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Native Australian Bees as Potential Pollinators of Lucerne

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by Katja Hogendoorn and Mike Keller

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Foreword

Many rural industries rely either directly or indirectly on the services of pollinators. Bees are among the most important crop pollinators, but the role of Australia's 1600 native bee species in the pollination of crops is largely unknown.

The use, maintenance and enhancement of native bees as crop pollinators can benefit both growers and the society as a whole. Native bees have the potential to increase both the yield of pollination-dependent crops, and their management ensures the resilience of an important ecosystem service. Enhancement of native bee densities can be conducive to more ecologically friendly crop management with consequential benefits for the health of consumers and agro-ecosystems. Native bees can provide continuing pollination service when densities of feral honeybees drop due to the anticipated incursion of the Varroa mite. To ensure these free pollination services, we need to identify the native bee species that are beneficial pollinators of crops and determine how to manage these bees in the crop environment.

This proof-of-concept study assessed the utility of several native bee species for pollination of lucerne and developed strategies to manage these bees in the crop. The key findings include (1) the identification of native species of resin bees that pollinate lucerne in captivity, (2) the development of artificial nesting substrate for these bees, (3) management practices for selected species, and (4) the identification of a common ground nesting bee as an efficient pollinator of lucerne.

This is only a first step towards the development of sustainable management systems for native crop pollinators in Australia. Further studies are needed to develop practical management methods for native bees as a way to ensure pollination of lucerne. We need to assess the performance of bee shelters in attracting and maintaining populations of native bees in lucerne and other crops. We need to investigate the possibilities of working with 'seed populations' of native bees. It will be critical to verify the performance of these bees outside protected cages. And finally, the management of native bees must be integrated with other aspects of crop management, such as the use of systemic pesticides and irrigation techniques. Nevertheless, this study provides a foundation for further development of native bees as reliable pollinators of lucerne.

This project was funded from RIRDC core funds which are provided by the Australian Government, and profited from in-kind support from the Pastures Group of the South Australian Research and Development Institute (SARDI).

This report is an addition to RIRDC's diverse range of over 2000 research publications and it forms part of our pasture seeds R&D program, which aims to maximise opportunities and minimise risks for a profitable and sustainable pasture seeds industry based on reputation for reliable supply, domestically and internationally, for a range of quality pasture species.

Most of RIRDC's publications are available for viewing, free downloading or purchasing online at www.rirdc.gov.au. Purchases can also be made by phoning 1300 634 313.

Craig Burns
Managing Director
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About the Authors

Dr. Katja Hogendoorn is a research associate at the University of Adelaide, with more than 25 years experience in the study of behaviour and ecology of bees, specifically in the context of crop pollination. Her current research focuses on the importance of native bees in agro-ecosystems and for the pollination of native plants. She also investigates how we can manage native bees to enhance their ecosystem services.

Dr Michael Keller leads the Plant Protection Group at the University of Adelaide. His research is focused on beneficial insects that contribute to biological pest control and pollination.

Acknowledgments

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In addition, we are grateful to Dr Remko Leijs (South Australian Museum) for intellectual encouragement and practical support, to Dr Jennifer Gardner (Waite Arboretum) for opportunities and support in building a bee wall in the Arboretum, to Faerlie Bartholomaeus and Arthur Mark for technical assistance. James De Barro donated some alfalfa leafcutter bees used during the first year of the project, and Charlie Hilton allowed us to catch bees on his property. Steve Hughes, Jake Howie, Trevor Wicks and Alan Humphries (SARDI) shared insightful suggestions during the project.

Abbreviations

°C	degrees Celsius
A.	*Amegilla*
L.	*Lipotriches* or *Lasioglossum*
M.	*Megachile*
mm	millimetre
p.a.	per annum
RIRDC	Rural Industries Research and Development Corporation
SA	South Australia
SARDI	South Australian Research and Development Institute
SAM	South Australian Museum
WINC	Waite Insect and Nematode Collection

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Executive Summary

What the report is about

The seed lucerne industry in Australia produces around $AUD 38 million worth of seeds annually. It is generally recognised that improved pollination would increase yield. This report summarises a proof-of-concept study to investigate the efficacy of some common Australian native bee species to pollinate lucerne. Over the course of two years, the project evaluated the utility of some bees for the pollination of lucerne in captivity and in the field, and investigated methods to enhance these bees in the crop environment.

Who is the report targeted at?

This report is relevant to the pasture seeds industry in particular and more generally to growers of crops that profit from bee pollination (e.g. lucerne, canola, cotton, and many horticultural crops).

Where are the relevant industries located in Australia?

About 83% of lucerne seed is produced around Keith, Naracoorte, Tintinara and Bordertown in South Australia, with the remaining produced in smaller areas near Crystal Brook (SA), Forbes, Wagga Wagga and Deniliquin (NSW), Shepparton (VIC), Merriden and Esperance (WA) and in Tasmania. The industry directly employs around 234 people on a full time basis.

Background

Australia produces an average of 7,500 tonnes of lucerne seeds annually (Carter and Heywood 2008). This production depends on pollinators. Although part of the seed lucerne industry relies on feral honeybees for pollination services, many growers pay for honeybee pollination, which costs the industry $600,000 p.a. (Downes 2002). Overseas, several species of solitary bees have been shown to be more efficient pollinators of lucerne than honeybees, and management strategies have been developed for the deployment of these bee species in the crop. One of these species, the alfalfa leafcutter bee, has repeatedly been introduced into Australia, but successful commercial breeding of this species has not been achieved here. Native species are likely to be better adapted to local conditions. Australia has more than 1600 species of native bees. Several of these can pollinate lucerne flowers. Harnessing this potential will benefit both seed lucerne production and the environment.

> ‘Australia has more than 1600 species of native bees. Several of these can pollinate lucerne flowers. Harnessing their potential will benefit both seed lucerne production and the environment.’

Aims/objectives

The overarching objective of this study was to supply the seed lucerne industry with methods that allow the enhancement of native bees that pollinate lucerne. The two specific aims of the project were to identify common native bee species that are useful for pollination of lucerne, and to investigate nesting structures that allow useful native bees to nest in or adjacent to the cropping area.

Methods use

Replicated pollination trials in enclosed tunnels were used to evaluate the efficacy of a blue-banded bee and two species of resin bee for the pollination of lucerne. Their efficacy was compared to that of the alfalfa leafcutter bee. Nesting walls containing various nesting substrates for native bees were designed and built, and their use was monitored. Native bee species that visited open pollinated lucerne crops were caught and identified. For one species, the flower tripping frequency was assessed and compared to that of honeybees.

Results/key findings

With a set of 48%, the common native resin bee *Megachile nigrovittata* is an adequate pollinator of lucerne in captivity. By contrast, blue-banded bees and another resin bee species turned out to be ineffective in pollinating lucerne.

> ‘With a set of 48%, the common native resin bee *Megachile nigrovittata* is an adequate pollinator of lucerne.’

The nesting substrate developed in this project was used by resin bees and leafcutter bees and in the second year, more than 100 nests had been provisioned at a single location. However, successful breeding requires protecting the nests from ants. Furthermore, winter management of resin bee nests is needed to prevent the build-up of parasitic wasps. Paper nesting substrates were shown to be unsuitable for resin bees. Solid wood, bamboo and waxed paper straws are suitable substrates.

Small, ground nesting nomiine bees were identified as a locally important pollinator of lucerne. These bees tripped 32 times more flowers per minute than honeybees.

> ‘Small, ground nesting nomiine bees tripped 32 times more flowers per minute than honeybees.’

Implications for relevant stakeholders

The identification of native pollinators of seed pasture crops, and the development of nesting substrate for useful pollinators, can help growers of pasture seeds to increase free pollination services. This is important per se, but should be more so when feral honeybees are inevitably eliminated after the anticipated foreseen incursion of the Varroa mite.

The development of nesting substrate and management techniques for native bees allows the promotion and exploitation of native pollinators in agro-ecosystems. This will increase resilience by reducing the reliance on a single pollinating species, and will lead to more environmentally friendly crop management. This will benefit producers of pollination-dependent crops in general and the community as a whole.

> ‘Promotion of native pollinators in agro-ecosystems will increase resilience and will lead to more environmentally friendly crop management.’

This report may serve as an eye-opener for policy-makers, as they are often unaware of the existence of native bees, or do not realise that these insects contribute to agricultural and horticultural production. Thoughtful management and regulation can enhance this contribution.

