early performance of queen bees – some factors affecting success
RIRDC Project No.:	DAN-182A
Start Date:	01/09/99
Finish Date:	31/07/01
Researcher:	Mr. John Rhodes
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Objectives	<ul style="list-style-type: none">• To determine whether the age of the queen bee at introduction influences introduction success.• To identify factors affecting sperm counts of sister queen bees mated under the same mating conditions and to investigate relationships between low sperm counts and queen failure following introduction.• To examine the effects of temperature and humidity during transport of queen bees on their introduction success.• A comparison of sperm counts between sister bees mated in a commercial queen bee breeder`s apiary and between those mated in a commercial honey producer`s queen bee breeding apiary.• Development of a method for preserving adult drones which would allow accurate sperm counts to be carried out on those drones.
Current Progress	<p>Field and laboratory work for objectives 1 to 4 have been completed and relevant data are being analysed. Compared to the 1999-2000 season, seasonal conditions were harder on bees than the 2000-2001 season however higher numbers of queen bees survived for each age group for both the 14 day and 15 week survival counts. The trend of increased numbers of queens surviving corresponding to increased age queens were caught from their mating nucleus continued for both seasons.</p> <p>Work on objective 5 is continuing, small trials in which whole drones were stored in preserving liquids and under refrigeration have been unsatisfactory .</p>

RESEARCH IN PROGRESS REPORT 2001

Project Title	Device for finding queen bees in managed beehives
RIRDC Project No.:	PFP-1A
Start Date:	01/02/01
Finish Date:	01/11/01
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Objectives	To design and prove an electronic device capable of pinpointing the position of queen bees in managed beehives.
Current Progress	The project has only recently started. We have completed a literature survey of possible electronic techniques and have been assembling and sourcing equipment for the first experiments to determine the attenuation by the beehive material at the electromagnetic frequencies of interest.

Project Title	Molecular taxonomy of <i>Nosema apis</i>
RIRDC Project No.: Researcher: Organisation: Phone: Fax: Email:	CSE-3H Robert N. Rice CSIRO Division of Entomology GPO Box 1700 CANBERRA ACT 2601 (02) 6249 3069 (02) 6249 5573 r.rice@mailbox.uq.edu.au
Objectives	To study genotypic variation in the large subunit (LSU) ribosomal RNA (rRNA) of <i>Nosema apis</i> , and determine if variation detected was suitable as a genetic fingerprint for strain identification. To investigate if thymol is likely to suppress Nosema disease in honeybees.
Background	<i>Nosema apis</i> is an economically important pathogen of honeybees and yet little is known about its genetic diversity. Analysis of genetic diversity requires suitable genetic fingerprints that define strains or isolates. This study attempts to identify useful <i>N. apis</i> genetic fingerprints. Some essential oils are known to control some pathogenic bacteria and fungi. Observations by a beekeeper suggest that thymol may suppress nosema disease in honeybees. <i>N. apis</i> cause Nosema disease.
Research	A partial gene sequence from the LSU rRNA of nine <i>N. apis</i> isolates was determined and compared for intraspecific variation. Interspecific comparisons were also made to two other species of microsporidia. The efficacy of thymol as a control for nosema disease in honeybees was investigated in a model system. The closely related species <i>Nosema vespula</i> and host caterpillars of the moth <i>Helicoverpa armigera</i> were used. A dietary regime containing thymol was fed to caterpillars orally inoculated with <i>N. vespula</i> spores. Ten days post-infection, the total number of spores per caterpillar was determined and the data statistically analysed.
Outcomes	Genetic variation was determined amongst the nine <i>N. apis</i> isolates and between <i>N. apis</i> and two other species. The level intraspecific variation detected was not sufficient to be useful as a genetic fingerprint. The efficacy of thymol as a possible control for Nosema disease was demonstrated.
Implications	Further research is required to identify suitable genetic fingerprints for strain identification amongst <i>N. apis</i> . It is likely that thymol will suppress Nosema disease in honeybees when included in artificial diets. The data presented warrants further research.
Publications	Rice. R.N., 1999. An Evolutionary Study of Two Microsporidia. A thesis submitted for the degree of Doctor of Philosophy of the Australian National University, Canberra, Australia. Other papers in preparation. Nosema Disease in Honeybees – Genetic Variation and Control, RIRDC Publication No. 01/046

