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Improving lucerne pollination with leafcutter bees – stage 2

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Foreword

Lucerne seed production is an important sector of Australia's pasture seed industry. However, lucerne seed yields in Australia are only about one-third of those achieved in Canada and the United States (US), with the higher overseas yields attributed to improved seed-set, achieved from using the leafcutter bee (*Megachile rotundata*) as a pollinator. The establishment of this bee in Australia would benefit local lucerne seed producers by increasing seed yields.

The complex quarantine and environmental issues involved with importing leafcutter bees from Canada and getting them established in the field were resolved during an earlier stage of the current project. The aim of the current project was to refine the importation processes to allow for very large numbers of leafcutter bees to be safely imported from Canada in single shipments, in order to facilitate the rapid establishment of the bees.

Approximately one million leafcutter bees were imported from Canada during each of the 2002-03, 2003-04 and 2004-05 Australian lucerne growing seasons. Transportation of the bees from Canada to Australia was improved during the first year of the project, reducing losses. The Australian quarantine handling procedures were also refined each year, resulting in bees spending less time in quarantine and being released on to crops faster, and in a healthier state. Approximately 70% of the bees imported each year were released from quarantine to lucerne crops in NSW or South Australia. The subsequent recovery of cells (leaf-covered cocoons containing immature new bees) from each release was below expectation, being adversely affected in some cases by poor weather following release, and in other cases by inappropriate on-farm management practices employed by farmers once the bees were established on the crops.

The improved importation processes now in place as a result of this project are safe and robust enough to deal safely with the importation of very large numbers of leafcutter bees in single shipments from Canada. However, local lucerne seed producers still need to improve their on-farm management practices to facilitate the build-up of bees. It is now up to the lucerne industry to move towards establishing the leafcutter bee in Australia.

This project was funded from industry revenue which is matched by funds provided by the Australian Government

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Executive Summary

Lucerne seed yields in Canada and the United States (US) are significantly higher than those in Australia and are achieved mostly through superior pollination provided by the leafcutter bee, *Megachile rotundata*. The establishment of this bee in Australia would significantly improve lucerne seed yields which, at present, rely on pollination by the European honeybee *Apis mellifera*, which is less efficient at pollinating lucerne than the leafcutter bee.

During Stage 1 of the current project (1999-2002), the complex quarantine and environmental issues involved with importing the leafcutter bee from Canada and getting it established in the field were resolved. The current Stage 2 project was aimed at improving the import procedure to deal with large importations of the bee. The project objectives were:

a) To develop safe and cost-effective quarantine procedures for importing large numbers of Canadian leafcutter bees.
b) To improve methods for managing leafcutter bees under Australian conditions.

When developing the present Stage 2 project it was anticipated that the research would be jointly funded as in Stage 1, by RIRDC and GRDC, with in-kind assistance from seed companies and independent growers. However, shortly before the project commenced, GRDC withdrew support for the leafcutter bee initiative. The subsequent reduction in funding led to a tightening of the scope of the project. Hence, no bees that emerged in quarantine could be managed by the project, as was the case in Stage 1. Instead, industry partners purchased the bees and managed them in the field once they were released from quarantine. This allowed project work to focus on streamlining the quarantine procedure for dealing with large numbers of bees, checking for pests and diseases, and making simple observations on released bees in the field.

Importations of large numbers of Canadian leafcutter bees were made during each of the 2002/03, 2003/04 and 2004/05 Australian lucerne growing seasons.

In December 2002 approximately one million leafcutter bee cells were imported from the Peace River District of Alberta, Canada on behalf of Pioneer Hi-bred Australia and two independent lucerne seed producers based at Keith in South Australia. Upon arrival in Australia the bees were introduced into the high security quarantine centre at CSIRO Entomology in Canberra and handled using the equipment and methodologies developed in Stage 1. However, improvements were made to the way that new bees emerged in quarantine, which more than halved the quarantine processing-time. A total of 711,802 adult leafcutter bees emerged from the imported cells giving an emergence rate of 71%. Of these, 533,752 were released on to a large lucerne seed crop near Cowra, NSW, 89,025 on to a single lucerne seed crop near Keith, South Australia, 44,512 on to a second lucerne seed crop near Keith, and 44,513 on to a mixed lucerne/clover field near Bordertown, South Australia. A further 200,000 leafcutter bee cells, that were recovered from bees that has been released at the same locality during the 2001/02 season as part of RIRDC project CSE-86A were also placed into shelters at the Cowra site. The recovery of cells from bee released at the Cowra site was below expectations, but cells recovered from the Keith and Bordertown sites almost equalled the numbers of bees released.

In December 2003 approximately 1.2 million leafcutter bee cells were imported from the Peace River district on behalf of Pioneer Hi-Bred Australia. These were again processed through quarantine, where 800,000 adult bees emerged, giving an emergence rate of 67%. All these bees were released on to a large lucerne crop near Cowra, together with 300,000 cells carried over from the previous year. The released bees, and those that emerged from the previous year's cells, showed excellent flight and forage activity. However, most were subsequently killed by an insecticide treatment of the crop.