Recommendations

For growers of pollination-dependent crops, including pasture seed crops, the provision of cheap nesting substrate around paddocks has the potential to enhance densities of native resin bees on the crops. This will only prove successful if two conditions are met. Firstly, the crop needs to be managed in ways that allows the continued presence of these bees. Secondly, the nesting sites will need to be maintained to achieve parasite and predator control and allow population build-up of these native species. The compatibility of lucerne growing practises with native bees, the choice of nesting substrates and bee shelters, and the strategies for maintenance of healthy populations require further investigation.

Introduction

Solitary bees in Australia

Australia has 1600 described and nearly as many undescribed solitary native bee species (Batley and Hogendoorn 2009). They range in body length between 1.5 mm and 25 mm, and can nest in the soil, mudbrick walls, stems and hollows and solid wood. Many native plants depend on solitary native bees for seed production and propagation.

Solitary bees and crop pollination

Solitary bees can play a substantial part in pollinating crops, in particular when on and off farm conditions are conducive to their presence (e.g. Delaplane and Mayer 2000, Blanche et al. 2006, Kremen et al. 2007, Winfree et al. 2007, Ricketts et al. 2008). In addition to free ecosystem services, overseas practices have shown that solitary bees can be managed for use as crop pollinators. Examples include the leafcutter bee *Megachile rotundata* (Richards 1984) and the ground-nesting halictine bees *Nomia melanderi* (Cane 2008) and *Rhophitoides canus* (Ptacek 1989, cited in Bosch and Kemp 2002) for the pollination of lucerne; *Osmia* species for the pollination of cherries, apple and almonds (e.g. Bosch and Kemp 2001, 2002), and carpenter bees (*Xylocopa*) for passionfruit pollination (Freitas and Oliveira Filho 2003). The management of these bees has been developed because their pollination gives better returns than using honeybees or unmanaged pollination. In Australia, no solitary bees are as yet used in crop pollination.

Reliance on a single pollinating species is inadvisable

Now is an important time to investigate the potential and importance of native bees for crop pollination. The imminent incursion of the honeybee mite *Varroa destructor* poses a substantial threat to Australian horticulture (Keogh et al. 2010). The mite will devastate feral honeybee populations (Kraus and Page 1995), thereby eliminating the free pollination services they provide (Keogh et al. 2010). This will cause a substantial rise in prices of pollinator-dependent fruit and vegetables (Gordon and Davis 2003, Cook et al. 2007), due to a shortage of commercial honeybee hives and increased management costs. Examples of crops that depend for more than 90% of production on bee pollination, and are likely to be affected are almond, apple, blueberry, cherry, lucerne, pumpkin, rockmelon, watermelon, white clover, sunflower and zucchini (Keogh et al. 2010).

As opposed to the reliance on a single pollinating species, diversification of crop pollinators can ensure sustainable production of horticultural crops in the future (Batra 1994). In addition, because native bees will not be affected by the Varroa mite, their role in crop pollination will become relatively more important after the loss of free pollination by feral honeybees, in particular for those crops that have a large pollinator reliance (e.g. Cunningham et al. 2002). Thus, we need to know which native bees are suitable candidates for the pollination of our crops, and how to manage and increase their pollination services.

The pollination of lucerne

Lucerne is a pollination-dependent crop. Australia produces around $AUD 38 million worth of lucerne seeds annually (estimate from 2008, Carter and Heywood 2008). The production per hectare is low compared to the US, and inadequate pollination is thought to be a major factors limiting productivity in Australia (Doull 1961, Morthorpe et al. 1990, Downes 2002, Bitner and Peterson 2003). Lucerne florets require tripping to produce pods and seeds and although some tripping of florets happens automatically, more than 90% occurs through visitation by bees (Cane 2002, Downes 2002).

Honeybees as pollinators of lucerne

Lucerne pollen has a low nutritious value (Standifer 1967), so when there is an abundance of other pollen sources, honeybees will not collect pollen on lucerne (Vansell and Todd 1946, Hobbs and Lilly 1955, Stephen 1955). In the lucerne growing areas in south east Australia, flowering Eucalyptus species provide such a distraction (Doull 1961). While collecting nectar from lucerne, honeybees trip only 1 – 2% of the florets they visit (Tysdal 1940, Reinhardt 1952, Bohart 1957). Therefore, recommended stocking rates of honeybee hives on lucerne are relatively high (Somerville 2002).

The costs of the placements of managed hives are considerable. In 2000, lucerne seed growers spent $600,000 to hire honeybees for pollination (Downes 2002). The economy of this expense has been questioned (Downes 2002), on the basis of the absence of a correlation between the density of managed hives and pod set (Maelzer and Pinnock 1983, Downes 2002). It has been suggested that the absence of such a correlation was a consequence of pollination services performed by feral honeybees in proximity of the paddocks (Maelzer and Pinnock 1983, Downes 2002). A high reliance of the industry on feral honeybees is further suggested by the substantially lower stocking rates recommended for Australia (3-5 hives per hectare Somerville 2002) than for the USA (7.9 hives per hectare Delaplane and Mayer 2000). If this high reliance on feral honeybees bears out for the Australian seed lucerne industry, then this implies that a future loss of feral honeybees could have a high impact on its productivity.

Solitary bees as pollinators of lucerne

In the USA, the alfalfa leafcutter bee (*Megachile rotundata*) and the alkali bee (*Nomia melanderi*) are widely used (e.g. Free 1993), and in the Czech republic, the ground nesting *Rhophitoides canus* (Ptacek 1989, cited in Bosch and Kemp 2002) is managed for the pollination of lucerne. These solitary bee species are more efficient pollinators than honeybees (Bohart 1957, Bohart 1972, Batra 1976, Delaplane and Mayer 2000), because they trip a large number of the flowers they visit (Bohart 1972, Woodward 1996).

Attempts to introduce the alfalfa leafcutter bee into Australia

Out of these species, the alfalfa leafcutter bee is the easiest to manage and transport in large numbers, and therefore several attempts have been made to introduce and breed *M. rotundata* in Australia (Bitner and Peterson 2003, Anderson 2006). However, despite considerable investments and overcoming several problems (Anderson 2006), the development of a breeding program has so far not been commercially successful. A variety of explanations are given for this lack of success, including high parasitisation rates by the parasitoid wasp *Melittobia australica* (Woodward 1994), predation of nests by ants (Woodward 1996), consumption of overwintering pupae by mice (Bitner and Peterson 2003), severe storms during the critical early nesting period of the bees, cool weather during foraging, hot weather during foraging, and incompatible use of insecticides (Anderson 2006, Manser pers. com). Considering the fact that these bees originate from central Europe and are much more successfully bred and used in Canada than in California (Parker and Tepedino 1982) it seems possible that the climate is not the most suitable for breeding this bee species where lucerne is grown in Australia.

Does Australia have native solitary bees that can pollinate lucerne?

Australian native bee species are an alternative to imported bees for pollination of lucerne, as they are adapted to the local climate. To date, investigations into the potential use of native Australian species for lucerne pollination have been limited to cage experiments using six megachilid bees (Bray 1973). Although not quantified in any way that allows comparisons to be made, all six species turned out to be efficient pollinators of lucerne in captivity (Bray 1973). However, no follow-up of Bray's study has so far occurred due to a lack of suitable techniques for the breeding and management of these bees, which precluded providing bee populations at the right time of crop flowering, and high parasitisation rates by an unidentified wasp (Bray 1973).

Recent experience with the breeding of Australian native bees provides a timely opportunity to re-visit their use in lucerne pollination. A breeding program has been developed for a ground nesting blue-banded bee (*Amegilla murrayensis*) at the University of Adelaide (Hogendoorn et al. 2006, Hogendoorn et al. 2007, Hogendoorn et al. 2010). Blue-banded bees are generalist species that visit both native and introduced plants (Batley and Hogendoorn 2009). They are adapted to the Australian environment and they actively provision nests in the period that the lucerne is in flower. In the main lucerne cropping areas, they are found from mid-late spring until early autumn (Leijs et al in preparation). Blue-banded bees have been reported to visit a suite of vegetable and seed crops including tomato (Batley and Hogendoorn 2009), eggplant (Hogendoorn pers. obs.), zucchini (Hannah pers. com.), pumpkin (Altmann pers. com.) and lucerne (De Barro pers. com.). Furthermore, preliminary data collected from small cages on the Waite Campus of the University of Adelaide in 2007 indicated that blue-banded were efficient pollinators of lucerne (Hogendoorn and Powell, pers. obs.).

