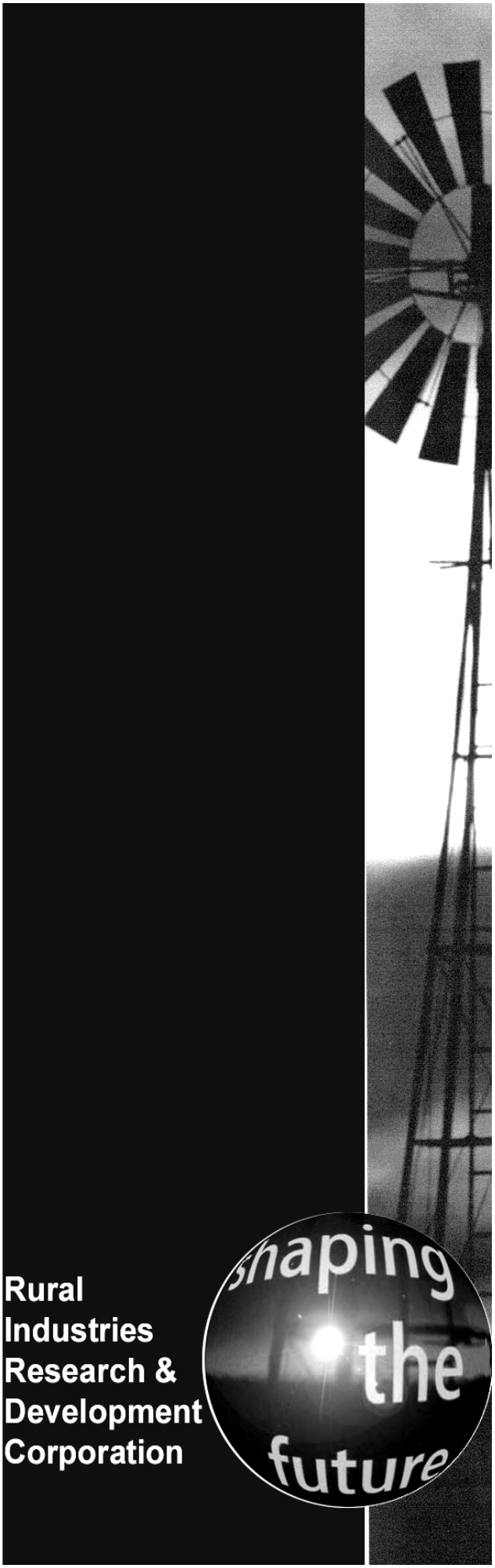

RURAL INDUSTRIES RESEARCH
& DEVELOPMENT CORPORATION

RIRDC Completed Projects in 1998-1999
and Research in Progress as at June 1999

Sub-Program 3.3

Honeybee

August 1999

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Foreword

This year RIRDC has produced *Research in Progress, June '99*, which contains short summaries of continuing projects as well as those that were completed during 1998-99 for all of the Corporation's 20 program areas.

The complete report on all the programs is only available in electronic format on our website at http://www.rirdc.gov.au

The following report is a hardcopy extract covering Sub-Program 3.3. It contains all entries from continuing and completed Honeybee research projects funded by RIRDC. This program aims to improve the productivity and profitability of the Australian beekeeping industry.

This report is the newest addition to our extensive catalogue of almost 400 research reports, videos and CD-Roms of projects supported by RIRDC. Please contact us for the latest publications catalogue or view it on our website.

Peter Core
Managing Director
Rural Industries Research and Development Corporation

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3.3 Honeybee
Completed Projects

Project Title	Honeybee Industry Disease Reduction Workshop

RIRDC Project No.:	AHC-1A
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Objectives

- To allow all industry participants, from the producer through to the packer, including relevant state government departments, to work in a unified manner developing strategic plans to manage the reduction in the level of AFB and EFB in Australia's honeybee population.
- Coupled with the strategic plan are guidelines for the use of chemicals to ensure that their usage does not present an unacceptable residue level in Australian honey.

Background:

With the introduction of European Foulbrood (EFB) into the Australian Honeybee population in the 70's the decision was made to use chemotherapy to control this disease. The decision has lead to the masking of American Foulbrood (AFB). During the 1988 2nd Australian and International Bee Congress, this chemical masking effect was reiterated. Unfortunately, industry now finds itself with unacceptable levels of AFB and the risk of chemical residues.

Research

To take the recommendations of various disease forums through to a national workshop representing all industry sectors including state and federal departmental personnel. This national workshop fine-tuned these recommendations into 31 action points.

Outcomes

The 31 action points have been categorised into 4 groups- **Bee Health, Food Safety, Quality and Marketing and Administration.** Drivers were appointed to establish committees to develop strategies to progress one or more of the 4 groups and to report back to industry for the development and implementation of these strategies.
Drivers were challenged to report to the various state and sector annual conferences.

Implications

The biggest challenge identified is how to fund this disease reduction program. Potential options identified were: AAHC Pty Ltd, NFF and RIRDC – Industry Coalition. Funding of all options would need to be via the formation of a statutory disease levy.

Publications

The results in the form of the final report with appendices have been circulated to the AHBIC Executive Board (*in confidence)*. The final report will be published by RIRDC prior to the July 1999 AHBIC Annual Meeting.

Project Title	Introduction and Performance of Queen Bees - A Survey of Queen Bee Breeders' Apiaries

RIRDC Project No.:	AQB-1A
Researcher:	Colin Wilson
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Objectives

- To identify critical areas during the production of queen bees which influence introduction and early performance levels by sampling and recording conditions under which queen bees are reared during spring 1997 and autumn 1998.
- Data from this project and from the project DAN-164A "Introduction of queen bees - introductory apiary status and post introduction results" to be analysed together.

Background:

During the 1996-97 season lower acceptance levels of queen bees was experienced than normal and some queen bees showed poor early performance. This was reported in Queensland (QLD) and New South Wales (NSW).

Only members of the AQBBA agreed to participate in the survey.

Research

Approximately one month before field trials were to commence the commercial queen bee breeders supplying queen bees to the pilot project (DAN-164A) had apiaries at each stage of queen bee production examined by government apiary officers.

Nutrition, disease and population strength were recorded and samples taken for examination.

Outcomes

Cell builders, drone mother hives and mating nuclei were examined for nectar and pollen stores and associated supplementary feeding programs.

All apiaries and stages were considered to have adequate nutritional stores.

All apiaries and stages were considered to have adequate population for the purpose required.

All mating yards examined were considered to have adequate numbers of drone mother hives in close proximity. The drone mother hives were regarded to have the quantity of drones required.

No brood diseases were reported.

The survey of queen bee breeder apiaries did not reveal any obvious areas in the production of queen bees that would influence the quality of queen bees produced.

Nosema disease was found in one queen out of 59 examined.