In January 2005, approximately 1.2 million leafcutter bee cells were imported on behalf of Seedmark. A total of 701,124 adult bees emerged from the quarantined cells, giving an emergence rate of 58.4%.

This was the lowest emergence rate recorded for imports handled during Stages 1 and 2 of this project, and resulted from a higher than normal level of parasitoid infestation of the imported cells. Only 70,000 cells were recovered from the released bees. This disappointing return was due to the very cool weather conditions that persisted from the later than normal release date until the removal of nesting blocks from the field, which prevented most of the bees from foraging and producing offspring.

In other project work, a DNA fingerprinting method was developed for distinguishing between imported and indigenous leafcutter bees.

The three large successful importations of leafcutter bees made during this project showed that the quarantine procedures were safe and robust enough to deal with very large importations. Such importations will be needed in the future if a leafcutter bee industry is to become established in Australia. It is now up to the lucerne seed industry to exploit the opportunity provided by this project (and the previous Stage 1 project), and move towards establishing the bee.

1 Introduction

Lucerne seed yields in Canada and the United States (US) are two-thirds higher than those in Australia and are achieved mostly through higher seed-set resulting from superior pollination provided by the leafcutter bee, *Megachile rotundata*. This bee, which is not present in Australia, arrived in North America from the Mediterranean during the mid-1930's, and showed up in Canada soon after (Stephen, 1961). Local lucerne growers soon realized that the bee was a very efficient pollinator of lucerne flowers, and constructed artificial nests to encourage larger numbers of bees on to lucerne crops. Over time, bee numbers increased and a leafcutter bee industry developed. Today, the leafcutter bee is an integral part of lucerne seed production in Canada and the US and efficient management systems ensure that more than enough numbers of the bee are available on demand throughout the lucerne-growing season. Establishment of the leafcutter bee in Australia would significantly improve lucerne seed yields, which, at present, rely on pollination by the European honeybee *Apis mellifera*, which is less efficient at pollinating lucerne than the leafcutter bee.

Even though leafcutter bees are solitary, they possess 'social-like' characteristics that have allowed them to be developed into highly efficient managed pollinators of lucerne. For instance, adult female bees always nest above ground, aggregate at nesting sites and forage close to those nesting sites. Hence, artificial nests can be constructing for thousands of females inside shelters, protected from the weather. What is more, in nature, the offspring of leafcutter bees enter diapause (a 'sleep time' that is different from hibernation) before the start of winter and break diapause the following spring and summer. This characteristic has allowed for diapause to be artificially induced by exposing prepupae to lower temperatures in cool rooms and then breaking the diapause by holding the prepupae at high temperatures.

Since 1996 efforts have been made to establish the leafcutter bee in Australia. During Stage 1 of the current project (1999-2002), the complex quarantine and environmental issues involved with importing the bee from Canada and getting it established in the field were resolved. The current Stage 2 project (2002-2005) was aimed at improving the import procedure to deal with large importations of the bee.

2 Background

2.1 Management of the leafcutter bee in Canada and the USA

Systems for managing leafcutter bees have gradually improved in the US and Canada as materials and machinery and knowledge of the bees' biology have simultaneously improved. These systems are now so sophisticated they can be manipulated to cater for differences in seasonal conditions, crop flowering times and effects of parasites and diseases. The two most common systems now in place are the '*closed cell system*' and the '*open cell system*'.

The closed cell system has dominated American leafcutter bee management for decades. It involves drilling tunnels in wooden blocks for the bees to nest in. The cells constructed in the tunnels (usually about 7/tunnel) are not removed at the completion of the flowering season but, instead, are left in the blocks, and the blocks are stored through winter in complete darkness at 4-5^{o}C. The following season the blocks are removed from cold storage and placed on lucerne crops for bees to emerge from the tunnels. Following emergence, the bees then reuse the tunnels to nest. A major drawback with this system is that the incidence and spread of disease is increased, particularly that of chalkbrood disease caused by the fungus *Ascosphaera aggregata*, through the action of newly emerging bees chewing through cells containing dead bees further towards the entrances of the tunnels.

The open cell system is a recent development that has now become popular throughout most of Canada and some parts of the US. In this system, pine laminates or blocks of polystyrene with tunnels completely bored through are used for the nesting sites. A removable backing is attached, as leafcutter bees will not nest in open-ended tunnels. At the end of the flowering season the backings are removed and the leafcutter bee cells containing the bee cocoons are stripped or punched from the tunnels, rolled in screened tumblers to remove excess leaf material and placed in cold storage over winter in bags (Richards, 1989). When bees are needed the loose cells are removed from cold storage and incubated for about 24 days at 30^{o}C, after which the new bees begin to emerge. The emerged bees are then chilled (at about 12-15^{o}C) and moved into shelters in the field. The open cell system reduces the need for large cold storage space, limits the spread of pests and diseases and allows for better sanitation (Bohart, 1972; Baird and Bitner, 1991). However, it requires a higher level of management than the closed cell system.