Project Title	Can the technique of 'shaking bees' and antibiotic therapy be used as a means of controlling American Foulbrood?
RIRDC Project No.:	DAN-176A
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Objectives	To determine whether the technique of shaking bees from hives with American foulbrood onto either foundation or irradiated equipment, followed by treatment with oxytetracycline hydrochloride (OTC) is an effective alternative to current control strategies
Background	AFB is a major disease of honeybees in Australia and it attracts more funds for its control than any other bee disease. The current control strategies for AFB on mainland Australia are destruction of diseased colonies or the gamma irradiation of infected hive material once the bees have been destroyed. These measures are costly especially when hives are destroyed. The losses incurred from diseased hives not only include the value of the hives but the loss of production, which occurs as a result of the destruction of bees. An alternative strategy, which does not involve destruction of the bees, may decrease the economic impact of this disease on the beekeeper. At State Brood Disease Forums and the National Workshop on Honeybee Brood Diseases held in recent years a consensus was reached that the shaking and OTC treatment technique be tested as an alternative control strategy.
Research	One hundred and one hives in five apiaries under field conditions were used to determine whether the technique of shaking bees is an effective alternative AFB control strategy. Five shaking treatments were used.
Outcomes	None of the treatments was 100% successful. The most successful treatment appeared to be shaking bees onto foundation with one dose of OTC. Of the 29 hives treated in this way 19 (65.5%) were successfully treated although the adult bees from two hives still carried <i>Paenibacillus larvae</i> (AFB) spores at the end of the trial. Two hives (6.8%) developed AFB and 8 (27.6) died out. The second most successful treatment appeared to be shaking bees onto foundation without OTC treatment where 10 (41.7%) of 24 hives were successfully treated although the adult bees from two of these hives also carried <i>P. larvae</i> spores. However, 2 (8.3%) developed AFB and 12 (50.0%) died out. Of the 32 hives, which had been prepared by shaking bees onto irradiated equipment and one OTC treatment 14 (43.8%), were successfully treated. Three hives (9.4%) developed AFB and 15 (46.9%) died out. AFB developed at varying times in the test apiaries. In one apiary AFB developed in hives after 3 months, 12 months, 14 months and 17 months after the shaking procedure. No further examinations were carried out after the 17-month examination. This indicates that further breakdowns could have occurred after this date.
Implications	There is no doubt that the three treatment methods: (a) shaking bees on to foundation (b) shaking bees onto foundation with one OTC treatment and (c) shaking bees onto irradiated material with one OTC treatment can successfully eliminate AFB in some hives. However, it is not certain how many more hives would have broken down with AFB had hive examinations been continued. This

Publications

uncertainty coupled with the facts that one or more hives developed AFB in each shaking treatment procedure and 39 (41.1%) of the remaining 95 trial hives (6 had been mistakenly killed by a beekeeper) died, suggests that shaking bees is unlikely to be an effective alternative AFB control strategy.

Controlling American Foulbrood – Assessing Effectiveness of Shaking Bees and Antibiotic Therapy Strategies, RIRDC Publication No. 01/048

Project Title	Literature review of chalkbrood
RIRDC Project No.:	DAN-190A
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Objectives	To prepare a literature review of chalkbrood (a fungal disease of honeybees)
Background	Chalkbrood is a major disease of honeybees in Australia. A literature review of all aspects of this disease would be useful to facilitate the identification of productive research areas for the control of this disease.
Research	Computer data bases, journals and books were used to compile this literature review
Outcomes	A broad range of literature dealing with the cause, occurrence, multiplication and spread, diagnosis and control of the disease was examined. This data was compiled into one document which also includes abstracts of the more important papers.
Implications	This document is an up to date review of chalkbrood including an outline and assessment of current control strategies.
Publications	-

Project Title	Sterilisation of beehive components using hot wax dipping and case studies of honey bee disease barrier systems
RIRDC Project No.:	DAV 167A
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Objectives	<p>To assist the Australian apiary industry control and reduce the spread of the notifiable honey bee brood disease, American Foulbrood, by identifying and documenting 'Best Practice' used in:</p> <ul style="list-style-type: none"> • sterilisation of beehive components using hot wax dipping techniques • beehive barrier management systems.
Background	<p>The honey bee brood disease American Foulbrood is caused by the bacterium <i>Paenibacillus larvae</i> and is endemic in all Australian States. This notifiable disease slowly kills honey bee colonies and if not controlled can cause significant economic losses in apiary enterprises.</p> <p>The frequent interchange of beehive components from hive to hive, and from apiary to apiary can cause significant spread of the disease. Barrier management systems restrict the interchange of hive material to individual hives, small groups of hives or entire yards of hives. The barrier aims to prevent the spread of disease. Barrier management is widely practised in Western Australia and to a large degree in South Australia but rarely in the eastern States.</p> <p>Hot wax dipping is used to both preserve wooden beehive components and also to sterilise beehive components contaminated with <i>P. larvae</i> spores.</p> <p>This project was funded to provide the apiary industry with detailed information about best practice in barrier management and hot wax dipping techniques.</p>
Research	<p>A literature search was conducted to identify recent developments in barrier management and hot wax dipping techniques.</p> <p>Selected apiarists using barrier management systems and/or hot wax dipping techniques were visited and interviewed to determine 'best practice' methods currently in use. Occupational health and safety issues relating to the use of hot molten wax for dipping were also identified.</p>
Outcomes	<p>The information obtained from these cooperating apiarists was compiled in two illustrated documents entitled:</p> <ul style="list-style-type: none"> • Hot wax dipping of beehive components for preservation and sterilisation • Case studies of honey bee disease barrier management systems.
Implications	<p>The two documents will provide the apiary industry with detailed information about 'best practice' in hot wax dipping and barrier management techniques.</p> <p>The information will enable apiarists to:</p> <ul style="list-style-type: none"> • use hot wax dipping safely and effectively to extend the life of wooden

Publications

hive components and sterilise American Foulbrood contaminated hive components which would otherwise have been destroyed

- incorporate barrier systems into disease management plans to minimise spread of American Foulbrood disease and reduce loss of hives and honey production in apiary enterprises.