Some sperm counts were lower than expected even though drone mother hives when examined were considered to have adequate numbers of drones present. (spring survey)

Project Title	Identification and application of the aggregation pheromone of Apis cerana.
RIRDC Project No.:	CSE-74A
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Objectives	• To isolate and identify the ingredients of the natural attractant that the Asian hive bee releases as a homing signal (aggregation pheromone) for other members of its species; • To make a synthetic mixture that mimics this pheromone; and • To show that the blend can specifically attract this pest.
Background:	Swarm boxes baited with synthetic aggregation pheromone have been shown to capture feral colonies of *A. mellifera*. The identification of the aggregation pheromone from the Asian hive bee will enable the development of a specific lure and therefore facilitate the detection and monitoring of this threat to Australian rural industry
Research	Successful strategies were specially developed in this project to import the aggregation pheromone of the Asian hive bee without deterioration or risk to quarantine. Floral-like odours were identified as constituents but they were found in the bees in extremely small amounts. Lures were made that allowed the odours from synthetic sources to be released very slowly in the field and these lures were tested, as well as natural extracts, with colonies of the Asian hive bee in Indonesia. Queens released their own pheromone which proved to be superior in these trials to that from workers for promoting aggregation. Progress has been made on identifying both pheromones so that a combination can be incorporated in swarm boxes as a specific bait for the Asian hive bee.
Outcomes	The outcomes of this research will be an increase in the effectiveness of protective monitoring measures and the safeguard of the Australian honey bee industry against this potentially serious competitor.
Implications	The Asian hive bee has spread throughout Irian Jaya and Papua New Guinea and has infiltrated Australian islands of the Torres Strait. If it eventually succeeds in establishing itself on the mainland, it will seriously damage the Australian honey bee industry by competing aggressively with *A. mellifera* and will disrupt pollination worth over a billion dollars a year. It will also introduce parasitic mites and will threaten native bee populations.

Project Title	Oxytetracycline Sensitivity Diversity and Study of *melissococcus pluton* (European Foulbrood)
RIRDC Project No.:	DAN-136A
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Objectives

- To determine the oxytetracycline hydrochloride sensitivity of Australian *Melissococcus pluton* isolates.
- To determine the diversity of Australian *M. pluton* isolates.
- To determine whether *M. pluton* isolates contain plasmids (antibiotic resistance transfer vectors).

Background:

EFB is endemic in all Australian States except for Western Australia. The impact of this disease necessitated the introduction of antibiotic therapy using OTC. Hence, its control is dependent on the effectiveness of OTC against *M. pluton* for which there is no current published data. An investigation to determine the sensitivity of *M. pluton* is needed to determine whether resistance to OTC is occurring and if so, where this is occurring so that alternative steps can be taken to control this disease.

In studies carried out in the early 1960s strains of *M. pluton* were determined to be closely related to each other in spite of their widely separate geographical origins. In recent years molecular typing technologies have advanced the capability of differentiating bacterial, viral and parasite isolates within the same species or genus. The DNA analysis of *M. pluton* isolates will be useful in determining whether the isolates from areas where EFB appears to be more severe than in other areas have different DNA profiles. If severity of disease can be associated to a particular DNA profile it may be possible to predict increases in disease severity if more virulent strains are detected in areas where they had previously not been recognised. It would also be useful to know whether there is a correlation between certain DNA types and antibiotic resistance (if it exists) as this information may identify *M. pluton* isolates "susceptible" to accepting resistance factors and identify areas where resistant may develop.

Industry Significance

European foulbrood (EFB) is a major cause of production losses to the beekeeping industry in Australia. The widespread nature of the disease in all States except Western Australia necessitated the introduction of antibiotic treatment in 1976 using oxytetracycline hydrochloride (OTC). OTC is the only antibiotic recommended for EFB treatment and although in place 21 years little is known regarding the current sensitivity of *M. pluton* to this antibiotic. There is, however, anecdotal evidence that OTC treatment is not always effective. This indicates that there is a need to determine the sensitivity of *M. pluton* so that beekeepers can be confident that treatment protocols are effective. If resistance of *M. pluton* to OTC has developed alternative control measures can then be pursued without unnecessary delay.

The eradication of EFB is unlikely due to its widespread nature and its ability to exist as a latent infection in hives. Hence, investigations of the organism to better understand its ability to develop resistance and to characterise isolates in terms of DNA profiles will provide State Departments of Agriculture and Industry with useful information which will guide them in future control strategy development.

A literature search conducted by the library at this Institute has demonstrated that information in this area is lacking.

Research

Each State Department of Agriculture (except Western Australia and the Northern Territory) will be requested to send at least 20 brood samples, each infected with EFB, from various areas within each State. The principal investigators will also collect samples as required. *M. pluton* will be cultured from these samples and the minimum inhibitory concentration of OTC will be determined by incorporating OTC at various concentrations in the prescribed medium. The technique employed will be based on that described by Hornitzky (1985) which reflects standard procedures for this type of work.

DNA restriction endonuclease profiles and typing of geographically diverse *M. pluton*

isolates and their plasmid content will be determined using methodologies based on the work described in our previous project. This section of the project will provide information regarding the diversity of *M. pluton* in Australia and whether *M. pluton* has the ability to develop resistance via plasmids.

Outcomes

This project has been successfully completed. One hundred and four *M. pluton* isolates were obtained during the course of the project. The number of isolates obtained from each state were South Australia (30), New South Wales (48) Queensland (9), Victoria (16) and Tasmania (1). OTC sensitivity assays were conducted on all isolates. All isolates were shown to be sensitive to OTC at 1 or 2 μg/mL. These findings indicate that *M. pluton* has not developed resistance to OTC even though this is the only antibiotic that been used to treat EFB over the past 21 years.

Following the examination of 49 isolates from a wide range of geographical areas it became clear that Australian *M. pluton* isolates belong to a very homogeneous group of organisms with insignificant variation in DNA restriction endonuclease profiles. All these isolates were also examined for the presence of plasmids. All isolates were plasmid-free.

In examining the brood samples submitted for this project *Paenibacillus alvei* was commonly encountered. Twenty eight isolates were examined. In direct contrast to *M. pluton* the *P. alvei* DNA restriction endonuclease profiles showed this group of organisms to be very diverse. A biochemical analysis of the reactions produced by these isolates also showed a great deal of diversity between isolates.

Implications

EFB seems to be more severe in the southern areas of Australia. There are a number of reasons why this may occur:
OTC resistant strains of *M. pluton* in areas where EFB seems more severe.
Differences in virulence of *M. pluton* strains.
Environmental factors eg rainfall, weather patterns in various areas having different impacts on EFB expression.
Nutritional factors.

The outcomes of this project indicate that reason 1 is not applicable and that reason 2 is unlikely to play a role in differences in disease severity. The most likely causes are (3) environmental factors and/or (4) nutritional factors. These are the two areas that need to investigated. Studies in these two areas may provide the means of minimising the effects of EFB which may result in a reduced need to use OTC for the control of EFB.

Publications

Hornitzky, M.A.Z. & Smith, L. (1998) Procedures for the culture of *Melissococcus pluton* from diseased brood and bulked honey samples. *Journal of Apicultural Research* 37(4): 292-294.

Djordjevic S.P, Smith L, Forbes W and Hornitzky M.A.Z (1999) Geographically-diverse Australian isolates of *Melissococcus pluton* are genetically homogeneous. *FEMS Microbiological Letters* 173(2): 311-318.

Djordjevic, S.P., Noone, K., Smith, L. and Hornitzky, M.A.Z (1998) Development of a hemi-nested PCR assay for the specific detection of *Melissococcus pluton. Journal of Apicultural Research*. 37(3) 165-173.

Hornitzky, M.A.Z & Smith, L.A. (1999) Sensitivity of Australian *Melissococcus pluton* isolates to oxytetracycline hydrochloride. *Australian Journal of Experimental Agriculture* (submitted)

Djordjevic, S.P., Forbes, W.A. , Smith L.A. and Hornitzky, M.A.Z. (1999) Geographically-diverse Australian isolates of *Paenibacillus alvei* from diseased brood (*Apis mellifera*) and honey are genetically and biochemically heterogenous (in preparation)

Project Title	Introduction and Performance of Queen Bees – Introductory Apiary Status and Post Introduction Results

RIRDC Project No.:	DAN-164A
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Objectives

- To identify critical areas in queen bee production and introduction which may be contributing to low acceptance and poor early performance being reported with commercially reared queen bees

Background:

Beekeepers have not been satisfied with the introduction success rate and early performance of commerically reared queen bees for a number of years. The problem is resulting in significant economic loss for some beekeepers.