The popularity and continued use of the closed cell system in the US has seen American lucerne seed producers become more reliant on the importation of Canadian leafcutter bees produced under the open cell system. Anderson (1997) recommended that the open cell system be adopted in Australia.

2.2 Early attempts to establish the leafcutter bee in Australia

Attempts to establish leafcutter bees in Australia prior to the current project have all been unsuccessful. Two importations of leafcutter bees from New Zealand during the 1980's (from stock that originated from the US) were destroyed in quarantine due to the presence of parasites and diseases. However, in 1987 another importation, also from New Zealand, was successfully released on to lucerne crops near Keith in South Australia. A further release of New Zealand bees was also made near Keith in 1989/90. These releases failed to establish the bee, principally because of high levels of parasitism by parasitoids among their offspring (82%), followed by a gradual decline through mismanagement of the remnant population (Woodward, 1994; 1996).

2.3 Stage 1 of the current project (1999-2002)

Despite the lack of success with early importations, interest in establishing leafcutter bees in Australia nevertheless remained high and, in 1996, an application for further importations was submitted to the Australian Quarantine and Inspection Service (AQIS) by Pioneer Hi-Bred Australia.

The Pioneer Hi-Bred application proposed the importation of large numbers of leafcutter bees from Canada, where bee surpluses are produced each year. AQIS subsequently commissioned CSIRO Entomology to conduct a risk assessment of Canadian leafcutter bees. At the same time a consultative committee of stakeholders comprising representatives from AQIS, Environment Australia, CSIRO, the Australian beekeeping industry, the lucerne seed industry, commercial seed companies, the Rural Industries Research and Development Corporation (RIRDC) and the Grains Research and Development Corporation (GRDC) was formed to discuss issues associated with possible importations.

The CSIRO risk assessment concluded that Canadian leafcutter bees could be imported into Australia with minimal risks of introducing unwanted pests, parasites or diseases, provided certain precautionary steps were followed. In brief these included:

- only importing leafcutter bees as prepupae in leaf covered nesting cells;
- testing shipments for pests and diseases prior to departure from Canada and immediately after arrival in Australia;
- processing imported cells in an AQIS approved high security quarantine facility prior to releasing adult bees to the field. The recommended processing involved: (a) surface sterilising imported cells with sodium hypochlorite, (b) incubating the cells in specially constructed incubators to allow the bee prepupae to develop into adults and also to allow unwanted pests to be 'bled' off and, (c) dipping newly emerged bees in a solution of 2.0% sodium hypochlorite prior to their release to field crops (Anderson, 1997).

These recommendations were endorsed by the consultative committee, accepted by AQIS and included in the *Permit to Import Live Material Into Australia*, which can be found on the AQIS web site (AQIS, 2002).

RIRDC and GRDC agreed to jointly fund a 3-year Stage 1 project (1999-2002) to develop quarantine procedures for importing Canadian leafcutter bees in Australia, and to identify pests and pathogens on imported bees and their offspring. Initial shipments from Saskatchewan consisted of individual cells, each containing a leafcutter bee prepupa inside of a leaf-covered cocoon. Upon arrival in Australia, the cells were surface sterilized and incubated in quarantine in specially constructed incubators to allow the prepupae to develop into adult bees. The adults were then dipped in a sterilizing solution as a preventative treatment against the accidental introduction of the fungus *A. aggregata*, which causes chalkbrood disease. After drying, the bees were released on to lucerne crops, mostly in western NSW. However, even though the bees survived the quarantine processing, they did not survive for more than a few days when released on to the crops, nor did they produce offspring. Tests carried out in the US subsequently showed that, contrary to popular belief, the dipping procedure was deadly to young bees. To eliminate the need for dipping, cells were thereafter sourced from the Peace River region of Canada, which AQIS approved mid-project as being free of chalkbrood disease. This move led to an immediate improvement in the survival and recovery of bees released from quarantine. At the same time several pests had been identified on imported bees, while a potentially serious Australian native parasitoid was found to affect the offspring of bees released to the field. These issues were addressed separately by the research team with positive outcomes. Hence, by the completion of the project the major hurdles for importing Canadian leafcutter bees had been overcome and, in recognition of these achievements, the project was awarded the prestigious '*Owen J Newlin Business Excellence Award*' (a US-based international award sponsored by DuPont).

During the final year of the Stage 1 project (2002) the first attempt was made to import a large shipment of Canadian leafcutter bees and process them through quarantine. This failed due to the emergence of very high numbers of parasitoids (*Pteromalus venustus*) in quarantine. This resulted from an overheating of the cells during their shipment from Canada. As a result, the entire shipment was destroyed. This loss could have been avoided if the cells had been incubated immediately on the arrival in quarantine instead of being placed in a cool room for 8 months prior to incubation. Hence, for the current project, modification were made to the way leafcutter bee cells were shipped from Canada that eliminated the need for cool storage after their arrival in Australia.