Hot wax dipping of beehive components for preservation and sterilisation, RIRDC Publication No. 01/051

Honey bee disease barrier management systems – case studies, RIRDC Publication No. 01/052

RESEARCH IN PROGRESS REPORT 2001

Project Title	European Foulbrood - investigating control measures
<p>RIRDC Project No.: Start Date: Finish Date: Researcher: Organisation:</p> <p>Phone: Fax: Email:</p>	<p>DAV-157A 07/12/98 10/07/02 Mr Russell Goodman Department of Natural Resources & Environment (Vic) Institute for Horticultural Development Private Bag 15 SOUTH EAST MAIL CENTRE VIC 3176 (03) 9210 9324 (03) 9800 3521 russell.goodman@nre.vic.gov.au</p>
Objectives	<ol style="list-style-type: none">a) To protect the apiary industry's continued access to domestic and export honey markets by reducing or eliminating industry's dependence on oxtetracycline hydrochloride (OTC) for the control of the bacterial honeybee brood disease, European Foulbrood (EFB) (<i>Melissococcus pluton</i>).b) To determine the efficacy of reduced doses of OTC and use of OTC extender patties for the control of EFB and to determine if these measures reduce or eliminate the occurrence of OTC residues in honey.c) To identify and develop alternative, non-antibiotic measures for control of EFB by investigating, primarily, the effect of enhanced honeybee colony nutrition and changed pH of honeybee larval guts.d) To obtain a greater understanding of active and latent infections of <i>M. pluton</i> and <i>Paenibacillus alvei</i> (a common secondary invader) in honeybee larvae and to develop new Polymerase Chain Reaction (PCR) methodologies for detection of <i>M. pluton</i> as a necessary prerequisite and support of the preceding aim c).
Current Progress	<p>During the first spring of this two-year sub-trial, 120 honey bee colonies with no previous history of oxytetracycline hydrochloride treatment were allotted to 3 groups of 40 hives and treated with the following:</p> <ol style="list-style-type: none">1. pollen supplement protein cakes during late autumn and spring on a 'as needs basis'2. oxytetracycline hydrochloride (1g active) in early spring3. controls - no treatment. <p>Hives were regularly monitored for incidence of European Foulbrood disease from early spring to early summer.</p> <p>European Foulbrood disease (EFB) symptoms were detected in only four hives. At first, this lack of infection presented a problem and continuation of this work was questioned. However, hemi-nested PCR assay of larvae sampled from colonies belonging to each treatment group confirmed the presence of the causal organism, <i>M. pluton</i>, in all cases. The low number of hives expressing EFB symptoms was attributed to anti-microbial properties (ie high fatty acid composition including linoleic acid) of the pollens foraged by the bees throughout the spring season. The results therefore provide valuable anecdotal evidence of the value of pollens with anti-microbial properties.</p>

Current Progress
cont.

At the time of writing, hives were being prepared for the second season of this work. Hives were moved to an apiary site in northern Victoria to access an Ironbark honey flow and to provide field conditions which might encourage development of EFB and expression of symptoms during the 2001 spring. In late autumn, hives belonging to treatment group 1. will be fed pollen supplement protein cakes. In late winter, all hives will be moved to an almond orchard because of the reputed association of EFB outbreaks with this blossom. For the remainder of spring, the hives will be deliberately located where the bees can only access pollen with low anti-microbial properties.

Project Title:	Export package bees - evaluation of a lupin flour based feed for increased live bee production
RIRDC Project No.:	DAW-75A
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Objectives	To evaluate the use of lupin flour as a substitute for torula yeast or soyflour in patties for supplementing the diets of honey bees.
Background	To optimise the amount of honey bees shaken from beehives for packages supplementary feeding of beehives has to become part of the hive management process. The primary supplementary foodstuff fed to honey bees is soyflour, an imported feedstuff into Australia, thus not freely available and relatively expensive. Other ingredients used in the mixes (e.g. torula yeast) are also expensive. Lupin flour, as an ingredient, is very cheap when compared to soyflour or torula yeast as Western Australia is the world's leading exporter of lupins with production exceeding one million tonnes annually. Lupin flour has not been used in a scientific experiment on feeding honey bees and as lupin is the largest crop grown in Western Australia it makes for a potentially cheap and freely available alternative source of protein for honey bee feedstuffs Australia wide.
Research	<p>Two separate series of experiments were carried out to evaluate the methodology of feeding feedstuffs to honey bees to gauge their preference and palatability to the different feeds in tests using lupin flour.</p> <p>The standard test of preference, where five feeds are placed into one hive (Stace and Hayter 1994) was used in this experiment. Initial concerns (Jane Speijers, Agriculture WA - Biometrician) about this method centred around whether the different feeds placed together would affect the honey bees behaviour towards particular feeds and thus affect their palatability. Based on this concern, another experiment on testing the palatability of feedstuffs was carried where each of the five patties were placed into five hives at the same time.</p> <p>The preference and palatability tests were carried out simultaneously over 137 days, with each trial (of three replicates) taking about 27 days to complete.</p>
Outcomes	<ol style="list-style-type: none"> 1. A mix of lupin and sucrose fed as a patty was most preferred by honey bees over similar mixes containing soyflour or torula yeast. Consumption of the lupin-sucrose mix was ranked third and behind a soyflour and torula yeast-sucrose mix in palatability tests. Torula yeast was the most palatable feed. 2. Lupin based feeds still need further development with different ingredients to achieve higher consumption rates and increased brood rearing. 3. Supplementary feeding of beehives suppressed pollen intake when pollen traps are used. 4. The rate of consumption of patties by honey bees declined, the longer they are left in the hive for testing. 5. Statistically, measurements of the brood frames and pollen intake showed that the brood frame development and the amount of pollen trapped had no