Research

Queen bees from five commercial queen bee breeders produced in spring and in autumn were introduced into honey production apiaries belonging to three commercial beekeepers. Survival rates of test queens and older control queens were monitored at 4 week intervals for 16 weeks. Data considered critical to the survival and performance of test and control queens were recorded.

Outcomes

A significant loss of 30% of spring reared queens occurred compared to a loss of 13% of autumn reared queens. Control queen losses were 17% during the spring trial and an average of 5% for the autumn trial. The age of the queen at introduction, numbers of spermatozoa stored in the queen's spermatheca, Mosema disease, physical damage to the queen during transport, and external hive conditions were identified as factors which may have contributed to the queen bee failures.

Implications

Improved management practices in the areas outlined are expected to improve queen bee quality and increase introduction and early performance of queen bees.

Project Title	The Development of a Natural Resource Database for the Queensland Apiary Industry

RIRDC Project No.:	DAQ-199A
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Objectives	• To document current natural resources of the Queensland apiary industry by surveying and recording honey production, economic value and important floral species of apiary sites within Queensland. • Areas currently under utilised as bee forage areas to be possibly identified
Background:	The beekeeping industry for a number of decades has registered concerns at the decreasing availability of suitable bee forage areas. This has been due to clearing of native flora for grazing and agriculture, and more recently the threat of exclusion from traditional sites through gazettal into protected crown lands. The lack of documented and evaluated information on melliferous resources has restricted the apiary industry in arguing effectively to retain access to some valuable bee forage areas.
Research	Queensland beekeepers owning more than 50 hives were surveyed and information on apiary sites, honey production, prime flora and other relevant details entered on a computer database. Tables and maps were prepared to present the resources and their value.
Outcomes	Returns of questionnaire forms supplying useful data were received from 248 (59.2%) of the 419 beekeepers able to provide data. The total annual value of the 8,779 Queensland apiary sites currently in regular use was calculated to be $16,461,169.00, with each apiary site valued on an average of $1,875.00. Figures were also produced on the production of beeswax, queen bees, pollen, package bees and the number of apiary sites currently in use on Crown land, lease-hold land and freehold land. Significant honey flora for each area of the State was identified. Other information that was collected relates to paid and unpaid pollination of cultured crops, effects of fire and American Foulbrood on the industry, and feral bee densities in areas of Queensland. Areas, particularly in State forests, were identified that are expected to be future useful beekeeping resources. Data has been presented in formats able to be used by the beekeeping industry, and by Government Departments when matters concerning honeybees and land use requires discussion.
Implications	Greater security for the Queensland honeybee industry through assured access to proven valuable flora for honeybees.

Project Title	**Promotion of Native Bees in the Torres Strait**
RIRDC Project No.:	DAQ-203A
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Objectives	• New Guinea to mainland Australia, via the Torres Strait islands. • To prevent the movement of Tropilaelaps clarae and Varroa jacobsoni mites from Papua

Background:	Both of these mites occur in Papua New Guinea, where they have had a devastating effect on the populations of European honey bee (*Apis mellifera*). The Asian honey bee (*Apis cerana*) is the natural host of Varroa mite. Asian honey bee, and the Varroa mite have spread from Irian Jaya into Papua New Guinea and onto three of the island of the Torres Strait. Currently the nearest population of European honey bees is on Hammond Island, in the Torres Strait, about 100 kilometers to the south of these three islands. Our best prospect of keeping mainland Australia free of these mites is to maintain this separation of the two populations of honey bees. However, there is a perception, among some islanders,that honey bees are essential to the production of food plants. In fact the bulk of traditional crops either do not require pollination agents, or are pollinated by native bees and/or other agents.
Research	The variety of native bees occurring on the islands has been determined Also, the variety of fruit trees (native and introduced) that are commonly utilised has been catalogued, along with the range of food crops currently under production in the region. The benefits of the native bees in production of the fruits and crops have been promoted via various booklets and promotional material.
Outcomes and Publications	An illustrated booklet outlining which of the food crops require pollination, and which are pollinated by the native bees and other insects. Two thousand of these were delivered to the Torres Strait in January 1997, for distribution through the region. An activity book for school has been produced and distributed to Primary Schools in the Northern Peninsula Area (NPA) around Bamaga.. The book promoted the native (stingless) honey bees (*Trigona* spp and *Austroplebeia* spp.) as the local hero, "Trigon", fighting off the invading Asian honey bees, as well as other quarantine themes. Heavy duty, plastic baggage tags featuring the "heroic" Trigon, produced in May 1996, have been distributed. Two thousand of these were produced. The label also features the Top Watch logo which was promoted by Mal Meninga in 1993. A twelve page booklet detailing the life histories of selected native bees from The region has been produced. The text is illustrated with line drawings and a four page colour 'center-fold' shows some of the native bees, as well as a resting swarm of Asian honey bees. Two thousand booklets, plus an extra thousand copies of the colour insert, have been produced and are ready for distribution in the Torres strait, via the schools extension programme.

Project Title	**Investigations into Disappearing Disorder, a Problem of Honeybees in Southe East Queensland**
RIRDC Project No.:	DAQ-205A
Researchers:	Fraser Trueman; Hamish Lamb; Patricia Greer; Peter Warhurst; Wendy Ward; Hugh Mawhinney
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Objectives

- Further investigate outbreaks of disappearing disorder in south east Queensland to define areas involved, seasonal occurrence and severity.
- Attempt to identify any particular floral resource that may be involved.
- Examine the possible role of heavy metals identified in pollens, and attempt to reproduce the disorder with a heavy metal feeding trial.
- Investigate if hive supplementary feeding can reduce or prevent disappearing disorder, and measure any production effects resulting from disappearing disorder.
- Investigate the possible role of flowering cat's claw creeper with outbreaks of disappearing disorder.
- Attempt to transmit disappearing disorder with a frame of affected brood.

Background:

From the spring of 1995 beekeepers in southern Queensland were requested to report any signs of disappearing disorder to apiary staff. Outbreak information was gathered and a brood sample examined to exclude known brood diseases. When possible apiaries were visited to collect further specimens and note floral resources available.

Experimental feeding, supplementary and transmission trials were undertaken at the Animal Research Institute, Yeerongpilly or at cooperating apiarist's sites. The expertise of botanists, toxicologists, nutritional biochemists and microbiologists was recruited where necessary to support the investigations.

Research

- Twenty six outbreaks of disappearing disorder were recorded during the two year period occurring from Rockhampton in central Queensland to Grafton in New South Wales. All cases were restricted to coastal areas. Outbreaks commenced after spring rains, and in one season extended to the following April, but this was reported as an abnormally long outbreak period. Cases varied from fleeting and mild, up to prolonged and severe, resulting in marked reductions in brood and adult bees.
- Botanical examination, and identification of pollens from affected larvae and hives, failed to incriminate any particular floral resource associated with disappearing disorder. The absence of cat's claw pollen from all samples argued against the involvement of this suspected plant.
- Heavy metal examination of hive pollens revealed elevated levels of aluminium, copper, iron and zinc in several samples, but the levels were considerably lower and similar in affected and unaffected larvae. A feeding trail using aluminium and zinc in pollen patties produced a small number of affected larvae with aluminium patties at 200 mg/kg, but the results were not conclusive.
- The preventative value of supplementary feeding on disappearing disorder was also inconclusive, as disappearing disorder outbreaks did not occur in control hives at either site under observation. Supplementary feeding did not improve hive production when measured at one site.
- While outbreaks of disappearing disorder coincide with flowering of cat's claw creeper, an attempted net feeding trial and location of hives adjacent to abundantly flowering creeper, failed to incriminate this plant.
- Transmission of disappearing disorder by inserting a frame of affected brood into a healthy hive could not be achieved. Microscopic examination of affected brood samples from outbreaks did not reveal any known brood pathogens.