3 The Current Project – Stage 2

The objectives of the current project were:

a) To develop safe and cost-effective quarantine procedures for importing large numbers of Canadian leafcutter bees.
b) To improve methods for managing leafcutter bees under Australian conditions.

When developing the present Stage 2 project it was anticipated that the research would be jointly funded as in Stage 1, by RIRDC and GRDC, with assistance from seed companies and independent growers. However, shortly before the project commenced, GRDC withdrew support for the leafcutter bee initiative. The subsequent reduction in funding meant that a Post Doctoral position was lost from the project and the scope of what the project could achieve with reduced funding was scaled down. As a result, no bees could be managed by the project, as was the case in Stage 1. Instead, industry partners purchased the bees and managed them in the field once they were released from quarantine. This allowed project work to focus on streamlining the quarantine procedure for handling large numbers of bees, checking for pests and diseases, and making simple observations on bees released to the field.

4 Results

Importations of large numbers of Canadian leafcutter bees were made during each of the 2002/03, 2003/04 and 2004/05 Australian lucerne growing seasons.

4.1 Leafcutter bee importation - 2002/03

In December 2002 approximately one million leafcutter bee cells were shipped from the Peace River District of Alberta, Canada, to the quarantine facilities at CSIRO Entomology in Canberra on behalf of Pioneer Hi-Bred Australia and two independent lucerne seed producers in South Australia. These cells had been removed from lucerne fields in July 2002 and then kept in cold storage at 5^{o}C for 4.5 months in Canada prior to shipping. This period of cold storage was long enough to allow the bees to enter diapause and for diapause to be broken immediately upon incubation at 30^{o}C upon arrival in Canberra (a minimum of 4 months of diapause is thought to be required before diapause can be broken).

Upon arrival in Canberra, AQIS staff inspected the cells, and a sub-sample of 4,500 cells was removed and X-rayed to determine the levels of parasitoid (*Pteromalus*) and chalkbrood (*Ascosphaera* spp.) infested cells. The remaining cells were then surface-sterilized by dipping in a 2% solution of sodium hypochlorite (bleach) for 30 seconds, followed by three water rinses, then spread out on trays to dry overnight at 30°C. Once dry, the cells were placed in incubators at 30°C to break bee diapause and allow the young bee prepupae to develop into adults.

The incubators were essentially the same as those used throughout Stage 1. Each accommodated approximately 100,000 cells. However, a major change was made to the 'collection boxes' in incubators, which collected the adult bees as they emerged from the cells. During Stage 1, shredded paper was placed into the collection boxes to allow emerging bees to separate themselves from each other and thus reduce the level of fighting and damage. However, removing the bees from the shredded paper was time-consuming because each shred had to be pulled between thumb and index finger to remove clinging bees. To simplify this task, the shredded paper was replaced with thin long strips of suede material that were permanently attached to the underside of the lid of each collection box. The removal of newly emerged bees from the suede strips then involved removing the lids from the collection boxes (in a cool room at 15^{o}C), shaking the lid over a large collection tray to remove the bees, then scooping up the bees that fell. This simple modification more than halved the quarantine processing time.

Results from X-ray analysis on the imported cells showed extremely low infestations of the parasitoid *Pteromalus*. No chalkbrood-infected cells (caused by the fungus *A. aggregata*) were present. As with cells imported in previous years, small numbers of a cuckoo bee species (*Coelioxys* sp.) a megachilid bee (*Megachile relativa*) and larvae of the chequered flower beetle (probably *Trichodes ornatus*) were also detected.

AQIS and Environment Australia approved the release of the first batches of newly emerged adult bees from quarantine on 6 January 2003.

Approximately 711,802 adult leafcutter bees emerged from the one million imported cells (emergence rate of 71%). Of these, 533,752 were released on to a large lucerne seed crop near Cowra, NSW, 89,025 on to a single lucerne seed crop near Keith, South Australia, 44,512 on to a second lucerne seed crop also near Keith and 44,513 on to a mixed lucerne/clover field near Bordertown, South Australia (see Table 1 below).

Once on the crops, collaborating industry partners managed the bees. As previously stated, this contrasted with previous releases in Stage 1, which were managed within the project by project staff.

At the Cowra site the bees were managed by Pioneer Hi-Bred Australia (Dr Ron Bitner and Mr Wayne Coleman), the 89,025 bees released near Keith were managed by De Barro Agricultural Consulting (Mr James De Barro), while the two single lots of 44,513 and 44,512 bees released near Keith and Bordertown were jointly managed by Mr Tony Campbell (a local lucerne seed producer) and Mr Jeff Smith (a commercial apiarist).