impact on the rate of consumption of the feedstuffs used in this experiment.

6. Experiments on supplementary feedstuffs can be done throughout the year if pollen traps are used to restrict the amount of pollen entering hives.

The experiment was terminated when it was well into its second round of testing when chalkbrood was discovered by the researcher (1st time in WA) and as a consequence State quarantine orders were initiated and a [premature] decision was made to destroy the apiary, which was subsequently burnt on the 29th April 1998. With the prolonged quarantine measures, and other projects undertaken, a decision was made to abandon the project to some future date.

Implications

1. Preference testing should be replaced by palatability tests as more qualitative information about each of the feeds can be gathered. The advantage is that the actual effect of the feedstuffs can be seen from hive measurements of burr-comb and live-bee production.
2. Fixed feeding times of short duration (about 5-7 days) should be part of the methodology in any experiment testing supplementary feeds.

Publications

Nil

Project Title	Pollen analysis of <i>Eucalyptus patens</i> (Blackbutt), <i>E. accedens</i> (Powderbark) and <i>E. wandoo</i> (Winter Wandoo variety) in Western Australia
RIRDC Project No.:	DAW-91A
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Objectives	Provide an analysis of six commercially important species of eucalypt pollens, four of which will be new to science and provide a fatty acid profile of the lipid fraction for six species of eucalypt for the first time in Australia.
Background	<p>Western Australia has the largest number of endemic eucalyptus species identified in Australia (Chippendale and Wolf, 1981). Nectar (as honey) and pollen from these eucalypts play an important part in the health and sustainability of the beekeeping industry and both products are extensively used by the community and contribute to Western Australia's export market.</p> <p>This project examined six commercially important beekeeper-targeted species: <i>E. accedens</i> (powderbark), <i>E. diversicolor</i> (karri), <i>E. marginata</i> (Jarrah), <i>E. patens</i> (forest blackbutt) and <i>Eucalyptus wandoo</i> (wandoo) for their protein composition, fatty acid and mineral profile and vitamin content. Redgum pollen (<i>Corymbia calophylla</i>) was used as a base to measure differences between the other species because it is considered by beekeepers to be excellent pollen for honey bee nutrition.</p>
Research	Pollen from six endemic Western Australian eucalypt species were examined for amino acid, fatty acid, mineral and vitamin composition (though incomplete).
Outcomes	<ol style="list-style-type: none"> 1. The lipid fraction of eucalypt pollen is high in C-18 fatty acids, most notably linoleic acid. 2. Amino acid deficiencies were found in powderbark (iso-leucine); jarrah (iso-leucine, histidine & possibly threonine) which also had total protein below the minimum of 20%; winter, spring and summer wandoo (iso-leucine). 3. Nutrition balance needs to be adjusted for the above species by supplementary feeding of soyflour or yeast (as examples).
Implications	<ol style="list-style-type: none"> 1. The honey flows from jarrah, powderbark and wandoo will reduce the lifespans of honey bees. If there are no pollens from other species being collected by honey bees then the effect on the honey production (for example) should be significant. 2. The high levels of C-18 fatty acids, particularly linoleic and linolenic in pollens assist in inhibiting of bacteria and yeasts within the hive. Of particular importance for beekeepers is the control of the bacteria that causes American foulbrood, European foulbrood and botulism.
Publications	Pollen Analysis of Eucalypts from Western Australia, RIRDC Publication No. 01/053