Outcomes

The cause of disappearing disorder in south east Queensland remains unknown. The outbreak area and prevailing seasonal conditions have been further identified, allowing apiarists to be alert to risk factors associated with outbreaks. While heavy metal toxicity could not be proven, some doubt still remains over the role of elevated aluminium levels in pollen. Naturally occurring acid soils in south east Queensland are directly linked with high aluminium soil levels, and this has been exacerbated by changing land management practices.

Project Title	A Quality Survey of Australian Honeys

RIRDC Project No.: DAQ-231A
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Objectives

- To survey the quality (chemical residues honeybee disease agents) of honey samples produced and packed in all states and territories in Australia.
- To examine the honeys for microbial quality (bacteria, yeasts and molds).
- To compare the results with those obtained in a previous report DAQ 202A Introduced Honeys – a Quality Survey.
- To research standard methodologies for assessing the microbial quality of honeys.
- To establish acceptable standards in terms of chemical residues bacteria, yeasts and molds for honey quality.

Background:

The Australian honeybee industry is committed to producing a high quality natural product free of residues and contaminants. To date few measurable standards have been adopted or examined across the Australian honey industry. A quality survey was conducted of some international honeys in 1995/96, and the opportunity now arises to compare the quality of Australian honeys with the results of that survey.

The further development of acceptable standards for honey quality will be a basis for industry to reassure consumers of the purity of honey. The ability of Australian honey producers to meet requirements in terms of residues, honeybee disease agents and microbial levels provides opportunities for increased national and international marketing.

Research

Sixty honey samples representing all states and territories of Australia were examined in this survey. Fifty two samples were collected from the honey drums of individual apiarists and 8 samples were purchased from retail outlets.

All samples were cultured for honeybee disease agents namely American foulbrood (AFB) and chalkbrood. All honeys were also examined for microbial flora (bacteria, yeasts and molds) and for chemical residues.

Two separate diluent preparations were trialled to determine the most sensitive methods for assessing numbers of bacteria yeasts and molds.

Outcomes

Recommendation on standard techniques for determining numbers of bacteria, yeasts and molds in honey can now be made.

- It is recommended that honey should contain <500 CFU's/gram bacteria and a total of <10 CFU's/gram yeasts and molds to be acceptable as a food product.
- This work provides useful information for honey packers who now have scientific information to guide their overall HACCP plans.

Only 8% (13.3%) of Australian honeys returned positive culture results for AFB disease agent compared to 47.2% of International honeys in a previous report DAQ 202A. This result appears to justify the AFB control programs which are in place in Australia.

Implications	• Thirty one or 51.7% of honey samples screened contained either AFB or chalkbrood disease agents and returned greater then recommended numbers of bacteria yeasts and molds. • A diluent preparation of peptone water with 40% glucose recovered greater numbers of yeasts and molds then peptone water diluent. • High levels of lead were detected in 2 honey samples. Immediate action was taken to determine the sources and reason for this contamination. Investigation revealed that soldering in honey extracting equipment was responsible for leaching of lead into honey which was left in extracting equipment. Coating the soldering with food grade paint is believed to reduce the risk of this contamination.
Publications	Verbal reports on the results of this survey have been presented to: AHBIL in October 1998. QBA Annual Conference June 1998. A final written report for RIRDC is in progress.

Project Title	**Strategic Planning and Action Meeting for Honey Bee Nutrition**
RIRDC Project No.:	GK-2A
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Objectives	• To develop and underpin honey bee nutrition with R, D & E proposals for further advances.
Background:	Australia is endowed with a range of climates and flora that necessitate varied management procedures. The inability of apiarists to match program with environment or to modify research results to suit specific situations has slowed industry application of nutrition.
Research	Prior to the meeting 120 randomly selected apiarists were surveyed to determine current general nutritional practices and the problems that inhibit increased use of nutrition strategies. Fifteen workshop delegates discussed past practices, survey results, participant statements and quality assurance implications and recommended an eight point action plan.
Outcomes	Two draft research proposals have been received. HBRDC has decided on the composition of a committee that will progress the development of the proposals in parallel with the proposed queen bee program. Three 1998/99 mid-term projects are expected to be recommended to RIRDC. Discussions are being held with Agriculture NSW regarding upgrading nutrition publications.

Implications	The projects in commercial apiaries will require active apiarist participation in the programs and appropriate remuneration included in the project budgets.

Project Title	**Development of a National Program for the Control of AFB**
RIRDC Project No.:	KHM-1A
Researcher:	Keith McIlvride
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Objectives	• To develop suitable management programs to control and reduce the level of AFB.
Background:	In 1977 an outbreak of European Foulbrood (EFB) (*melissococcus pluton*) occurred in South Australia. The treatment of this disease was to use antibiotic. This in a number of cases masked AFB, making the destruction of AFB bacteria difficult. In 1993 the then industry peak body the Federal Council of Australian Apiarists' Associations (FCAAA) decided it was time to setup an industry committee. FCAAA then asked the Animal Health Committee could it assist the industry to develop a national control program. The AHC set up a AHC working party to look at the issue. A face to face meeting took place of this committee in Sydney on October 1996 and a number of requests and suggestions were made to industry. From all this HBRDC set up a workshop to further develop the plans put forward by the AHC working party. The set of guidelines from the AHC working party became the agenda for the 29th July 1998 workshop. Industry will make the final decision.
Outcomes	The workshop group reports explain fully the meeting recommendations. The following is a brief summary of those. • To have all beekeepers honey testing for AFB. • To a national database program set up to maintain levels of AFB. • To have uniform standards with the laboratories carrying out the honey testing and have these labs audited. • To develop codes of practice for honey and queen producers. • To develop a comprehensive quality assurance for beekeepers with packers. An essential part of this is a differential pricing for honey to encourage a quality product. • Develop a standard national method to quantify the level of AFB that exists in the industry. If we do not develop a uniform statistical way of measuring the number of hives infected we can never show the industry is on top of it. • The words amateur and commercial should be taken out, only refer to beekeepers. • Australia should be divided into four zones when it comes to movements of bees/product etc. Western Australia, Kangaroo Island, Tasmania and the rest of Australia. Letting the first three to set their standards and the balance to be supported by vendor declarations, that is free movement.

Project Title	Laboratory and field trials relating to heat treatment of AFB infected bee equipment

RIRDC Project No.: QBA-2A
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Objectives

- To determine if heat treatment can be used to sterilised beekeeping equipment that has been infected with *Paenibacillus larvae* subsp *larvae*, the spore-forming bacterium which causes American foulbrood (AFB) disease of honeybees.
- To examine the possibility of using powder-coating kiln to heat sterilise beekeeping equipment infected with spore of *P. larvae* subsp *larvae*.

Background:

At present the only proven method of sterilising bee boxes and other beekeeping equipment contaminated with *P. larvae* subsp *larvae* is by irradiation. However, apiarists in Queensland who elect to retain their AFB infected beekeeping equipment are forced to travel to Sydney, NSW to access the irradiation plant. This is a costly process in terms of money and time. Therefore, there is a need for the establishment of a local, efficient sterilisation unit in this state. As an alternative to irradiation, attempts have been made to sterilise bee boxes using heat treatment.

A previous report (RIRDC Project QBA-1A) indicated that spores of *P. larvae* subsp *larvae*, can be destroyed by heat, but further work was required to accurately determine a temperature and time frame which would result in complete sterilisation of beekeeping equipment. This report also indicated that there were a number of variables affecting the ability of heat to destroy spores of *P. larvae* subsp *larvae.* These variables included heterogeneity of spores with respect to heat tolerance, time of exposure to heat, and the number of spores which may be present on infected equipment.