More recently, some expertise has been obtained with attracting native *Megachile* bees to artificial nesting substrate, and with winter management of parasites (Hogendoorn 2011, Hogendoorn et al submitted). As these species belong to the same genus as *M. rotundata*, and six elated native bees are efficient pollinators of lucerne in captivity (Bray 1973), these bees could have potential for the pollination of lucerne.

Because the utility of native bee species critically depends on our ability to maintain and enhance their numbers on the crop, the focus of this study was to investigate (1) whether blue-banded bees and resin bees are useful as pollinators of lucerne, and (2) the nesting substrates and management strategies required for maintaining and enhancing native bee populations of useful species at lucerne crops.

Objectives

The overarching objective of this project was to contribute to the development of improved, sustainable pollination services for the pasture seed industry by investigating the potential of native bee species for the pollination of lucerne, and by exploring ways to harness this potential.

Specific project aims were twofold:

(1) To investigate the ability of blue-banded bees (*Amegilla*, Anthophorini), and of resin bees (*Megachile*, Megachilini) to pollinate lucerne in captivity.

(2) To develop permanent nesting structures for the management of useful bee species that allow enhancements of the presence of these bees in the crop environments, and to investigate the management strategies needed to maintain these bee populations.

Methods

Experiments in captivity: can blue-banded and resin bees pollinate lucerne?

To investigate the bees’ ability to pollinate lucerne, replicated experiments were performed in greenhouse tunnels that contained individual bee species. The pollination efficacy was quantified as percentage pod set (Doull 1961) and evaluated against a negative control, i.e. no pollination, and against a positive control, i.e. pollination with the alfalfa leafcutter bee, *Megachile rotundata*.
A total of three replicated experiments were performed to establish the efficacy of the blue-banded bee *Amegilla murrayensis*, and of the resin bees *M. nigrovittata* and *M. erythropyga*.

Plants

A total of 180 lucerne plants were grown in enclosed greenhouses from single batches of scarified lucerne seed (“SARDI 5”). The plants were grown in University of California potting mix in pots of diameter 230mm. The plants were watered by hand every other day and fertilized using liquid fertilizer every two weeks. A total of 60 plants were used per experiment. By growing plants under artificial light, and cutting them back whenever appropriate, the flowering time of the crop could be manipulated to coincide with the activity of the bees. Furthermore, five plants open pollinated plants were monitored.

Tunnels and nesting substrate

Just before the onset of flowering, 60 plants were moved into three experimental flight tunnels (6 m x 4 m), which were covered by thrips netting. Each tunnel was assigned to a control or experimental treatment. Plants were moved into the control and experimental tunnels simultaneously, 20 plants per tunnel. Here, they were watered automatically once a day for 10 minutes using drip irrigation. The tunnels were further supplied with suitable nesting substrate for the different bee species. Nesting substrates used were i.e. unlined Binderboards™ (www.pollinatorparadise.com) for leafcutter and resin bees, and clay blocks (40x40x100mm) for blue-banded bees (Hogendoorn et al. 2007). Sugar water feeders containing a 30% sugar solution (Hogendoorn et al. 2007) were placed in the tunnels, to ensure sufficient carbohydrates. The feeders were replaced once a week, or more often during periods of hot weather (>38 °C). Both nests and feeders were protected from ants by using ant-excluding polybutene (Tanglefoot®, Contech Enterprises, Victoria BC, Canada) on the stands.

Bees

Blue-banded bees
Fresh, inexperienced blue-banded bees were harvested from a breeding culture at The University of Adelaide and raised to adulthood in incubators according to existing protocols (Hogendoorn et al. 2006, Hogendoorn et al. 2007) The newly eclosed bees were introduced in the tunnels after the crops had started flowering. Eleven bees were introduced (4 males and 7 females).
To check whether motivation to collect pollen influenced visitation and flower tripping frequency, we placed 10 lucerne plants in a greenhouse that contained 5 actively nesting females. The bees in this greenhouse suffered from a shortage of pollen, due to the fact that these bees can work many more plants than can be provided in the space available (Hogendoorn et al. 2007, Hogendoorn and Mark pers. obs). Hence, it was assumed that, compared to the females in the tunnels that were not provisioning cells, the females in this area would be more motivated to collect pollen and trip flowers.

Resin bees

Native resin bees (*M. erythropyga* and *M. nigrovittata*) were obtained from nesting substrate (Hogendoorn et al. 2011) that had been placed at various locations around Adelaide, SA in November 2010. The nests of the resin bees (5 nests of *M. nigrovittata* and 7 of *M. erythropyga*) were harvested in October 2011. One nest of each species was opened, the contents were recorded, and the prepupae were reared in wax cups. The remaining nests and the larvae were placed in cool conditions (14 °C) at 70% humidity, in incubators until early December. After this, incubators ran at a daily cycle of 16h at 25 °C and 8h at 18 °C. The development of the brood was checked weekly.

Early January 2012, the first males eclosed, and the nests were moved into separate greenhouse tunnels, one for *M. nigrovittata*, and another for *M. erythropyga*. The *M. nigrovittata* tunnel had only five bees (two females and 3 males), while the *M. erythropyga* tunnel contained 9 bees. The bees remained active for a period of approximately 5 weeks.

Controls: no pollination and alfalfa leafcutter bees

Two controls were used: a negative control (no pollinators present) and a positive control (pollination by the alfalfa leafcutter bees). To achieve the positive control, 40 cocoons of alfalfa leafcutter bees, donated by J. de Barro (Keith SA) from his personal breeding stock in December 2010. The cocoons were placed in incubators at 27 °C. Eclosion of young adults was checked daily. As soon as the first males had emerged, the remaining cocoons were placed in individual nesting holes in a binderboard, which was then positioned in a greenhouse tunnel on 24 December 2010. At that stage, the lucerne plants in the tunnels were flowering. A total of 15 males and 10 females eclosed. Five females started to provision nests and produced 13 brood cells.

Unfortunately, no leafcutter cocoons were available for the project in the following year. It was therefore decided to rely on the positive control data from the first year, and to use the freed tunnel space to evaluate the performance of two rather than one native resin bee species. The rationale for this was that the experimental design was the same in the two years, and that the information about an additional native bee species would be of more value to the industry than a repeat of the positive control.

Behavioural observations

In all bee tunnels, flower visitation was recorded weekly. Noted was whether the bees visited and tripped flowers, whether they collected nectar and/or pollen and visited sugar water feeders. Furthermore, the nesting behaviour of females was recorded. Because both the blue-banded and resin bees seemed to prefer the feeders over flowers, the feeders were removed between 10 AM and 4 PM, three days a week.

Pod set

After pollination, plants were left to grow until the pods started to turn brown. Following Doull (1961), set was quantified as % of pods set per raceme, for 200 racemes. Pod set was assessed on randomly selected racemes on randomly selected branches. For each raceme the number of pods was divided by the number of stipules per raceme. Ten racemes were counted per plant per treatment in the tunnels, but 20 racemes per plant were counted in the *Amegilla* trial in the greenhouse, and in the plants near the Waite bee wall.

Before statistical tests, the data on % pod set were arcsine transformed to meet distributional requirements for the performance of parametric statistics. For each treatment (i.e. blue-banded bees with and without alternatives, *M. nigrovittata*, *M. erythropyga*, positive control, negative control), the set in the replicates were compared using *t*-tests. As the replicates did not differ for any of the treatments, the results were combined.

Overall comparison between treatments was performed using a non-parametric Kruskall-Wallis test, as homogeny of variances could not be achieved. Subsequent post-hoc comparison of set per treatment were done using the arcsine transformed set data with Bonferroni correction for multiple comparisons.

Development of permanent nesting structures for native bees

Bee nesting walls were designed and built in October 2010 at two locations. The first location was the Waite Arboretum of the University of Adelaide, at the Waite Campus, Urrbrae South Australia, in the proximity of the main area used by the SARDI Pastures Group. The second location was at a private property in Eden Hills SA. Many flowering plants were available at both locations. The Waite wall was built in the Eucalyptus section of the Waite Arboretum that harbours many species of *Eucalyptus* and *Acacia* and borders on the area where pasture crops (cullen, lucerne, lotus and clovers) are grown. The private property at Eden Hills is maintained to accommodate native bees, and has a large variety of local native plant species that have been selected based on their attractiveness to native bees.