RESEARCH IN PROGRESS REPORT 2001

Project Title	Production of a publication on honeybee nutrition in Australia - 'Fat bees/skinny bees'
RIRDC Project No.:	DAN-186A
Start Date:	01/01/00
Finish Date:	31/10/02
Researcher:	Mr. Doug Somerville
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Email:	doug.somerville@agric.nsw.gov.au
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	<p>Information on the use of carbohydrate supplements to increase pollination efficiency has been gathered and was presented to a Crop Pollination field day in South Australia on the 16 February 2001.</p> <p>The field day focus was on lucerne pollination, yet the published evidence would indicate that there is a strong role for the use of sugar feeding to stimulate colonies to increase foraging flights in general, increasing visits to blossom and thus increasing the pollination benefit from honey bees in a range of crops. There are some strong cases for the use of sugar feeding in the provision of a pollination service, particularly for kiwifruit, some lucerne crops and perhaps avocados. Other horticultural or agronomic crops may also benefit, particularly via the stimulation effect of regular small amounts of sugar syrup (1 to 2 litres) every one to three days per hive. Open or bulk feeding next to, or feeders distributed throughout the crop may also have benefit, and this was demonstrated at the SA field day on lucerne.</p> <p>A paper on minerals in bee collected pollen has been drafted and is in the process of being refereed. Of the 10 elements tested relating to pollen samples, K, P and S have the highest levels with means of 5530, 4600 and 2378 ppm. Ca, Mg and Na with mid range means of 1146.4, 716 and 92 ppm and Fe, Zn, Mn and Cu having very low level means of 67, 58, 33 and 12 ppm.</p> <p>A sample of onion weed pollen demonstrated an extremely high level of K at 38,000 ppm. This has similarities with K levels in onion nectar which has been identified as causing a repellency effect on bees.</p> <p>One interview with a queen breeder, Greg Mulder, took place in the Hunter Valley. Supplementary feeding was critical to his operation and business, particularly strategic sugar feeding.</p>

Project Title:	Breeding hygienic disease resistant bees
RIRDC Project No.:	US-39A
Researcher:	Dr Ben Oldroyd
Organisation:	School of Biological Sciences, University of Sydney
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Email:	boldroyd@bio.usyd.edu.au
Objectives	<ol style="list-style-type: none"> 1. To determine the number of genes influencing hygienic behaviour, their relative level of influence, and their location in the honey bee genome by construction of a genetic map and quantitative trait loci (QTL) analysis. 2. To develop DNA markers for hygienic behaviour genes so that hygienic breeding stock may be rapidly and cheaply identified 3. To train a PhD student in honey bee molecular biology and bee breeding 4. To establish facilities and protocols that will allow genetic markers developed in other laboratories (eg. for honey production) to be utilised by the Australian honey industry
Background	<p>Hygienic behaviour in the honeybee (<i>Apis mellifera</i>) has been shown to be an effective control mechanism against brood diseases such as chalkbrood and AFB. Rothenbuhler investigated the genetic basis of hygienic behaviour, proposing a two-gene model to explain the uncapping and removal of dead brood. His elegant experiment remains the textbook example of a behavioural genetic study. It is still generally agreed that a small number of unlinked genes produce a large effect on hygienic behaviour in honeybees, that these genes are recessive and inherited in a Mendelian manner. A more accurate determination of how many loci directly influence hygienic behaviour and their relative level of influence and location within the genome of <i>Apis mellifera</i> can be made using molecular techniques, linkage mapping and QTL.</p>
Research	<p>The collection of molecular data is complete and a comprehensive genetic map has been produced. Combined analysis of the behavioural and genetic data has identified seven putative quantitative trait loci for hygienic behaviour. A field study on 32 colonies derived from various lines was carried out in conjunction with Mr. Linton Briggs between December 15-17, 1999. Samples of both drone and worker brood were taken from each of these colonies for testing of the candidate markers identified by our study. DNA was extracted from these samples, along with others identified as either highly hygienic or non-hygienic in an earlier backcross experiment.</p>
Outcomes	<p>This study has utilised molecular techniques to elucidate the genetic mechanisms involved in honeybee hygienic behaviour. Experimental backcross colonies were established and assayed for expression of the behavioural phenotype. Statistical analyses of the field data indicated that the genetic basis of the trait was more complex than either the simple Mendelian and widely accepted two-gene or three-gene models that have been proposed previously. A genetic map of the honeybee genome (25 linkage groups, a total map distance of 3406 cM) was constructed by full multipoint linkage analysis of 358 segregating marker loci. QTL analysis has identified seven putative genetic markers associated with hygienic behaviour indicating that there are many genes of small effect rather than few genes of large effect involved in this complex behavioural trait.</p>

Outcomes cont.

The project was responsible for the importation of new genetic material into Australia from the United States. This hygienic stock has been well received by industry, has been widely disseminated, and incorporated into local breeding programs. We hope that it has led to a general improvement in the level of disease resistance in Australian commercial bees.

Implications

Four-five candidate markers investigated in this study were shown to be related to the hygienic phenotype. However, as we have now shown that hygienic behaviour is a polygenic, quantitative trait, simple diagnostic markers for Rothenbuhler's 'uncapping' and 'removal' genes are unlikely to be achieved. Our results show that the most likely way to improve disease resistance in Australian stock is via traditional methods of recurrent selection.