Research

Spores of *P. larvae* subsp *larvae* were collected from brood samples sent for disease diagnosis to the Department of Primary Industries Honeybee Diagnostic and Laboratory at Yeerongpilly, Brisbane. These spores were collected from widely diverse areas of southern Queensland. Spores from New South Wales were also included to ensure that a wide variety of strains of *P. larvae* subsp *larvae* were contained in the spore pool.

Laboratory Experiments

Spores from the pool were seeded on the surface of small pieces of pinewood and heated in a small laboratory oven. Exposures to dry heat at temperatures of 110^0C and 130^0C were trialled. At the conclusion of the heating regimes culture tests to determine the viability of the spores were performed.

Kiln Trial

A trial in a commercial powder coating kiln at Sumner Park in Brisbane was undertaken. An air hose was passed into the kiln to try to enhance the spread of heat through the heating chamber.

One hundred and four boxes were seeded with 3×10^8 spores per site of *P. larvae* subsp *larvae* from the stock pool. Six additional boxes were seeded with spores and left out of the kiln to act as controls. The test boxes were exposed to dry heat at 130^0C for 4 hours.

As for la boratory experiments the heated spores were harvested and cultured to determine viability.

Outcomes	**Laboratory Trials** The laboratory work showed that at 110^0C, spores of *P. larvae* subsp *larvae* could be destroyed after exposure for 5 hours. At 130^0C. the spores were killed after 3 hours exposure. These experiments were conducted under conditions of dry heat. **Commercial Kiln Trial** Powder coating plants are fairly common in cities and towns throughout Australia. It was reasoned that if the trials were to be us use to the beekeepers, there had to be premises that could be easily accessed. The powder coating plant could achieve the desired temperature of 130^0C and hold for the time required, 4 hours. After heating at this time and temperature, there were 31 out of 104 sites (30%) which still showed viable spores of *P. larvae* subsp *larvae.*
Implications	It is concluded that failure to destroy all of the spores in this trial could be due to: • Cold spots near the floor of the chamber which did not reach the required temperature. • Lack of super heated steam during the heating process. • Time of heating was too short to be effective. The authors are aware that some beekeepers are presently using powder coating kilns in attempts to sterilise beekeeping equipment contaminated with spores of *P. larvae* subsp *larvae.* Our results show that it is unlikely that complete sterilisation is being achieved using these kilns.
Publications	A final report written has been submitted to RIRDC.

Project Title: Australian liquid honey in commercial bakery products

RIRDC Project No.:	UQ-50A
Researchers:	Dr Bruce D'Arcy, Ms Nola Caffin, Dr Bhesh Bhandari, Ms Nicole Squires, Mr Paul Fedorow and Mr. Darren Mackay
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Objectives	• To investigate the use of Australian liquid honey in commercial bakery products, and • To determine the effect of the incorporation of honey on the quality and functional properties of bakery products; • To communicate research findings to the Australian food processing industry.
Background:	*Dough rheology:* In the production of breads, it is essential that the bread dough is fully developed during the mixing process. When honey is added to dough, the system exhibits different molecular and physical properties. To a bread manufacturer, the addition of honey ideally should not increase the time to reach peak dough development, and should still produce a strong and stable dough matrix once the dough has reached peak development. *Gelatinization of bread doughs:* In the production of breads, it is important that

gelatinization is not adversely affected. Too great an increase in the gelatinization temperature may yield a bread that is not fully baked in a standard oven, although, a small increase in gelatinization temperature is thought to slow the rate of staling. A previous RIRDC funded study suggested that adding honey to bread retards staling better than sugars alone.

Research

Dough rheology: Bread doughs were formulated using honey added at varying levels. Dough development was measured using a National Mixograph, and the mixing time, dough strength and dough stability were determined. Doughs with added honey were compared against doughs formulated using a model sugar solution (same sugars as in honey) and a control with no honey or sugar. *Gelatinization of bread doughs:* Bread doughs were formulated using honey added at varying levels. Gelatinization was then measured using a differential scanning calorimeter, and the gelatinization temperature and energy were determined.

Doughs with added honey were compared against doughs formulated using a model sugar solution and a control with no honey or sugar. *Viscosity of batters:* Batters were formulated using honey added at varying levels. Viscosity was then measured *versus* temperature using a visco-amylograph. Batters with added honey were compared against a control (0%) batter and batters containing a model sugar solution. *Commercial optimization:* Breads were made using a commercially available formulation with honey (manufacturing grade ironbark honey, courtesy of Capilano Honey Ltd.) at 2%, 3%, 4% and 6% levels.

All treatments were compared against bread loaves incorporating comparable levels of a model sugar solution (the same sugars as the honey sample) as well as a control, containing no honey or model sugar solution. The effect of honey on the following properties were studied: dough proofing volume and time, crumb fineness and cellular elongation, crust thickness and contrast, and crumb texture (firmness) and water activity over a four-day period.

Outcomes

Experiments of this project studied rheology and gelatinization of bread doughs in model systems. The rheology found that when honey is added at 6% through to 10%, the mixing times were significantly shorter than when using a sugar solution at the same concentration. Additionally, honey produced stronger and more stable doughs at the higher levels of addition. The gelatinization study found that the gelatinization energies for 6% and 8% honey were significantly lower than all other treatments, whilst the gelatinization temperature changed little if at all.

As these results were in model systems, it was necessary to apply this knowledge in a commercial bread formulation to ensure that the model system studies were valid. Here it was found that honey had little effect on the properties of the baking process. However, reanalysis of the results of an earlier study confirmed that adding honey at a level of 3% (w/w flour) significantly retarded the staling of white pan bread by at least 12 h relative to loaves containing 3% sugar and no sugar or honey.

Implications

The data produced by this project, when combined with the data produced earlier by Ms Nicole Squires (1994-1996), suggest there is a definite trend for bread loaves formulated with honey to perform better during the baking process than those bread loaves containing sugars or a model sugar solution.

There is a strong indication that the mode of action of honey is unique to the honey itself (ie. the minor components of the honey), rather than being due to the sugars of honey alone. This data will convince the Australian baking industry to seriously consider the use of honey at levels of up to 3%, since this level may retard staling, can not be detected when tasted (as sweetness), and does not adversely affect any stages of the baking process from dough development (mixing) to gelatinization.

Publications	1. Squires, N.K., D'Arcy, B.R. and Caffin, N.A. Australian honey as an ingredient in white pan bread to retard staling. *Cereals '97: Proceedings of the 47th Australian Cereal Chemistry Conference*, Perth, 14-18 September 1997, 42-46. Melbourne, R Aust Chem Inst, 1997. 2. Mackay, D.C., D'Arcy, B.R., Caffin, N.A. and Bhandari, B.R. Kinetics of gelatinization of plain bread doughs using honey. *Cereals '97: Proceedings of the 47th Australian Cereal Chemistry Conference*, Perth, 14-18 September 1997, 262-264. Melbourne, R Aust Chem Inst, 1997. 3. Squires, N., Ford, A., Nottingham, S. Caffin, N. and D'Arcy, B. The use of sensory evaluation to determine changes in bread texture during the staling process. *Culinary Arts and Sciences II Global and National Perspectives*, 411-419. Poole, UK, Worshipful Company of Cooks Centre for Culinary Research, 1998.

Project Title	**The Use of Honeybees to Deliver Biocontrol Agents to Geraldton Waxflower**

RIRDC Project No.:	UQ-65A
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Objectives

- The objectives of this project were
- To find microorganisms, which are potentially antagonistic to *Botrytis cinerea*, and
- To determine the effectiveness of honeybees to deliver biological control agents to plants susceptible to *B. cinerea* such as Geraldton waxflower.