The walls were built using large Besser blocks (190 x 190 x 390 mm) that were filled with clay (Fig. 3). They faced NE and received morning sun but were shaded in the afternoon. The rear of each wall was backfilled with soil, to provide a more stable temperature to the nests. Between the clay blocks and the back-fill, a layer of high quality shade cloth was placed to prevent large soil insects from digging through the wall.

Each wall contained 20 clay blocks, each with between 10 and 20 nesting holes of 6 – 8 mm in diameter and at least 80 mm in length.

In spring of 2010, the walls were seeded with nests of *Amegilla murrayensis,* derived from a combination of nests sourced from the breeding culture at the University of Adelaide, and from a natural population near Orroroo SA. This was done so that so young, inexperienced bees would emerge directly from their maternal nest in the walls. Female *Amegilla* often nests in aggregations which arise due to the fact that females nest near their place of emergence ('philopatry', Cardale 1968).

To investigate the importance of nesting substrate for resin and leafcutter bees, a small wooden Binderboard ("leafcutter board" Pollinator Paradise, Idaho, USA) with 130 holes (100 mm long with 5.5 mm holes), was placed in the Waite wall in November 2011. The holes in this board were lined with waxed paper straws that were made to fit. In addition, waxed paper straws (n = 40) were placed into some of the holes prepared in the clay blocks (Fig. 8).

The presence of bees and nest closures was monitored every two weeks between October and May. This was done by watching visitation to the wall for approximately 30 minutes, recording the bee species that were either returning to the nests with pollen or closing nests, and identifying the type of closure of all nests. Distinguished were: closures by small hyleaine bees (cellophane closures), resin bees (closures with resin, sometimes adorned with wood, clay, leaf bits or plant pulp), leafcutter bees (closures with leaf bits; Fig. 5).

Opening of nests

To check the suitability of the nesting substrate and the number of offspring produced in the nests, some of the used nest blocks were opened in October 2011(8 leafcutter bee nest and one resin bee nest), and early May 2012 (7 leafcutter and 27 resin bee nests). For the nests in clay, this was done by carefully chipping away the substrates from the sides of the blocks using a hammer and chisel, until a nest was reached. The nest contents of resin bees in paper tubes were first recorded by shining a light through the paper tube and recoding cell lengths. Subsequently, the paper was gently peeled off in a spiral fashion and the cell contents were documented. The larvae of evanoid wasps were readily recognised on the basis of their mandibles and general high mobility (Michener 1953). In 2011, the bee brood was kept at room temperature until 21 November 2011, and then moved into an incubator at settings described above.

Assessment of bee visitors of lucerne

Lucerne growing in experimental paddocks on the Waite Campus and Urrbrae High School were inspected for bee visitation between early December and end January, approximately fortnightly, whenever the weather allowed the bees to be active. Bees were caught using either a hand-net or an aspirator. Furthermore, the lucerne plants in the vicinity of the bee walls were inspected every two weeks for bee visitation. Here, visiting bees were not caught as this could affect the occupancy of the bee wall.

At the Waite Campus, the efficacy of a small ground nesting bee, *Lipotriches flavoviridis* (Nomiini) in tripping lucerne was assessed and compared to that of honeybees on three sunny mornings after 10 AM between 23and 30 December 2011, when temperatures were between 27 and 33 °C. We used focal animal observations with ad libitum sampling to quantify the number of flowers visited and tripped per unit of time. Bees were selected randomly, and their behaviour was recorded using a handheld event recorder. The following behavioural elements were recorded: Landing on an untripped flower, tripping the flower, and pollen collection. When the track of the focal bee had been lost, another bee was chosen. Over the course of 7 hours of observation, a total of 844 flower visits by 71 bees were observed, 503 visits by 23 workers of *A. mellifera* and 341 visits by 48 females of *L. flavoviridis*. *The Observer*® software (Noldus 1991) was used for scoring and analysis of these data. The number of flowers tripped was analysed per visit, and per unit of time, and comparisons between the species were made of the basis of averages per female, using t-tests.

In addition, the lucerne flowering crop of C. Hilton at Bordertown was visited on 27 December 2011 to inspect the activity of native bees in and around the crop. The property was chosen because the farmer is known for his limited and considerate use of insecticides. Bees were caught using blue vane traps (Stephen and Rao 2005), and by hand net both on the crops and on flowering weeds and native plants surrounding the crops.

All bees collected during the project were killed by freezing, pinned and identified to the species level whenever possible, by comparison to reliably identified specimens at the South Australian Museum and Waite Insect and Nematode Collection, and/or by using the following publications: (Michener 2000) to identify bees to the genus level, Halictini: *Lipotriches* (http://www.padil.gov.au/pollinators/), *Homalictus* (Walker 1986), *Chilalictus* (Walker 1995), Apini: *Amegilla* (Apini; Leijs et al. in prep), Megachilini (King 1994).

Results

Experiments in captivity: which native bees can pollinate lucerne?

Overall, there was a significant difference between the treatments in the percentage pod-set (Fig. 1; Kruskall Wallis test, P < 0.001). There was no difference in the percentage pod set between the following pollination treatments: “no pollination”, blue-banded bees with (*Amegilla* 1) and without (*Amegilla* 2) alternative food plants, and *M. erythropyga* (Figure 1, Table 1). In addition, the pod set did not differ between open pollinated lucerne and the lucerne pollinated in the tunnels by *M. nigrovittata* (Table 1). The highest pod-set was achieved by *M. rotundata* in tunnels (Fig. 1).

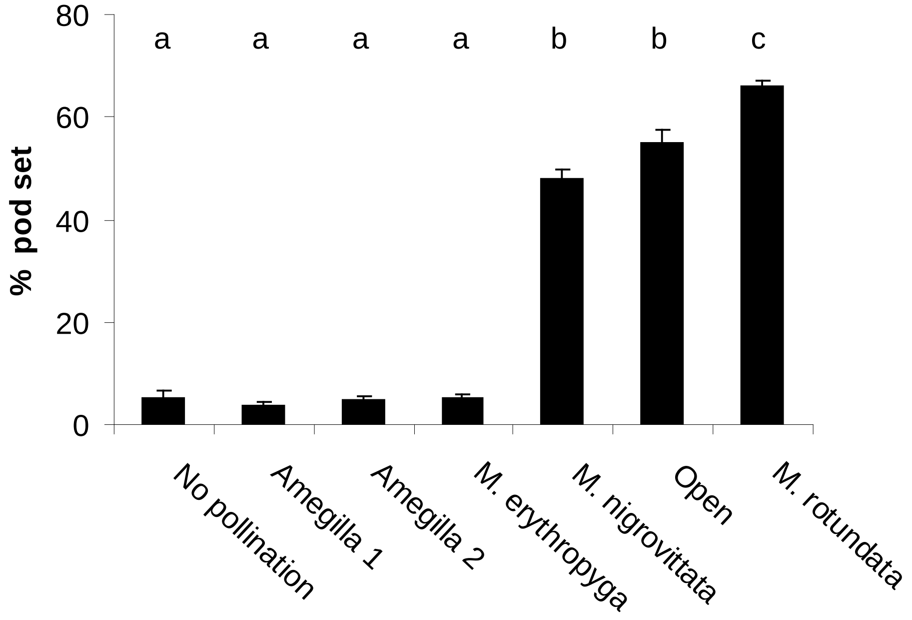

Figure 1. The average number of pods per flower per raceme for the different pollination treatments (n = 200 racemes per treatment). *Amegilla* 1 is in the presence, *Amegilla* 2 in the absence of alternative food plants. Significant differences are indicated by ‘a’, ‘b’, and ‘c’.

Table 1. The significance* of differences in pod set between different pollination treatments.

treatment	1	2	3	4	5	6
1. No pollination						
2. *Amegilla* 1	n.s.					
3. *Amegilla* 2	n.s.	n.s.				
4. *M. erythropyga*	n.s.	n.s.	n.s.			
5. *M. nigrovittata*	**<0.01**	**<0.01**	**<0.01**	**<0.01**		
6. Open near bee wall	**<0.01**	**<0.01**	**<0.01**	**<0.01**	n.s.	
7 *M. rotundata*	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**	**<0.01**

*Post-hoc comparisons were made using t-tests with Bonferroni correction.