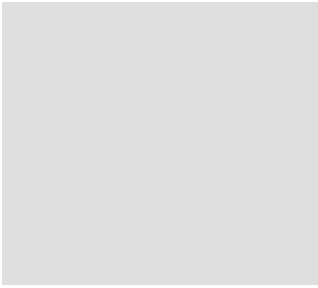
RESEARCH IN PROGRESS REPORT 2001

Project Title	Natural resource database for the South Australian apiary industry
RIRDC Project No.:	DEH-1A
Start Date:	05/07/99
Finish Date:	01/08/02
Researcher:	Dr. Chris Holden
Organisation:	Department of Environment, Heritage and Aboriginal Affairs (SA) Biodiversity Conservation Program Heritage and Biodiversity Division PO Box 1047 ADELAIDE SA 5000
Phone:	(08) 8204 8736
Fax:	(08) 8204 8889
Email:	holden.chris@saugov.sa.gov.au
Objectives	<p>To create a database of floral resource information for the South Australian apiary industry, which includes details of:</p> <ol style="list-style-type: none">1. the floral resource base on which the industry is dependent, including the reliance of the industry on native vegetation and the most valuable and reliable floral species for honeybees;2. the distribution of various native plant species as they relate to beekeeping;3. the frequency with which these resources are used, the land tenure on which they currently exist, and the relative values for honey and pollen as they relate to honeybee nutritional requirements and honey production; and4. estimates of the value of currently used apiary sites on both private and Crown lands in terms of dollar value or as a percentage of the State annual production.
Current Progress	<p>On behalf of the Department for Environment and Heritage, Dr David Paton, Lecturer with the Department of Environmental Biology, University of Adelaide is undertaking the survey of South Australian apiarists with more than 200 hives. Two part-time casual employees have been appointed to manage the incoming responses and enter data. In addition to information on production, the survey will seek information on native vegetation "health". As of March 2001, there was only a 10% response to the survey forms. Dr Paton is actively seeking a greater response by attending regional meetings of apiarists and has been informed that more forms will be returned once honey flows decrease during March. Reminder letters were posted in early March. There exists some confusion over the forms, indicating a need for direct interviewing.</p>

RESEARCH IN PROGRESS REPORT 2001

Project Title	Eucalypt regrowth thinning trails to optimise leatherwood honey production
RIRDC Project No.:	FTA-1A
Start Date:	21/01/99
Finish Date:	30/06/03
Researcher:	Ms. Frieda Heese
Organisation:	Forestry Tasmania 3 Crozier Place WARRANE TAS 7018
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Fax:	-
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Objectives	<ul style="list-style-type: none">• 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	<p>This project has been investigating the effect that non-commercial thinning of eucalypts in regenerated logging coupes has on leatherwood regrowth. Leatherwood is one of the main flora resources for the Tasmanian Beekeepers and its inability to regenerate well after logging has been a major concern for beekeepers. This research hopes to demonstrate that by reducing the eucalypt canopy cover the leatherwood flowering will increase.</p> <p>In March 2000 the non-commercial thinning of eucalypts by way of stem injection was completed. The success of the stem injection process was assessed and a 20% reduction in canopy cover was found after the 9 months. This survey also found that light to the forest floor had increased since the stem injection process was completed.</p> <p>Field inspections in February 2001 of the leatherwood found the occasional tree flowering tree at low intensity. The trees in the thinned plots did show a slight increase in the stem radius compare to the trees with in the controls, however when analysed this did not prove to be scientifically significant.</p>

Project Title:	Adulteration of pure honeys
RIRDC Project No.:	DPI-31A
Researcher:	Dr Wolfgang Korth
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Phone:	02 6272 4771
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Objectives	To evaluate the suitability of a method based on internal standard isotope ratioing mass spectrometry for detecting the presence of synthetic honey in pure honey at levels that would make the adulteration of pure honeys economically viable.
Background	In February 1999 the Australian HoneyBee Industry Council (AHBIC) first approached NRS to undertake a small project aimed at detecting the presence of honey substitutes such as Analog Honey, in pure honey. Following a feasibility evaluation, NRS received approval from AHBIC to commence the work.
Research	A technique based on the natural differences in isotopic ratios between plants utilising carbon in their respective photosynthetic pathways was used to detect the adulteration of pure honey with synthetic honey. As different floral sources of honey can have different isotopic carbon ratios, it was considered appropriate to include as many sources of pure Australian honey as possible in the initial study to establish a baseline range of values for Australian honeys. Consequently, honeys from 20 different floral sources were obtained and analysed.
Outcomes	This study has demonstrated the suitability of the isotope ratioing technique and established baseline levels for a number of Australian honeys. Three of the 20 honey samples studied consistently gave rise to calculated apparent adulterations of between 3 and 5.5 %. However, because these differences were below the internationally accepted threshold of - 1 ‰ (7 % adulteration), the samples were considered not to be adulterated. Although the technique can be used to detect lower levels of adulteration, these can only be detected if both the pure honey and the synthetic honey used for adulteration are available and a calibration curve is used to determine the extent of adulteration of the suspect sample.
Implications	This project has demonstrated that the isotope ratioing technique is suitable for the detection of adulteration of Australian or other pure honeys with synthetic honey derived from plant sugars such as cane sugar, corn syrup or pineapple sugar. However, levels of adulteration would need to be approximately 7 % or greater to be detected, unless both the synthetic and pure honeys are available for calibration. Furthermore, only pure honeys adulterated with synthetic honey derived from plant sugars such as cane sugar, corn syrup or pineapple sugar and not beet sugars can be detected.
Publications	AOAC Official Methods of Analysis (1998). Official Method 991.41, C-4 plant Sugars in honey. AOAC Official Methods of Analysis (1999). Official Method 998.12, C-4 plant Sugars in honey. Martin, G. I., Macias, E. M., Sanchez, J. S and Rivera, B. G. (1998). Detection of honey adulteration with beet sugar using stable isotope methodology. <i>Food Chemistry</i> , 61 (3), 281-286.