Background:

Preharvest infection of Geraldton waxflower with *B. cinerea* results in postharvest flower fall due to pathogen induced wound ethylene production. Current chemical control methods maybe considered as environmentally undesirable. There is also evidence that strains of *B. cinerea* are resistant to important fungicides. Biocontrol is an option and honeybees have been shown to successfully deliver biocontrol agents to strawberries for the control of grey mould (*B. cinerea)* in Canada.

Research

Saprophytic fungi, bacteria and yeasts were isolated from the surface of Geraldton waxflowers and screened as possible antagonists to *B. cinerea*. Two inoculum dispenser designs were tested using a commercial formulation of a biocontrol agent.

Outcomes

One bacterial isolate of 35 different microorganisms screened showed potential as an antagonist to *B. cinerea*. Honeybees were shown to successfully remove the commercial biocontrol agent from the hive however it could not be recovered from the flower surfaces of Geraldton waxflower plants foraged by the bees close to the hives.

Implications

Future research should be directed towards formulation of appropriate biocontrol agents such as a bacterial antagonist. Further research is also required to optimise the inoculum dispenser design as the honeybee delivery system shows real potential to be as effective as other control methods.

3.3 Honeybee
Research in Progress

Project Title	Development of a specific aggregation lure for *Apis cerana javana* (follow-on from CSE-74)

RIRDC Project No.:	CSE-81A
Start Date:	1/06/98
Finish Date:	16/07/99
Researcher:	Dr. Michael Lacey
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Objectives

- The outcome will be the validation of a blend of queen and Nasonov pheromones as a potent specific attractant for the Asian hive bee Apis cerana javana and the development of a controlled-release lure of synthetic chemicals for this exotic pest for incorporation in artificial nest cavities.
- The deliverables will be an increase in the effectiveness of protective monitoring measures and safeguard of the Australian honey bee industry against this potentially serious competitor.

Current Progress:

Most of the major and minor pheromone constituents of the queen mandibular glands and Nasonov glands of the exotic Asian hive bee *Apis cerana javana* have now been identified. It is clear that the molecular profiles of these particular pheromones differ from those of *A. mellifera*. Blends of components for the two *Apis* species, in proportions that were similar to those found in their natural queen substances, were synthesised and were subsequently evaluated in short-range bioassays in Papua New Guinea in November, 1998.

The assays showed that *A. cerana javana* workers aggregated around the synthetic blend for their own queen pheromone but were strongly repulsed by that for *A. mellifera*. The blends and additional components were then incorporated into controlled-release baits for evaluation in longer-range bioassays in Indonesia in March, 1999. The assays confirmed that the synthetic baits led to aggregation by workers and demonstrated the species-selectivity of the lure for *A. cerana javana.*

Project Title	Crude protein and amino acid levels of pollens collected by honey bees in southern NSW

RIRDC Project No.:	DAN-134A
Start Date:	1/03/95
Finish Date:	30/04/99
Researcher:	Mr. Doug Somerville
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Objectives

- To collect and test various pollens originating from NSW for crude protein and amino acid levels
- To measure any yearly variation in the levels of crude protein and amino acids from the one source of pollen
- To utilise this information to inform beekeepers of appropriate management strategies

Current Progress:

A total of 170 pollen samples have been collected for analysis from 54 species of plants. 89 samples of pollen have thus far been tested for crude protein, amino acids and fat levels, and a further 81 samples are currently being analysed. The crude protein is tested using the Macro Kjedahl method; amino acid, using high pressure liquid chromatography; and fat, using extraction with petroleum spirits.

Twenty eight samples of Patersons curse pollen were trapped and analysed for the 1995 season. There was a significant variation in crude protein, ranging from 28% to 33% of the 28 samples. The amino acids (Valine, Leucine, Iso-Leucine and Lysine) showed a significant variation in their levels in these Patersons curse pollens. The number of samples of Patersons curse pollen was significantly less in the 1996 and 1997 seasons, due to dry and drought conditions. These results will be compared for each year and the variations that occur from year to year on the one site. Some of the results have been published as follows.

Seven (7) articles in NSW Agriculture publication, *"Bee Briefs"*. Pollens covered include:

- Woollybutt *(Eucalyptus longifolia)*
- Lupins *(Lupinus angustifolius)*
- Willows *(Salix species)*
- Black thistle *(Cirsium vulgare)*
- Gorse *(Ulex europaeus)*
- Alpine ash *(Eucalyptus delegatensis)*
- Maize/corn *(Zea mays)*

Somerville, DC (1997). *"Value of Pollens Collected from Agricultural Crops"*. Proceedings Crop Pollination Association Conference. Tatura, Vic, 14-15 August, 1997. Somerville, DC; Peasley, W (1996). *"Eucalyptus Pollens & Their Value to Honey Bee Nutrition"*. Proceedings 3rd Asian Apicultural Association Conference. Hanoi, Vietnam, 6-10 October, 1996. (In Press)

Project Title: Floral resource database for the NSW apiary industry

RIRDC Project No.:	DAN-155A
Start Date:	1/02/96
Finish Date:	30/04/99
Researcher:	Mr. Doug Somerville
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Objectives

- To create a database of floral resource information for the NSW apiary industry.

Current Progress:

Floral resources are the basis of the Australian beekeeping industry. There is increasing pressure on the beekeeping industry from:

- reduced physical resources due to forestry, land clearing, urban expansion, firewood cutting and biological control of weed species;
- reduced health of vegetation due to lack of regular flooding of western rivers, dieback, salt inundation, drought and fire;
- policy adverse to the farming of honey bees on National Park estate, Water Board and State recreation areas.

There is little data available regarding the productivity, economic value and geographic significance of apiary sites in NSW. The objective of this project is to overcome the shortfall in information available on beekeeping activities and resource reliance in NSW. The project began with a mailing of census forms in April 1997 to all commercial beekeepers registered in NSW, with 200 or more hives. As at the completion of information gathering, an 81% response had been achieved through postal and personal interview techniques to gather the relevant information.
Information is now being correlated on the values of various floral species, the size of labour requirements of the industry and the gross income reliance of the industry on various enterprises within the apiary industry. The final report is currently being compiled. Published references relevant to the project include:

Somerville, DC (1998). *"State Forests—A Valuable Beekeeping Resource"*. The Australasian Beekeeper, September 1998. Vol 100, No 3; pp 96-101.
Somerville, D (1998). *"Floral Resource Database"*. New South Wales Apiarists' Association Newsletter, September-October 1998. pp 9-12.
Somerville D (1997). *"Bee Sites & Rural Lands Protection Boards in NSW—A Major Resource"*. The Australasian Beekeeper, October 1997. Vol 99, No 4; pp 142-143.
Somerville, DC; Moncur, MW (1997). *"The Importance of Eucalyptus Species for Honey Production in New South Wales, Australia"*. Paper for XXXVth International Apicultural Congress, Antwerp, Belgium. 1-6 September 1997.
Somerville, DC (1999). *"NSW National Parks & Beekeeping"*. The Australasian Beekeeper, April 1999. Vol 100, No 10.

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
Start Date: 1/12/98
Finish Date: 31/01/00
Researcher: Dr. Michael Hornitzky
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Objectives

- To determine whether the technique of shaking bees from hives with AFB onto foundation or irradiated hive equipment followed by treatment with oxytetracycline hydrochloride is an effective alternative to current control strategies.