The Cowra Site

The Cowra site was located on a property managed by Mr John Bruce approximately 40km from Cowra on the Cowra-Forbes road. The crop on to which the bees were released was a 28-hectare seed lucerne crop. It was divided into bays and each bay had previously been mowed at slightly different time intervals so that the released bees had a continuous source of flower over a 6-week period.

Seven bee shelters with nesting blocks were placed on the crop, 3 on one side of the crop and 4 on the other. An eighth shelter, also containing nesting blocks, was placed on a second lucerne crop approximately 1.5 km from the main crop to monitor bee movement off the main crop (bee 'drift'). Following-on from recommendations that came from Stage 1, metal containers were welded onto the bases of the shelter legs and filled with oil to prevent ant access. Tanglefoot®, a petroleum based sticky barrier gel, was also placed on the shelter tie-downs and on the wire brackets used to hold the nesting blocks in place inside the shelters to also prevent ant access. A sheet of wood was also added to the top entrance of the shelter to prevent direct sunlight and rain from entering (see Appendix).

533,752 bees were released into 4 shelters on one side of the crop. As well, a further 200,000 leafcutter bee cells, that were recovered from the previous years' releases, were placed into one of the 3 shelters on the opposite side of the crop. The remaining 2 shelters did not receive any bees for the purpose of monitoring bee drift within the crop.

The cells from the previous years' releases contained prepupae and had been stored at 5^{o}C over winter at CSIRO Entomology in Canberra. During the storage period (approximately 7.5 months) the cells had also been exposed to 2^{o}C temperatures for 4 continuous days, with the purpose of seeing whether the lower temperatures would kill *Melittobia hawaiensis* parasitoids (hereafter referred to as simply *Melittobia*) if present. Following cold storage, the cells were incubated at 30^{o}C for 20 days, and then placed in polystyrene boxes inside the single shelter.

After 6 weeks on the crops, no bees were found nesting inside the single shelter placed 1.5 km from the main crop. However, many bees were observed nesting in the 2 shelters on the main crop into which no bees had been released. This showed there was little (if any) drift of bees off the main crop, but a substantial amount of drift within the main crop. It also suggested that shelters should be placed around a particular crop, and not in a single row on one side of the crop, in order to catch drifting bees.

No *Melittobia* parasitoids were observed emerging from the previous years' recovered cells, even though it was known that 0.2% of these cells were infested with *Melittobia*. This indicated that the procedure of chilling stored cells at 2^{o}C for 4 continuous days controlled parasitoid levels.

At the end of the nesting season, before the nesting blocks were removed from the crop, an estimate was made of the levels of second generation bees by counting the numbers of open capped holes in the nesting blocks. The level of second generation was estimated at 30%, much higher than observed at any time during Stage 1, and much higher than observed at the end of the current season at the Keith and Bordertown sites (see below).

The nesting blocks were removed from the shelters at the completion of flowering (in April), transported to Pioneer Hi-Bred facilities at Narromine, NSW, and the cells removed, counted and stored. Approximately 300,000 cells were recovered from the 733,752 released bees, giving a recovery rate of approximately 41% (if all the bees emerged from the 200,000 cells, which is

unlikely). This disappointing recovery was put down to strong winds and severe storms during the critical early nesting period of the bees, as a significant drop in flying bees was observed after a period of very strong winds. During a rather severe storm, one shelter was completely blown over despite being tied down, and ants attacked the nesting blocks. During another storm, rain entered the shelter and submerged the carry-over cells from the previous season in their polystyrene boxes. These mishaps showed the need for improvement in shelter design. It was obvious that shelters needed to be more securely tied down and that containers used to hold cells with emerging bees needed to be made water-proof and to contain easy bee-escape routes.

A subsample of 3000 of the 300,000 recovered cells was inspected for pests and diseases. 0.1% were found to be infested with *Melittobia hawaiensis*. (a very low infestation). As well, a cuckoo bee, *Coelioxys* sp., was detected. No chalkbrood was detected.

The Keith and Bordertown Sites

The collaborating industry partners at the Keith and Bordertown sites provided crops for the bees, constructed bee-nesting shelters (see Appendix) and managed bee recovery at the end of the season and the subsequent storage of recovered cells.

The recovery rates from bees released at each site were extremely promising, almost equalling the numbers of bees released (see Table 1). The percentage of second-generation bees was also quite low at approximately 10%.

Very low levels of parasitoids (*Melittobia* sp.) were detected in cells recovered from each site, but no chalkbrood was detected. An unidentified Australian native leafcutter bee was also observed nesting in the nesting blocks at one of the Keith sites.

Table 1 Summarises the numbers of leafcutter bees released at the different release sites during the 2002/03 season, the numbers (and percentages) of cells recovered from the released bees and the percentages of observed second-generation bees.