White, J. W. (1992). Internal standard stable carbon isotope ratio method for determination of C-4 plant sugars in honey: collaborative study, and evaluation of improved protein preparation procedures. *JAOAC*, **75** (3), 543 – 548.

White, J. W. and Winters, K (1989). Honey protein as internal standard for stable carbon isotope ratio detection of adulteration of honey. *JAOAC*, **72** (6), 907 –911.

Project Title:	Flavour quality assurance of Australian floral honeys by chemical fingerprinting
RIRDC Project No.:	UQ-67A
Researchers:	Bruce D'Arcy, Gavin Rintoul, Bregje Krebbers, Dave Jung and Mark Fedorow
Organisation:	The University of Queensland School of Land and Food Sciences, BRISBANE QLD 4072
Phone:	(07) 5460 1384
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Email:	bd@fst.uq.edu.au
Objectives	To increase the accuracy of the flavour quality authentication of Australian floral (straightline) honeys by developing a commercially available quality assurance procedure based on chemical fingerprinting by 2000.
Background	There was no reliable, objective test, as part of quality assurance programs, available in Australia for authenticating the flavour quality of floral honeys. In addition, there was a need for better control of honey flavour quality to satisfy government and consumer requirements related to labelling of floral honeys. These deficiencies can be overcome by the development of a chemical test that objectively identifies the floral source of species-specific types of Australian honey.
Research	Significant chemical data on the natural volatile substances in multiple samples of 16 species-specific floral types of Australian honey were collected. These floral types were the eucalypts, yellow box, blue gum, red gum, yapunyah, pilliga box, Caley's ironbark, spotted gum, bloodwood, grey iron bark and mugga ironbark; and the noneucalypts, leatherwood, crows ash, jelly bush, heath, tea tree and brush box. Two extraction techniques were developed: solvent extraction using ethyl acetate, and headspace solid-phase microextraction (SPME). In addition, gas chromatographic (GC and GC-MS) analysis methods were developed to separate, identify and quantify the extracted substances. The quantitative chemical data were then analysed by the multivariate statistical analysis technique, principal components analysis. This grouped individual samples of each honey type based on their composition of natural volatile substances, so that identification of the floral type could be done.
Outcomes	Based on the reference honey samples supplied by the Capilano Honey Ltd., and the Tasmanian Leatherwood Honey Exporters Group, a floral certification test has been developed for authenticating the floral source, and thus the flavour quality of 14 species-specific floral types of Australian honey. This test involves a three-step procedure: (1) solvent extraction of naturally occurring volatiles; (2) GC analysis of the extracts; and (3) multivariate statistical analysis of the chemical data. An integral part of this test is the established data bank of mass spectra and GC 'chemical fingerprints' of naturally occurring volatiles in greater than 16 species-specific floral types of Australian honey. Limited trials of this test on industry samples produced interesting and unexpected results, but showed the power of the test for identifying honeys samples, based on data for the reference samples originally supplied by the industry as part of this project.
Implications	This project has shown that a rapid chemical procedure for sourcing the floral origin of specific-specific floral (straightline) honeys can be used to authenticate the flavour quality of these honeys. When the developed floral certification test becomes a part of the quality assurance programs of honey packing companies and beekeepers, there can be an extension of the range of boutique floral honeys that are sold at premium prices.

Publications

Rintoul, G., D'Arcy, B., Sancho, T.M. and Garson, M. Authentication of unifloral heath (*Banksia ericifolia*) honey by chemically fingerprinting natural volatiles. *Abstracts 33rd Annual Convention of Australian Institute of Food Science and Technology*, Brisbane, 20-23 August 2000, 58 (P9). Abstract. Brisbane, Aust Inst Food Sci Technol, 2000.

D'Arcy, B.R., Rintoul, G.B., Krebbers, B. and Garson, M. Authentication of Australian unifloral honeys using chemical fingerprinting of volatile substances. *10th World Congress of Food Science & Technology*, Sydney, 3-8 October 1999, 116-117 (P26/08). Abstract. IUFoST, 1999.

RESEARCH IN PROGRESS REPORT 2001

Project Title	The use of Australian honey in moist wound management
RIRDC Project No.:	DAQ-232A
Start Date:	01/07/1997
Finish Date:	30/09/2000
Researcher:	Dr. Craig Davis
Organisation:	Department of Primary Industries (Qld) Centre for Food Technology 19 Hercules Street HAMILTON QLD 4007
Phone:	(07) 3406 8611
Fax:	(07) 3406 8677
Email:	davisck@dpi.qld.gov.au
Objectives	To develop a set of guidelines for the commercial production of honey as a therapeutic agent.
Current Progress	The recent registration of Jellybush honey as a "Drug" with the Therapeutic Good Administration has been the project highlight. In 1999, Capilano Honey Limited completed the registration of their product - "Medihoney" - which is pure, sterile <i>Leptospermum</i> honey packaged in a 50gm tube and promoted as a "high-potency antibacterial honey" which can be used as "a topical application for the treatment of minor cuts, abrasions and minor wounds". The listing of this product (AUSTL69532) is the first of its kind in the world, and represents an acceptance of the therapeutic benefit of natural products by the TGA. Capilano have recently added a second product to their range – Medihoney Active+ Honey in a 375 gm jar – which is again registered with the TGA (AUSTL77311) and has the claim of being an "oral therapeutic". The routine screening of honeys from apiarists and processors is now complete, and over 5000 honeys have been screened. An area in Northern NSW has repeatedly produced the "active" honey. Beekeepers are now receiving a premium in excess of 10 times the traditional price for these <i>Leptospermum</i> honeys if they are identified as florally-"active". A final report and two PhD theses are currently in preparation.