Blue-banded bees do not pollinate lucerne

The set in the tunnels and in the greenhouse with blue-banded bees was below 5% (Fig. 1). However, this was not because the bees did not visit the flowers. In the tunnel without alternative forage, blue-banded bees were regularly observed to visit the flowers and take nectar (Fig. 2c). However, during visitation, the bees did not trip the flowers. Comparison of the behaviour of alfalfa leafcutter and blue-banded bees showed that while the leafcutter bees use their heads to prize the flowers open (Fig. 2a, thereby tripping the flowers, the blue-banded bees used their long tongues (Fig. 2b) to reach the nectar, and could do so without tripping the flowers. Moreover, the blue-banded bees often approached the flowers from the top (Fig. 2c), i.e. landing on the flag rather than on the keel. Approaching the flower from that side may have further reduced tripping events.


(a) (b) (c)

Figure 2. The alfalfa leafcutter bee (*Megachile rotundata*) lands on the keel and uses its head to prize the flower open (a), tripping it in the process. The blue-banded bee (*Amegilla murrayensis*) can use its long tongue (b) to reach the nectar without tripping the flower, and often lands on the standard or flag (c).

The bees remained active for 5 weeks. As none of the females in the tunnel without alternative food started to nest, they did not actively collect pollen. To check whether this affected the propensity to trip flowers, lucerne plants were placed in a greenhouse compartment that contained actively nesting females and alternative food plants. Despite the obvious shortage of pollen in this arena (pers. obs. K. Hogendoorn and A. Mark), the actively nesting females did not collect pollen from the lucerne plants. In fact, in the presence of alternative nectar sources, they did not even visit the lucerne.

It is clear that *A. murrayensis* is not a pollinator of lucerne.

Some native resin bee species can be adequate pollinators of lucerne

The results for the resin bees were variable. While *M. nigrovittata* was an adequate pollinator of lucerne in tunnels, with a set of 48 ± 2 % (Fig. 1), the plants in the tunnel with *M. erythropyga* had very low pod set (5 ± 1%). *M. erythropyga* did not visit the lucerne flowers at all. Even when the sugar water feeders were removed for more than two hours, and the bees where clearly hungry, as judged from their increasing attempt to escape from the cage and from the fact that they jostled each other for access to the feeders after they were returned (Hogendoorn pers. obs), the adult *M. erythropyga* did not visit the lucerne flowers. The bees were active for six weeks, and none of the females started to actively provision cells.

Thus, the native bee *Megachile nigrovittata* seems to be a suitable pollinator of lucerne, but not *M. erythropyga*.

Permanent nesting structures for native pollinators of lucerne

Figure 3. Nesting wall for native bees built at the Waite Arboretum in October 2010. The irregularly shaped blocks are parts of natural blue-banded bee aggregations that were used to seed the walls.

Blue- banded bees

At the Waite wall (Fig. 3), the first female *A. murrayensis* was seen to enter the nesting block in October 2010. On 19 November 2010, two females of *A. (Notomegilla) chlorocyanea* and one *A.murrayensis* were actively nesting in the wall. Fresh diggings, indicative of nesting activity, were found under three nesting holes, and females were observed entering the nests with pollen on their legs. However, on 25 November, all activity had ceased. After this initial success, no more blue-banded bees were seen in this nesting wall. In the wall in Eden Hills, no blue-banded bees were observed at any time. Thus, the bee walls were not successful in promoting blue-banded bees.

Resin and leafcutter bees

Observations

Unexpectedly, both nesting walls were used by leafcutter bees and resin bees (Megachilini, Fig. 4). In the Waite wall, the first leafcutter and resin bees were observed on 9 December 2010, and the first nest entrance was closed five days later. At the end of the 2010/2011 season (April 2011), the Waite wall contained 22 closed and 4 open leafcutter nests and 6 closed and 1 open resin bee nests (Fig. 4). Eight of the leafcutter nests and one the resin bee nests were opened, while 14 closed leafcutter and 5 closed resin bee nests were left in place for emergence of bees in the next season.

On 6 December 2012, renewed activity of resin (Fig. 4) and leafcutter bees was observed at both walls. At the Waite wall, bees were seen during every subsequent visit until the end of March.

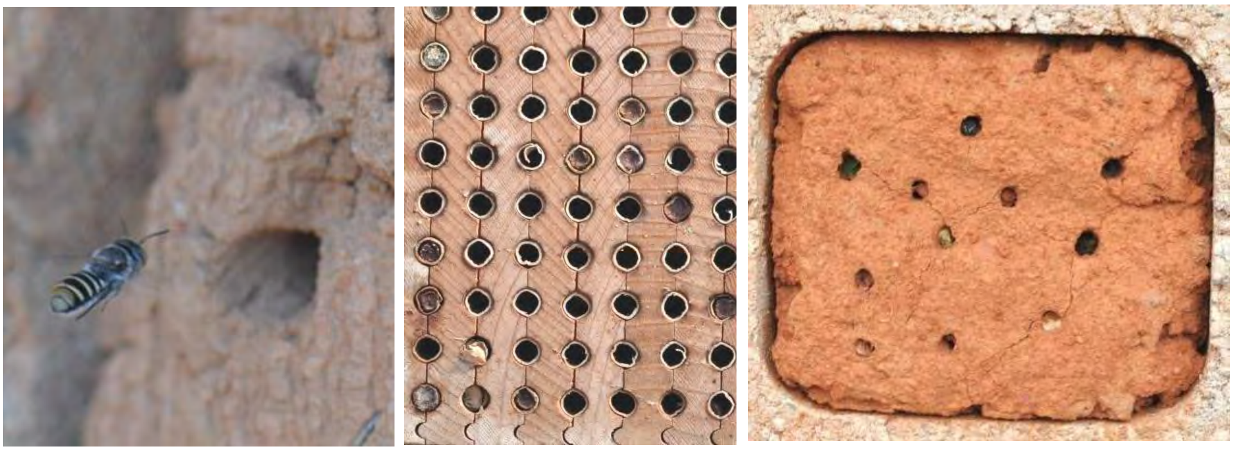
(a) (b) (c)

Figure 4. (a) A resin bee (*M. nigrovittata*) returning to a nest in a bee-wall; (b) detail of a binder board showing 12 resin bee nest closures in waxed tubes and two leafcutter bee closures; (c) a clay block showing nests closed by leafcutter bees.

After the end of the active season of 2011/12 (early May 2012), a total of 51 nests of leafcutter bees had been provisioned in the Waite bee wall. Of these, 95% were in clay blocks, and the remainder in two holes in the binder board that lacked waxed tubing. No leafcutter bee nests were found in the waxed paper tubes. The resin bees had provisioned a total of 52 nests, 71% of which were in waxed paper tubes, 29% in clay blocks. Furthermore, there were 5 nests of masked bees (Hylaeini) in clay. The lower nests got flooded between 6 and 19 March 2012 after a new ditch, dug by the maintenance staff from the Waite Arboretum to deal with water on the path, overflowed during torrential rains.

Thus, over the year, there was a substantial increase in numbers of nest provisioned in the Waite wall. For the leafcutter bee nests, this was an increase of 214% compared to the previous year, and for the resin bees, the increase in the number of nests provisioned was 750%. There is no doubt that the latter increase is due to the provision of waxed paper tubes as nesting material.

The resin bee species nesting in the bee wall were identified as *Megachile erythropyga* and *M. nigrovittata*, and the leafcutter bee species was *Megachile (Eutricharea) quinquelineata*.


(a) (b) (c) (d) (e)

Figure 5. The nest closures of the bees using the nesting walls. (a) Cellophane fibres made by the masked bee *Hyleoides concinna*; (b) Leafcutter bee nests of *M. quinquelineata*, both closed and raided by ants (lower right); (c) Closed leafcutter (up) and resin bee (down) nests in a binderboard; (d) Nests of a masked bee (*Hylaeus* sp.) after emergence of the offspring; (e) Nest closure of plant pulp and resin made by *M. aurifrons*

The nesting wall at the Waite arboretum had been used at a higher frequency that the one at Eden Hills where only 4 resin bees nests were observed each year, and one leafcutter bee nest in the second year. This is most likely caused by the presence of alternative nesting substrate for resin bees at this location, which will be discussed below.