Release Site	Number of bees released	Number (and percentage) of bees recovered	Percentage (%) of second generation bees
Cowra	733,752+	300,000 (41.0)	30.25
Keith (site 1)	89,025	70000 (87.0)	10
Keith (site 2)	44,513	35,000 (79.0)	10
Bordertown	44,512	50,000 (112.0)	10
TOTALS:	911,802+	475,000 (52.0)	

+ = 200,000 of these were cells containing emerging bees that had been recovered from the previous season.

4.2 Leafcutter bee importation - 2003/04

On 18 December 2003 1.2 million leafcutter bee cells were imported from the Peace River district of Canada into the CSIRO Entomology high security quarantine centre in Canberra on behalf of Pioneer Hi-Bred Australia. The cells had been removed from lucerne fields at Peace River during July 2003 and kept in cold storage at 5^{o}C for 4.5 months prior to shipping.

Immediately upon arrival of the cells in Canberra a sub-sample of 6,733 cells were removed and X-rayed to determine the general health of the cells and the incidence of parasitoid (*Pteromalus*) infestation and chalkbrood (*Ascosphaera* spp.) infection. The remaining cells were surface-sterilized by dipping in a 2% solution of sodium hypochlorite (bleach) for 30 seconds, dried and placed in incubators at 30°C to break diapause of the leafcutter bee prepupae inside and thus allow the prepupae

to develop into adults. The dipping and drying procedures were modified from the previous year to allow more cells to be dipped and dried at once in a single batch, thus reducing processing time.

Results of X-ray analyses showed that 0.59% contained immature larvae, 81.29% healthy live prepupae, 3.09% dead prepupae and 0.73% live pupae. A further 10.08% contained pollen balls and 4.2% had been machine damaged. There were no chalkbrood-infected cells and the level of *Pteromalus*-infested cells was 0.01%, well below the AQIS requirement of 0.25%.

Further processing of the developing bees in quarantine was essentially the same as that described for the 2002-03 season. During incubation very small numbers of cuckoo bees (*Coelioxys* sp.), the leafcutter bees *Megachile relativa* and chequered flower beetle larvae of the genus *Trichodes* (probably *T. ornatus*) were detected. Only 10 individual adult *Pteromalus* parasitoids were detected, indicating a high level of care had been shown in Canada in preparing the cells for export.

On 12 January 2004 AQIS and Department of Environment and Heritage (DEH) approved the release of newly emerged leafcutter bees from quarantine. All these bees were released on to a single lucerne crop near Cowra. As with last years' releases, all bees released during the current year were managed and cared for by Pioneer Hi-Bred Australia.

As well as monitoring the bees released to the Cowra site, bees that had been released during the previous season at 2 sites at Keith and 1 at Mundulla (near Bordertown) in South Australia were monitored. These bees had also been managed and cared for after their release by industry partners.

The Cowra Site

The Cowra site was the same as that used during the 2002/03 season, except that the lucerne crop on to which the bees were released was different. The site was located approximately 40km from Cowra on the Cowra-Forbes road.

Approximately 800,000 leafcutter bees were released from quarantine on to the crop from 18 January to 3 February 2004 (giving an emergence rate of 67%). As well, Pioneer Hi-Bred Australia placed 300,000 leafcutter bee cells on the crop in January 2004. These cells, which contained bees ready to emerge, had been produced by the 2003 quarantine-released bees and they had been over-wintered at 5^{o}C. During the over-wintering phase, the cells had also been held for 3 continuous days at 2^{o}C to kill any *Melittobia* parasitoids that may have been present. When introduced to the crop, the cells were placed on shelves inside small wooden boxes on the floors of shelters. The boxes allowed for emerging bees to escape but prevented them from re-entering to nest (see Appendix). Just prior to placing the cells on the crop, a sub-sample consisting of 400 cells was removed and examined at CSIRO Entomology for pests and pathogens. Given a 70% emergence rate for these cells (estimated from the number of live bees in the sub-sample), then the 300,000 released cells would have added a further 210,000 adult leafcutter bees to the crop, making for a grand total of 1,010,000 adult bees released on to the crop. No *Pteromalus* parasitoids or chalkbrood affected cells was detected in any of the 400 cells tested, indicating that the current quarantine procedures employed to prevent the introduction of these pest into Australia were working. As well, no *Melittobia* parasitoids were observed emerging from the remaining cells in the field, indicating that the procedure of quickly chilling stored cells at 2^{o}C during their over-wintering phase was controlling this parasitoid.