RESEARCH IN PROGRESS REPORT 2001

Project Title	Glycemic index of honey
RIRDC Project No.:	UNS-17A
Start Date:	01/12/00
Finish Date:	30/11/01
Researcher:	Dr. Jayashree Arcot
Organisation:	University of New South Wales Department of Food Science and Technology SYDNEY NSW 2052
Phone:	(02) 9385 5360
Fax:	(02) 9385 5931
Email:	j.arcot@unsw.edu.au
Objectives	<ul style="list-style-type: none"> • A clear understanding of the differences between the blood glucose responses of the different floral varieties of honey based on sugar and organic acid content. • The identification of various honeys with a low GI factor and to use it as a major marketing strategy by the honey industry to increase consumption of honey.
Current Progress	<p>Literatures pertaining to production of honey in Australia, composition of floral varieties available and any studies done relating carbohydrates in honey and effect on health have been collected. Honey producers across Australia, particularly in NSW, Queensland, Victoria, South Australia and Western Australia were contacted and the floral varieties namely, <i>Slavation Jane</i>, <i>Stringybark</i>, <i>Iron Bark</i>, <i>Yellow Box</i>, <i>Red gum</i>, <i>Yapunya</i> and two commercial blends were obtained and analyzed for available carbohydrate content (sugars) and organic acid content. The above varieties are now being tested on humans for the measurement of the glucose response in blood, which will estimate the glycemic index in relation to a standard glucose solution. A variation in individual sugar contents of the different floral varieties will influence the glucose response produced on consumption of honey, which will decide the glycemic index of the honey. Information on the exact source of honey; age of honey; date of packing; are also being sought from industry currently to be able to substantiate the results that will be obtained. It is envisaged that information such as the above will be needed to address the second objective of the study.</p>

RESEARCH IN PROGRESS REPORT 2001

Project Title	Improving the movement/use of liquid Australian honey within manufacturing processes
RIRDC Project No.:	UQ-84A
Start Date:	01/07/98
Finish Date:	30/11/01
Researcher:	Bruce D'Arcy, Peter Sopade, Nola Caffin, Bhesh Bhandari, Peter Halley
Organisation:	The University of Queensland Food Science & Technology School of Land and Food Sciences GATTON COLLEGE QLD 4345
Phone:	(07) 5460 1384
Fax:	(07) 5460 1171
Email:	bd@fst.uq.edu.au
Objectives	To increase the inclusion of honey in manufacturing processes, particularly commercial baking operations, by developing an understanding of the physical properties and flow characteristics of honey, and by developing key technology for the movement of liquid Australian honey in a number of commercial processes by 2001.
Current Progress	The rheological property of food ingredients is important, and this was studied in 10 honeys at temperatures from -15 to 0°C . All the honeys exhibited Newtonian behaviour. The oscillatory- and steady-viscosity is essentially the same in accordance with the Cox-Merz rule. The dependence of the viscosity on temperature appears to be better predicted by the William-Landel-Ferry model at both sub- and above-zero temperatures. With an increase in oscillatory frequency, the solid character of the honeys became more noticeable and this has implications (eg. blockage) during handling or pumping of honeys at high shear rates. Pumping studies on four commercial honey types at temperatures between 30 and 50°C are being conducted at flow rates up to 350 kg/h in simulated commercial conditions. Although detailed statistical analysis is pending, generally the higher the flow rate, the higher was the pressure drop due to frictional drag along the pipe. It is envisaged that the relationship between (Fanning) frictional factor and Reynolds number will be obtained for each honey as well as the power-law parameters using the Rabinowitsch-Mooney equation. The influence of temperature on the pumping behaviour of the honeys is to be analysed for better understanding of the pump requirements of the honeys.

New Projects –2001/2002

The following projects have been approved by RIRDC for commencement in the 2001/2002 year:

Clarification of aspects of Varroa reproduction - first stage of a possible new control method (HBE01-01)

Dr Denis Anderson
☎ (02) 6246 4148

Predicting the productivity of honeybees from the nutritional value of pollen (HBE01-02)

Mr Ian Wallis
☎ (02) 6249 2533

A study of Gluconobacter - gluconic acid producing bacteria, symbionts of bees: development of biological control for chalk brood (HBE01-03)

Dr Murali Nayudu
☎ (02) 6249 3643

Valuation of honeybee pollination services (HBE01-09)

Dr Jenny Gordon
☎ (02) 6248 6699

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 (includes postage), phone (02) 6391 3433 or 1800 028 374 to order

Enables beekeepers to identify external exotic parasites (varroa, tracheal 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