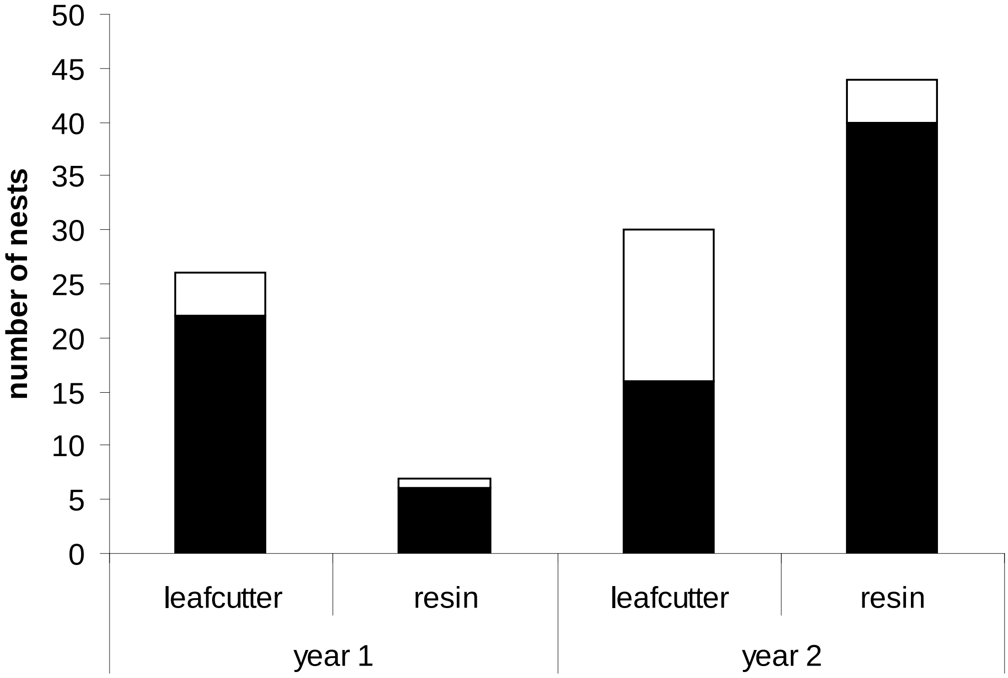

Figure 6. The number of closed and open nests of resin and leafcutter bees in the Waite bee wall at the end of the breeding season. In the second year, the large fraction of open leafcutter bee nests was caused by ant raids. The differences in the numbers of resin bees between the years reflect the addition of waxed straws to the nesting wall in the second year.

Several observations support the notion that predators and parasites are important factors in the success of these nests. Firstly, the nesting walls were not protected from ants. Ants were often observed walking on the nesting blocks and entering the nests. A large ant raid of the leafcutter nests by workers of a tiny *Monomorium* species was observed on 13 January at the Waite bee wall. The tiny ants bit their way into the leaf closures, and this led to a large number of irregularly opened nests for the leafcutter bees (Fig. 5b, Fig. 6), the increase in number of nests was low from then onwards, despite the continued presence of the leafcutter bees (Fig. 7). The resin bee nests seemed to be better protected from ants, which were only observed to enter active nests. Secondly, activity of both chrysidid and evanoid wasps were observed on the bee walls on numerous occasions and cells containing parasitised bee brood were found upon opening the nests (see below). Both parasites were observed to enter active nests, but they were not seen to parasitise closed nests.

Breeding success in nesting walls

To investigate the breeding success and effects from the presence of ants and parasites, every year a number of nests were opened.

In October 2011, 9 of the 28 closed overwintered nests were opened, one out of 6 resin bee nests, and 8 out of 22 leafcutter nests. The resin bee nest contained two cells, one with remnants of pollen and Psocoptera or book lice, which are consumers of debris and often found in cells containing dead bee larvae. The other cell contained a healthy prepupa inside a cocoon (Fig. 8a). The 8 leafcutter bee nests contained on average 2.6 cells. Healthy cocoons (Fig. 8c) were found in 12 out of 21 cells. Of the remaining 9 cells, 2 were empty, 4 cells had small holes, presumably as a result of parasitisation by a wasp (Woodward 1994), and three appeared to have suffered from fungal infestations. From seven of the healthy looking cocoons, adult *M. quinquelineata* (4 male, 3 female) emerged. The remaining cocoons suffered from a fungal infection in the incubator at later stage of development.

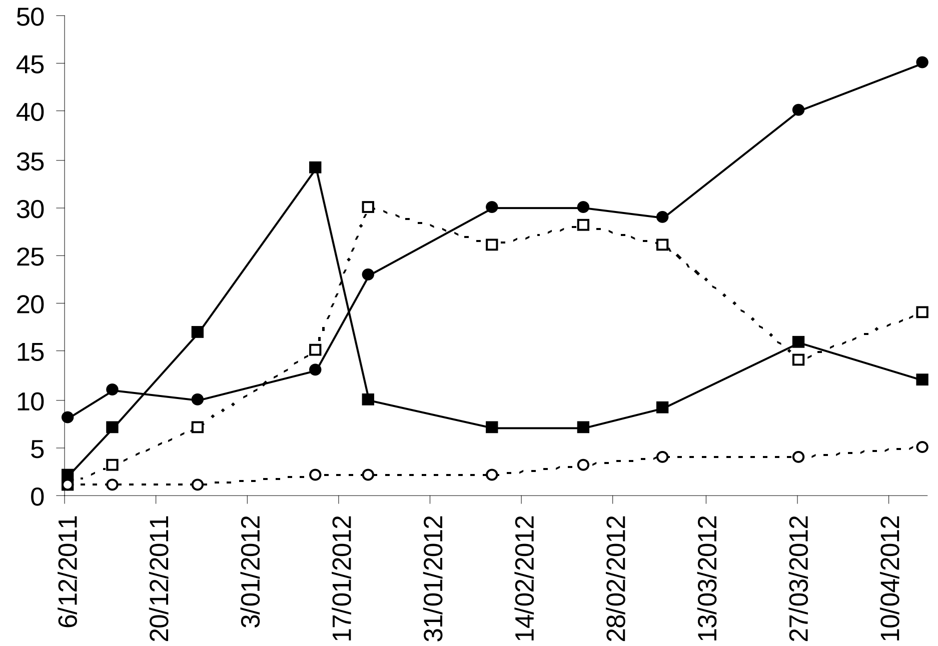

Figure 7. **The presence of open (dotted lines, open symbols) and closed (continuous lines, closed symbols) of leafcutter nests (squares) and resin bee nests (circles) at the Waite bee wall.**

In early May 2012, a total of 34 closed nests were opened, 27 out of 40 resin bee nests, and 7 out of 15 leafcutter bee nests.

Of the 27 resin bee nests, 24 were in waxed straws, and 3 were in clay blocks. The nests contained on average 1.2 ± 0.2 cells, but 22% of nests (8 out of 27) did not contain any brood. These nests had been closed with resin, and did not demonstrate any remnants of pollen. Parasitisation was evident in three resin bee nests. In one nest a single cocoon had been parasitised by a large number of wasp larvae, possibly *Melittobia australica* (Eulophidae, Fig. 8e), and in two nests larvae of evanoid wasps were found. The 27 resin bee nests contained 29 cells with viable bee brood.

The leafcutter bee nests (n = 7) contained on average 3.1 cells (± 0.9) with a range of between 0 and 6 (Fig. 8c). Out of the 7 nests, only one did not contain any brood cells. However, in four other nests, only empty cocoons were found. Some of these empty cocoons had small holes and had either been parasitised by eulophid wasps or had been raided by ants. I one nest, the cocoons were opened from front. Thus, the 7 nests had a total of 7 viable cells.