Current Progress:	The first trial involving 20 hives commenced in December 1998. Treatments involving "shaking" bees into irradiated hive boxes containing irradiated frames or foundation coupled with, or without antibiotic treatment were carried out using 5 hives per treatment. To date all 20 hives except one have remained disease-free. It is, however, too early to make any assessment as to the efficacy of any particular treatment. Further examinations will be carried out on these hives in spring. Similar trials also need to be carried out on hives in a variety of locations to determine the effectiveness of "shaking" bees as an American foulbrood control strategy under various conditions. The extent of these trials will be determined by the availability of diseased hives over the next few months.

Project Title: The use of Australian honey in moist wound management

RIRDC Project No.:	DAQ-232A
Start Date:	1/07/97
Finish Date:	30/06/00
Researcher:	Dr. Craig Davis
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Objectives

- To develop a set of guidelines for the commercial production of honey as a therapeutic agent.

Current Progress:

This project has been investigating the potential application of a group of Australian honeys for their antibacterial properties. Screening of over 2000 honeys from throughout Australia has identified a specific area in Northern New South Wales which produces a thick (thixotrophic) dark honey with particular antibacterial properties. Potent activity against a range of food pathogens (eg E. Coli) and medical pathogens (eg Golden Staph.) has been described in laboratory experiments.

Preliminary trials in hospitals for the treatment of infected leg ulcers (Wesley Hospital, Brisbane) and for the treatment of bedsores (Princess Alexandra Hospital, Brisbane) support these laboratory observations. Some severe and unresponsive ailments can be healed in 2 to 3 months. Most recently, the effectiveness of these honeys on the organisms that cause mastitis in cattle has been evaluated.

Test-tube experiments indicate that the organisms are susceptible to honey. Ethical approval is currently being sought to proceed to animal trials. Although the floral-derived chemical component in these honeys has so far eluded identification, it appears to have some of the chemical properties of small sugars (which make up over 80% of the weight of honey). The characterisation and identification of the responsible plant (a Leptospermum) continues during each honey flow.

Project Title	A comprehensive study of beekeepers' use of honey and pollen flora resource in Victoria

RIRDC Project No.:	DAV-109A
Start Date:	1/07/95
Finish Date:	16/07/99
Researcher:	Mr. Russell Goodman
Organisation:	Department of Natural Resources & Environment Institute for Horticultural Development Private Bag 15 SOUTH EAST MAIL CENTRE VIC 3176
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Objectives

- To identify Victorian apiary sites on public land.
- To list nectar and pollen production resources at each site and link these to the production of honey and other apiary products.
- To identify those sites which are currently under-utilised by apiarists and identify areas which may have a potential for beekeeping.
- To catalogue the information on a data base and adapt it for use by industry.

Current Progress:

Two questionnaires, one for apiary sites located on public land and the other for apiary sites on privately owned (freehold) land were designed with assistance from the Victorian Apiarists' Association and mailed to apiarists owning 50 or more hives. A total of 930 completed questionnaires were returned. A few promised and outstanding questionnaires were expected to be returned shortly.

A new data base using the Microsoft Access computer program was developed by the person employed to assist with the progress of this project. This has enabled efficient entry and analysis of data provided by apiarists in the returned questionnaires. At the time of writing, entry of data was in progress.

Project Title	Developing a communications and marketing strategy for honeybee crop pollination

RIRDC Project No.:	DAV-119A
Start Date:	1/07/96
Finish Date:	15/04/99
Researcher:	Mr. Russell Goodman
Organisation:	Department of Natural Resources & Environment Institute for Horticultural Development Private Bag 15 SOUTH EAST MAIL CENTRE VIC 3176
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Fax:	03 9800 3521
Email:	goodmanr@knoxy.agvic.gov.au

Objectives

- To improve and promote an effective honeybee crop pollination service by developing a national marketing and communications strategy and a technology transfer resource package to enhance the delivery and use of commercial honeybee crop pollination services.

Current Progress:

A total of twenty-seven documents for growers and apiarists were completed. Of these, seven illustrated papers provide general and basic information on the principles of pollination and the efficient use of honeybee crop pollination services.
Sixteen pamphlets address the pollination requirements of specific fruit and seed crops, or group of crops (eg pome fruits), and present guidelines for the efficient use of bees to maximise pollination and crop set. Each pamphlet has a comprehensive flow chart which details steps to be taken by the grower to obtain effective pollination. The steps commence from the planning stage of the crop through to harvest.
The remaining documents include:

- Honeybee crop pollination services business and marketing strategies for apiarists and crop pollination associations
- Honeybee crop pollination services draft code of practice
- Draft code of practice for the use of bee tubes
- Honeybee crop pollination services contact list. This contact data base is for use by growers and apiarists.

Throughout the project, various crops and beehives have been photographed, firstly to illustrate the above papers and secondly to provide a bank of ninety 35 mm colour transparencies for use by apiarists or crop pollination associations.

Project Title

Best practice use of oxytetracycline hydrochloride (OTC) in beehives

RIRDC Project No.: DAV-157A
Start Date: 7/12/98
Finish Date: 10/06/99
Researcher: Mr. Russell Goodman
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Objectives

- To identify causes for the occurrence of OTC residues which threaten industry access to export and domestic markets by conduct of field trials which mimic current apiarist OTC application practices.
- To provide an opportunity for consideration for change of current OTC use patterns to prevent the occurrence of residues, and for possible establishment of a Maximum Residue Limit (MRL) by presentation to the National Registration Authority of data detailing levels of OTC residues obtained in the above trials.

Current Progress:

Field trials
Beehives with no history of oxytetracycline hydrochloride (OTC) applications over the previous twelve months were selected and standardised in terms of brood area and adult bee populations. The hives were allocated to three OTC product treatment groups. Within each group, six hives were treated with OTC product in sugar syrup (wet treatment) and six were treated with the same product mixed in castor sugar (dry treatment). The hives were then moved to a honey flow.
When full, the super directly above the queen excluder was removed and the honey extracted. A replacement super of empty, dry combs and beeswax foundation also filled with honey and was later extracted. Analysis of honey from of each group of six hives and each extraction will provide an indication as to whether bees move OTC contaminated honey from the broodnest to the super. Analysis of stored honey from each group of six hives sampled at various time intervals will provide an indication of the rate

of degradation of OTC.

Laboratory trials
Six floral type honeys were inoculated with OTC at 20 ppm and placed in controlled environments of 22ºC and 35ºC for later sampling and analysis to determine the effect of heat and pH on degradation of OTC over several months.

Project Title	Export package bees - evaluating a lupin flour based feed for increased live bee production

RIRDC Project No.:	DAW-75A
Start Date:	1/01/97
Finish Date:	30/04/01
Researcher:	Mr. Robert Manning
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Objectives

- Develop a lupin flour feed that is more palatable than expeller soyflour and/or Torula yeast.
- To enhance live bee production by developing a lupin based feed that is cost effective in comparison to other high protein feeds.

Current Progress:

This project had to be put on hold because of the exotic disease (Chalkbrood) outbreak in the experimental apiary. It was placed in quarantine and subsequently destroyed by Agriculture WA vets in April 1998. With the destruction went 12 months of extensive data already collected where some analysis had been carried out. The apiary has not been re-established because of quarantine and industry politics. The experiment will recommence as soon as the 'air' clears.

The likely date of restart was to be January 1999, but as May (winter start) looms closer, the project start date will probably be about May 2000.