The released bees, as well as those bees that emerged from cells recovered during the 2002/03 season, showed good forage and flight activity and females soon began nesting in the blocks provided. Indeed the flight and forage activity rivalled that seen previously in shelters in Canada and the US. However, on the evening of 28 January, as bee emergence in quarantine was coming to an end, a decision was made by Pioneer Hi-Bred to spray the crop with an insecticide (Steward™, a product of Dupont, active ingredient indoxicarb) to prevent a build-up of insect pests. It was known that this insecticide was relatively harmless to honey bees but its affect on leafcutter bees was not known. The spray was applied in the evening when most of the bees had finished foraging. Seven days later, on 3 February

2004, it was estimated the spray had killed most of the foraging bee population. The nesting blocks were removed from the shelters in April 2004 and transported to Pioneer Hi-Bred facilities at Narromine NSW where approximately 300,000 cells were recovered (the majority of these would have been produced in a short period prior to the spray being applied). This unfortunate event demonstrated the level of care that importers must exercise during the critical build-up period of leafcutter bees on the crops.

The Keith and Bordertown Sites

The 70,000 cells recovered from one of the Keith sites in the 2002/03 season (see Table 1) were over-wintered at 5^{o}C. During this period they were also held for 3 days at 2^{o}C to kill parasitoids that may have been present. In late 2003 the cells were incubated at 30^{o}C and, shortly before bees were due to emerge, placed on to a lucerne crop near Keith. No *Melittobia* parasitoids emerged from the cells, providing further evidence that the brief chilling of cells controls these parasitoids. Shortly after bees began to emerge from the cells, the lucerne crop was affected by high winds and rain, which affected the survival of emerging bees. Approximately 45,000 cells were recovered, 10% of which produced a second-generation. This was considered a very positive return, given the extremely poor weather conditions that affected the newly emerged bees.

Cells recovered from both the Bordertown and second Keith site in the 2002/03 season (a total of 85,000) were amalgamated and over-wintered at 5^{o}C during which they were held for a 3-day period at 2^{o}C. In December 2003 they were incubated at 30^{o}C until adult bees were due to emerge, then placed on a lucerne crop near Keith. During the first 10 days after release, the newly emerged bees appeared healthy and showed good foraging and nesting behaviour. However, most of the bees then suddenly disappeared. It was difficult to pinpoint the exact cause of the loss of the bees. At the time they disappeared the crop had experienced a very high daytime temperature of 42^{o}C but, if this has an effect, it should only have affected the larvae inside the shelter, not the adult bees. The Keith site also had records that demonstrated the site had not been sprayed with insecticide within a 1 km radius of the bee shelter. Nevertheless, in this and previous projects, all sudden adult leafcutter bee deaths in the field have been associated with the use of insecticidal spray. In the present case it was concluded that insecticide spray drifting on the wind from outside the 1km radius was probably responsible for the death of the bees.

4.3 Leafcutter bee importation - 2004/05

At late notice during the final year of this project, Pioneer Hi-Bred Australia withdrew from a planned importation of Canadian leafcutter bees. However, Seedmart and South Australian lucerne seed producers stepped in, also at late notice, and supported the importation.

On 6 January 2005, 1.2 million leafcutter bee cells arrived into quarantine in Canberra (1 month later than initially anticipated). A sub-sample of 7,306 cells was removed from the shipment and the cells X-rayed to test their general health. Results showed that 1.05% contained immature larvae, 82.4% healthy live prepupae, 2.92% dead prepupae and 1.49% live pupae. A further 9.18% contained pollen balls and 2.72% had been machine damaged. There were no chalkbrood-infected cells and the level of *Pteromalus*-infested cells, although high at 0.23%, was still below the AQIS requirement of 0.25%. All remaining cells in the shipment were then surfaced sterilized in bleach and placed into incubators to allow the bee larvae to develop into adults, as described above. During incubation, small numbers of cuckoo bees, *M. relativa* and chequered flower beetle larvae (probably *Trichodes ornatus*) were detected but, towards the completion of incubation, very large numbers of *Pteramalus* parasitoids emerged from the remaining cells. Even though these parasitoids were manageable within the quarantine system, slightly higher numbers would almost certainly have led to an early closedown of quarantine and destruction of remaining cells. Thus the requirement by AQIS to restrict the level of *Pteromalus*-infested cells in a single importation to 0.25% of cells

Approximately 701,124 adult bees emerged from the quarantined cells, giving an emergence rate of 58.4%, the lowest emergence rate recorded for imports handled during Stages 1 and 2 of this project, and almost certainly a result of losses caused by the higher than normal level of parasitism by *Pteramalus* parasitoids. The Canadian exporter indicated that such high levels of parasitism could have been avoided if the planned shipment of cells by Pioneer Hi-Bred had taken place. It is unlikely that the high levels of parasitism of cells that occurred with this shipment will be seen again.

On 4 February 2005 AQIS and DEH approved the release of newly emerged bees from quarantine. From that time on, batches of newly emerged bees were released from quarantine and transported in refrigerated vans at 4-day-intervals to lucerne and clover crops south of Keith. Mr Tony Campbell, Mr Charlie Hilton and Mr Jeff Smith managed these bees on the crops.

Approximately 70,000 cells were recovered from the released bees. This disappointing return was due to the very cool weather conditions that persisted from the later than normal release date until the removal of nesting blocks in April and which prevented most of the bees from foraging and producing cells.