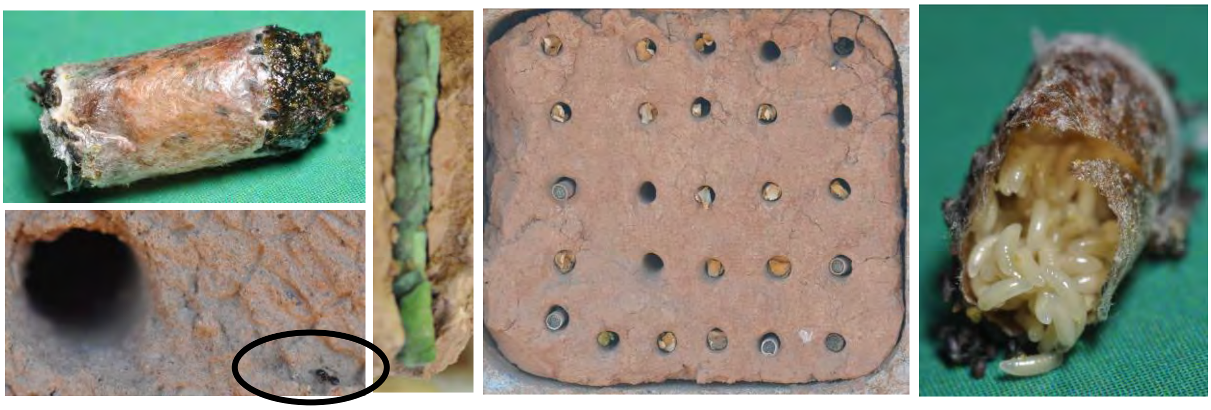

Figure 8. **From left to right: top: (a) a healthy cocoon of *M. nigrovittata*; (b) the tiny *Monomorium* species (circled) that raided the leafcutter nests;(c) a successful leafcutter bee nests with 5 cells; (d) nest block showing leafcutter nests that had been raided by ants (note that the resin bee nests in straws remained intact); (e) a resin bee cocoon infested by a wasp larvae (possibly *Melittobia australica*: Eulophidae).**

Assessment of bee visitors of lucerne

At the Waite Arboretum and Urrbrae High School, several bee species were observed foraging on lucerne seed crops maintained by the SARDI Pastures Group. These included the following ground nesting bees: *Lasioglossum (Chilalictus) lanarium*, *Lipotriches australica* and *L. flavoviridis.* Furthermore, *M. nigrovittata* and *M. quinquelineata*, which nested in the bee wall, were also observed on lucerne. The other resin bee species in the wall, *M. erythropyga*, was not seen on lucerne, but collected pollen on lotus and cullen. These crops also attracted the other Megachilini and the Halictini. At Urrbrae High School, *L. lanarium* was caught while collecting pollen and tripping flowers inside the bird cage.

The most abundant bee on the lucerne at the Waite Campus was *Lipotriches flavoviridis,* a relatively small species (body length 4 mm). This nomiine species belongs to the same taxonomic group as *Nomia melanderi,* the alkali bee, which is managed for lucerne pollination in the USA.

The tripping behaviour of this species was observed in the field and its efficacy was compared to that of honeybees. Of all flowers visited, the nomiine bees tripped 59% (200 out of 345, $n = 48$ females) while honeybees tripped 0.8 % (4 out of 475, $n = 23$ workers, $\chi^2 = 371.15$, $p < 0.001$, Fig. 9). The nomiine bees visited significantly less flowers per minute than honeybees (nomiine bees 3.71 ± 0.54, honeybees: 23.1 ± 1.77 flowers per minute, $t = 9.41$, $p < 0.001$). Two behaviours caused the nomiine bees to spend more time on each flower. First, the activity of tripping involved pushing their heads into the flowers, which seemed to take considerable effort. The time between landing on the flower and tripping it was on average 5.3 seconds per flower ($n = 200$ flowers). Second, the active pollen collection by the bees took on average 14.9 seconds per flower ($n = 200$). By contrast, the honeybees did not collect pollen, and when accidentally tripping a flower, immediately moved to the next one.

Combining all data, the average number of flowers tripped per minute was significantly higher for nomiine bees (3.25 ± 0.39, $n = 48$) than for honeybees (0.10 ± 0.07, $n = 23$, $t = 5.50$, $p <0.001$, Fig. 9). This implies that the nomiine bees were 32 times more efficient than honeybees. Importantly, the nomiine bees chose to collect pollen from lucerne, despite the presence of numerous other pollen sources in the close vicinity. The honeybees did not collect pollen from lucerne at this location.

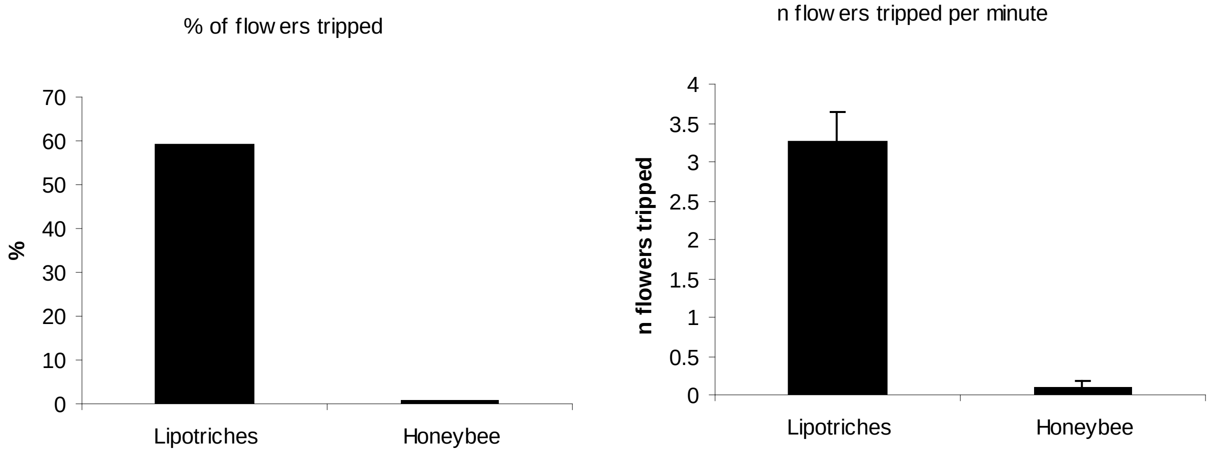

Figure 9. **(a) The percentage of visited flowers tripped by honeybees and native ground-nesting *Lipotriches flavoviridis* (n = 345 flowers visited by 48female *L. flavoviridis,* 475 flowers by visited 23 worker honeybees). (b) The number of flowers (± s.e.) tripped on average per minute by females of each species.**

A visit to Bordertown was made on 27/12/2011, to investigate the native bees foraging on seed lucerne in the seed growing area. A total of 44 specimens belonging to 10 species of native bees (three *Lasioglossum*, two *Homalictus*, one *Lipotriches*, three colletid bees and one megachilid species) were caught while foraging on native plants and weeds surrounding the crop. This collection included a number of species that had earlier been observed foraging on lucerne, such as *Lasioglossum lanarium*, *L. globosum* and *Lipotriches australica* (Hogendoorn pers. obs, Walker 2012). However, none of these species were observed to visit lucerne at this site.

Discussion

Experiments in captivity: which native bees can pollinate lucerne?

The study has identified that the native resin bee *Megachile nigrovittata* is a potential pollinator of lucerne, and has demonstrated that blue-banded bees (*Amegilla murrayensis*) and *Megachile erythropyga* will not pollinate lucerne. This confirms findings by Cane (2002) that candidate pollinators can differ dramatically in the proportion of flowers they trip. The differences between species in the potential to pollinate lucerne in captivity seem to be related to their ability to recognise the flowers as a food source and their propensity to trip the flowers while collecting nectar.

While blue-banded bees (*Amegilla murrayensis*) are known to collect nectar from lucerne, both in the field (De Barro pers. com., Hogendoorn, pers. obs.), and in captivity, they did not trip flowers as their long tongues allow them to reach the nectar without tripping the flowers. This result was unexpected, because a small pilot trial in field cages had shown promise (Hogendoorn and Powell, unpublished data). However, the earlier trials had been performed at the same locality where the ground-nesting nomiine bees were active. Thus, the earlier results could be explained if the cages included one or more nomiine nests. The results further serve as an illustration that a flower visitor is not necessarily a pollinator(Popic et al.2012).

Although in theory the blue-banded bees could still trip flowers when collecting pollen, our trials have clearly shown that this does not happen. Even when actively nesting and suffering from pollen shortage, the bees did not collect lucerne pollen. This may either be related to the inferior quality of lucerne pollen (Standifer 1967), or to an inability to recognise the flower’s structure. Either way, despite the fact that they visit the crop for nectar, it is very unlikely that blue-banded bees are useful bees for the open pollination of lucerne.

In contrast to the blue-banded bees, the larger resin bee *M. erythropyga* did not visit lucerne flowers at all, ignoring the flowers even when deprived of nectar for several hours and clearly hungry. Hence, it seems unlikely that *M. erythropyga* is a useful pollinator of open pollinated lucerne.