Project Title	Pollen analysis of Eucalyptus patens (Blackbutt), E. accedens (Powderbark) and E. wandoo (Winter Wandoo variety) in Western Australia

RIRDC Project No.:	DAW-91A
Start Date:	1/07/98
Finish Date:	15/07/00
Researcher:	Mr. Robert Manning
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Objectives	• Provide 'first-time' analysis of amino acids of two important melliferous eucalyptus species to the beekeeping industry which are major contributors to the profitability of Western Australian honey and pollen producers.
Current Progress:	Collection of all winter wandoo samples has been completed. The collection of the blackbutt and powderbark pollen samples is incomplete (ie not enough replicates) because of the poor season in summer 1999. Hopefully, these will be collected this coming season when environmental conditions may improve. Analysis of wandoo pollen is complete and shows some interesting north/south latitude differences in nutrient levels of pollen, presumably as a result of soil type. In comparison to Marri/redgum (my baseline pollen that builds bees extremely well) some amino acids are less than 'optimal' and may be the source of beekeepers concern (bee population decline) when their bees are on this nectar flow. The fatty acids also show differences in composition to Marri pollen. Marri pollen itself is showing higher levels of some essential fatty acids that may have some bearing on disease suppression.

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:	3 Crozier Place WARRANE TAS 7018
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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 main focus of this project has been in mixed forest regrowth that contains leatherwood at reduced numbers as a result of overstocking with eucalypts. Field inspections found low numbers of leatherwood in the 0.01 ha experimental plots. Afraid that this may affect the results, the research design was revised and modified. The revised research design is a number of sequentially paired plots – control and thinned. Which will be analysed by paired t-tests. Ten plots (5 controls and 5 thinned plots) have been established within one coupe. This will be thinned in early spring of 1999. More plots will be established in the following months that will be thinned at the same time. Field inspections to find coupes suitable for non-commercial thinning found a number of coupes where the forest regrowth contains leatherwood in large numbers. This occurs in forest where regeneration of eucalypts was not successful and are below 800 stems per hectare which is the level to which non-commercial thinning procedure intend to reduce overstocked coupes. It is for this reason that plots will be established in this forest type in which flowering intensity will be monitored. So far 5 plots have been established.

Project Title	Non-fungicidal and biological control of core rots in pome fruit

RIRDC Project No.:	TAR-1A
Start Date:	1/07/97
Finish Date:	30/06/00
Researcher:	Mr. Chris Archer
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Objectives

- Develop a low cost and efficacious method of management of the core rot diseases in susceptible pome fruit varieties by the year 2001.
- Value add to the honeybee industry by way of utilising bees for transfer of the antagonists to the apple blossom.
- Pesticide reduction in apple orchards.

Current Progress:

Core rots (also called mouldy core) of apples is a major problem for Australian apple growers and exporters. The disease is caused by a complex of fungal pathogens (*Alternaria* and *Pezicula* spp. predominate), and results in zone of macerated and rotting tissue around the apple core with.

The disease develops from infections that occur during flowering but remains latent until after harvest when the apples are held in controlled atmosphere storage. The discrete period (full bloom) and site (senescing apple blossom) at which infection occurs, allows a strong potential for control of the disease by way of competitive or antagonistic biological control agents. As bees hives are normally placed in apple orchards during the full bloom period for the purposes of pollination there is also great opportunity for use of bees as a transfer vector of any control material to the apple blossom. Trials conducted in the 1998 season demonstrated bees to be more efficacious in the transfer of material to the flowers than high volume inundative spraying. Material transferred by bees resulted in a similar concentration of biocontrol material at the flowers compared with spraying however the sprayed material declined rapidly in concentration over time compared with that transferred by bees. A fungal species (*Gliocladium* naturally occurring within apples), that demonstrates antagonism to the core rot disease has been isolated and is to be used in further trials.

An investigation of new apple varieties demonstrated that 3 (Johnagold, Royal Gala, Sundowner) have the morphology to promote core rot development with 2 (Johnagold and Sundowner), exhibiting similar levels of rots occurrence to Red Fuji (considered a susceptible variety).

Project Title	Flavour quality assurance of Australian floral honeys by chemical fingerprinting

RIRDC Project No.:	UQ-67A
Start Date:	4/08/97
Finish Date:	31/10/00
Researcher:	Dr. Bruce R D'Arcy
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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.

Current Progress:

There is no reliable, objective procedure, as part of quality assurance programs, available for authenticating the flavour quality of Australian floral honeys. Honey flavour quality and the floral origin of honey are closely connected. The need for chemical authentication of the floral origin of unifloral honey throughout the world rests mainly with the deficiencies of the presently used method, pollen analysis.

A three-step procedure has been developed in this study and involves: Firstly an extraction of volatiles from honey using solvent (ethyl acetate) extraction and solid-phase microextraction; secondly, quantitative analysis of volatiles using gas chromatography and mass spectrometry; and thirdly, multivariate statistical analysis of the data. The overall authentication procedure requires collection of data on the levels of volatile substances in a large number of Australian unifloral honeys; presently, the following 13 honeys have been, or are in the process of being collected: blue gum, yellow box, leatherwood, red gum, yapunyah, jelly bush, brush box, strawberry clover, clover, mallee, Caley's iron bark, crow ash, and tea tree.

The economic implications of being able to objectively authenticate flavour quality is an increase in sales of Australian floral honeys through a positive increase in the overall image and profile of these honeys.

Project Title	Improving the movement/use of liquid Australian honey within manufacturing processes

RIRDC Project No.:	UQ-84A
Start Date:	1/07/98
Finish Date:	30/11/01
Researcher:	Dr. Bruce R D'Arcy
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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:

This project involves research targeted at understanding physical properties of honey, which are important in honey handling, processing and storage.

Some of these properties are viscosity, glass transition temperature, hygroscopicity and crystallisation (granulation).

- Firstly, this project will study how honey viscosity is correlated to composition. The viscosity behaviour of honey for a wide range of temperatures (above or below ambient and sub-ambient temperatures) is being evaluated. Mathematical viscosity models will be developed for various Australian honeys. At the same time, crystallisation behaviour will be correlated to viscosity. Knowledge of viscosity and glass transition properties will enable evaluation of the granulation kinetics of honey during storage, particularly at sub-ambient temperature conditions. This information will provide a more scientific base to explain current subjective methods to evaluate crystallisation of honey.
- Secondly, honey pumping systems will be evaluated for commercial application. This study will explain how different types of honey behave during movement, particularly focussing on honey variability (eg. viscosity, hygroscopicity and sugar profile).

The economic implications of this study of the physical properties of Australian honeys and the movement of liquid honey in commercial processes are that the baking and other food industries may greatly expand their use of liquid honey.

Project Title: Breeding hygienic disease resistant bees

RIRDC Project No.:	US-39A
Start Date:	1/07/96
Finish Date:	30/06/00
Researcher:	Dr. Ben Oldroyd
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Objectives

- To develop DNA markers for hygienic behaviour genes so that hygienic breeding stock may be rapidly and cheaply identified.
- To train a PhD student in honeybee molecular biology and to establich facilities and protocols that will allow genetic markers developed in other laboratories (eg for honey production) to be utilised by the Australian honeybee industry.
- To develop protocols for molecular analysis of other economic traits.

Current Progress:

Field data collected from experimental backcross colonies (backcross A) has been analysed in detail and the results presented at the XIII International Congress of IUSSI (International Union for the Study of Social Insects) in Adelaide, Dec 98/Jan 99. Feedback from colleagues at this conference was both positive and encouraging.

Currently, molecular analysis of the mapping population is continuing. To date nerarly 300 RAPD primers have been screened and over 250 segregating loci scored. Preliminary linkage analysis of this data has identified several linkage groups spanning the honeybee genome. Addition of loci to the data set and more rigorous analysis will increase both the accuracy and informativeness of the linkage map. Once this is achieved, QTL analysis based on the field data will be carried out and genomic regions of major influence on hygienic behaviour identified.

Observation and analysis of reciprocal backcross colonies (backcross B) has begun with one colony observed over 5 days in February 1998. Individuals observed performing hygienic behaviour were identified and collected. These will be used both in cDNA subtraction experiments and to test identified putative QTLs. It is expected that more backcross B colonies will be established and observations undertaken.