No pests or pathogens were found in a sub-sample of 100 cells removed from the recovered cells.

4.4 Other project work

Canadian leafcutter bees are morphologically very similar to many Australian leafcutter bee species, and hence are difficult to distinguish from those species. Therefore, studies were undertaken to develop a DNA-based identification method to distinguish between imported and indigenous leafcutter bees. PCR primers were developed and a region of the mitochondrial COII gene of the Canadian leafcutter bee (*Megachile rotundata*) and a local Canberra leafcutter bee species (thought to be *Megachile maculata*) sequenced. The sequences obtained were unique for each species, showing that the *M. rotundata* sequence will be useful in the future for unambiguously identifying that bee.

5 Environmental Issues

Further monitoring was carried out during this project to determine whether leafcutter bees moved off lucerne crops after being released from quarantine. This was done by placing small 'trap-nests' around the perimeters of crops at varying distances from the crops just prior to, or on the same day, that leafcutter bees were released. The trap-nests were made from two pinewood grooved laminates that, when fitted together, would make 13 holes that could be used by the bees as nesting tunnels. The trap-nests were removed from the field at the same time that nesting blocks in shelters were removed. They were then dismantled and examined for signs of leafcutter bee nesting activity. Like in previous years, results indicated that the released leafcutter bees did not drift away from the crops, as only the traps close to the crops were utilised as nesting sites by the bees.

The import of live animals into Australia, including that of the leafcutter bee *Megachile rotundata*, is controlled by the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act), administered by the Department of the Environment and Heritage (DEH), and the *Quarantine Act 1908*, administered by the Australian Quarantine and Inspection Service (AQIS). Under the EPBC Act, a list of specimens taken to be suitable for live import is scheduled and the leafcutter bee is listed under Part 2. This means that the importation of leafcutter bees requires an import permit (with conditions) under the Act. In December 2003 a correction was made to the conditions of import for leafcutter bees under Part 2 of the schedule by removing the condition that leafcutter bees be imported for non-commercial purpose only excluding household pets, and that they can only be imported into a high security facility (not released from it). This now brings the DEH conditions of import for leafcutter bees into line with the AQIS permit conditions (AQIS, 2002). However, prior to any future importations, permits to import leafcutter bees will still have to be obtained from both DEH and AQIS.

6 Conclusions and Recommendations

The objectives of this project were achieved on time and within budget.

The loss of leafcutter bees during this project due to the effects of insecticide sprays used to control pests on crops, shows that more care is needed during the preparation of crops on to which leafcutter bees will be placed. It is recommended that crops on to which leafcutter bees will be placed be treated for pests at least 1-2 weeks before receiving bees and not treated again until at least 4-6 weeks after the last bees have been released.

The three large successful importations of leafcutter bees made during this project have shown that the current quarantine procedures used for importing leafcutter bees are safe and robust enough to deal with very large importations of bees. Such importations will be necessary in the future if a leafcutter bee industry is to become established in Australia. It is now up to the lucerne seed industry to exploit the opportunity provided by this and the previous Stage 1 project and move towards establishing the bee.

Efforts to establish leafcutter bees in Australia to date have been rather ad-hoc. However, if the lucerne seed industry is serious about establishing the bee, greater effort, focus and financial input will be required. During the initial establishment phase, growers will be unlikely to see significant benefits in the form of increased seed yields, and this may be discouraging. However, as bee numbers increase, bees will gradually become available for general pollination use, and growers will begin to see increased seed yields. During the initial establishment phase it will also be necessary to conduct research into best management practices for the bees under Australian conditions. This research will be crucial if the use of leafcutter bees is to succeed.

To move toward establishing the leafcutter bee in Australia it is recommended that the Australian lucerne seed industry develop a detailed business plan for establishing the bee. This plan should clearly state the desired outcomes, the time frames in which those outcomes will be generated, the steps required to achieve the outcomes, the parties that will deliver the outcomes, and the funding required. When developing the plan, crucial decisions will have to be made as to whether bees will be imported through the current CSIRO quarantine facilities, or whether new facilities perhaps closer to the bee release sites be utilized or developed. The development of a peak industry body would assist in lobbying support for the plan.

7 References

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8 Appendix – Bee shelters employed in the project

Top: The leafcutter bee shelter used at the Cowra (NSW) site during the 2003/04 season. Note that the legs of the shelter have been placed in pots of oil to prevent ant access, ties have been placed on the shelter to prevent it tipping over in strong wind, and a strip of wood has been placed along the top of the shelter to prevent rain and direct sunlight form entering. Similar features are built into the shelters used at Keith (SA) by Mr James De Barro (**bottom left**) and by Mr Tony Campbell and Mr Jeff Smith (**bottom right**). Note that the wooden box in the left half of the top shelter contained shelves that were used for placing cells carried over from the previous season, so that bees could emerge from the cells and be sheltered from the weather.