The native resin bee *M. nigrovittata* tripped flowers in captivity while collecting nectar and caused adequate set. As is the case for the alfalfa leafcutter bees (Cane 2002), the resin bees tripped the flowers while prizing them open with their heads. This may be the only way for these relatively small, short bees to reach the nectar. The importance of tongue length for tripping the flower was also been suggested by Cane (2002) who found that the long tongue of *Osmia aglaia* allowed nectar feeding on lucerne without tripping, while the short-tongued *Osmia sanrafaelae* was an adequate pollinator. Our data suggest similar explanations for the differences between species in the fraction of tripped flowers.

Because *M. nigrovittata* is one of the most common resin bees in the lucerne growing region (King 1994), developing management strategies for these bees in or adjacent to lucerne crops could be advantageous for the lucerne industry now and in the future.

The finding that a native resin bee species is an adequate pollinator of lucerne is not unexpected. An earlier study used six native Australian resin bee species in captivity (Bray 1973) and although the results were not quantified, the conclusion was that all six were able to pollinate lucerne, and some species even started nesting in captivity. Thus, it seems likely that a number of native resin and leafcutter bee species are useful as pollinators of lucerne. However, the fact that the bees in this study can pollinate lucerne in captivity does not necessarily imply that they will do the same excellent job outside, when alternative floral resources are available. This is clear from the behaviour of honeybees, who become distracted from lucerne, e.g. when *Eucalyptus camaldulensis* is in flower (Doull 1961).

Thus, the efficacy of M. nigrovittata, M. quinquelineata, and of the megachilid species suggested by Bray (1973), should be investigated on lucerne crops outside. To achieve this, we would need to maintain populations of these bees in the crop environment, and to document their behaviour on the crops, in the way that has been done in this study for a nomiine bee species. So far, the propagation and maintenance of Australian solitary bees has been deemed problematic, due to lack of insights in nesting needs, predators and parasites (Bray 1973). In the following section, we will use the data from the nesting walls to further investigate and address these problems.

Development of nesting substrate for native bees

The nesting substrate we provided was successfully taken up by native bees, and our study allows identification of several aspects of the properties and maintenance of artificial nesting substrate for native bees in the crop environment.

Identity of the bees in the walls

Although both walls were seeded with blue-banded bee brood, and some females started to nest in one of them, they disappeared within a month for unknown reasons. Subsequently, no blue-banded bee activity was observed. Blue-banded bees are known to nest in N.E facing clay wash-outs and mudbrick houses (Cardale 1968, Leijs et al in preparation). Establishment of the additional nesting requirements would be interesting for a number of horticultural industries, as these bees are adequate pollinators of various solanaceous and cucurbit crops. However, in the context of lucerne pollination, this is not of interest, as our data show that blue-banded bees were inadequate as lucerne pollinators.

The nesting walls were used by resin and leafcutter bees, and despite the lack of control of natural enemies, the nest numbers increased substantially in the course of two years. One of the resin bees in the bee wall, *M. nigrovittata,* was shown to be an adequate pollinator of lucerne. Another species that was abundant in the bee wall, *M. quinquelineata,* has in the past been caught collecting pollen on lucerne at Tintinara and Keith, as evidenced by the labels under 30 specimens in the Waite Insect and Nematode Collection. Therefore, even though their tripping behaviour was not tested in captivity, they can be safely assumed to trip lucerne flowers. The development of methods to promote these bees in the crop is likely to benefit the seed lucerne industry, and our study provides a first step in that direction.

Nesting preferences and requirements of leafcutter and resin bees

The most important and overarching conclusion is that the species that used the walls differed both with respect to the nesting substrate of choice, and in the strategies required to protect the nests from natural enemies.

The leafcutter bees significantly preferred clay substrate over waxed paper tubes. This may be in part related to the small diameter of he paper tubes in the binderboards, which were designed for *M. rotundata* and had a diameter of 5.5 mm. The nesting tunnels in clay blocks had various diameters, many between 6 – 8 mm. Because *M. quinquelineata* is a substantially larger species than *M. rotundata*, it is likely that their body size precluded the use of the binderboards. However, the larger waxed drinking straws with a diameter of 8 mm also failed to attract to *M. quinquelineata*. Therefore, it is possible that either the waxed surface of the tubes rendered them less attractive for leafcutter bees, or that the properties of the clay blocks were preferred over those of paper tubes. The clay blocks would be firmer, may provide more protection from natural enemies and extreme temperature fluctuations, and have better humidity conditions than paper tubes. Evaluation of the important properties of the different nesting substrates for these useful bees requires further study.

The resin bees, including *M. nigrovittata*, significantly preferred paper tubes over unlined clay blocks. The fact that the tubes were also used when inserted into clay blocks (Fig. 8) demonstrates that nesting blocks for these species do not necessarily need to be made out of wood. However, tubes that were presented lose and not in wood or clay blocks, seemed less attractive. A further important insight gained during this and another (Hogendoorn 2011) study of resin bee nesting behaviour is that paper tubes should be relatively impervious to water.

Protection from ants

Regarding protection from other natural enemies, it is clear that both resin bees (Hogendoorn 2011) and leafcutter bees (Woodward 1994, this study , Anderson 2006) benefit from protection from ants. Resin bee nests may be better protected than leafcutter nests, as ants were seen to chew their way through leafcutter cocoons, but not through resin caps. It is easy to underestimate the importance of such protection, because ant raids are not often observed. In this study, despite regular inspections, a raid was only observed once. However, it is well-known that ants can be a major threat to native bees (Gerling et al. 1981, Smith et al. 2003, Hogendoorn et al submitted). This may be more so in Australia, due to the relatively high ant densities. Two experimental studies on the effects of ants on native bees in Australia indicate that they have had a role in shaping the social and communal nesting behaviour (Kukuk et al. 1998, Zammit et al. 2008). Thus, we conclude that the successful maintenance of large local native bee populations should involve measures to exclude access by ants.

The nesting walls used in this study did not allow the exclusion of ants. Hence nesting shelters, such as used for alfalfa leafcutter bees, may be preferred, because these can be protected using Tanglefoot®. In this context, it is important to realise that resin bees have been observed to mistake Tanglefoot for resin, and will collect it to close their cells and nests (Hogendoorn pers. obs.). However, unlike resin, Tanglefoot remains sticky, and this badly affects the eclosing brood in the subsequent years. Therefore, special structures should be designed to make the Tanglefoot inaccessible to the resin bees, while still protecting the shelters from ants.

Protection from parasitic wasps

Parasitic wasps observed at the nest sites and inside the nesting walls were evanoid, chrysidid and eulophid wasps. Of these, the eulophid wasps are cited to cause the most damage to leafcutter bees (Woodward 1994). Experiences with resin bees in Victoria indicate that evanoid wasps can also devastate local populations (Hogendoorn 2011). Therefore maintenance of local solitary bee populations requires management strategies to reduce parasite loads. Such management strategies have been developed for alfalfa leafcutter bees (Richards 1984) and orchard bees (Bosch and Kemp 2001), and several measures have been recommended for the Australian situation (e.g. Woodward 1994). This involves removing the nests from the shelters into storage, cooling the nests to kill eulophid wasps (the effect of this measure on the health of native bee brood should be assessed), and removal of eclosed parasitic wasps before release of the bees (Woodward 1994, 1996, Anderson 2006). Such measures place additional demands on the design of nesting substrate, as it has to be removed from the field, stored in incubators and checked. For resin bees, waxed paper tubes of appropriate sizes inserted in wood, clay or possibly polystyrene boxes allow such measures, as do bamboo canes that have previously been split but have their halves securely fitted together. For native leafcutter bees, opportunities need to be explored further.

Further observations on the use of artificial nesting substrate

The wall at the Waite Arboretum had significantly more nests than the one at Eden Hills. This may reflect a locally lower density of bees at the latter locality. However, with respect to the resin bees, it is important to note that, at Eden Hills, no waxed tubes were inserted in the wall and a large amount of alternative nesting materials (waxed straws, wood blocks, bamboo) had been placed in the immediate surroundings. Considering the fact that the resin bees were significantly more attracted to straws than to the clay nesting substrate, it is likely that the lack of success of the nesting wall in Eden Hills reflects the fact that the resin bees had a choice.

Interestingly, a relatively high percentage (22%) of the resin bee nests found did not have any brood or signs of provisioning. It seems unlikely that these nests were the result of ant raids, as their contents were completely clean and closed off. It is possible that these nests were accidentally closed by females, i.e. that they had mistaken them for the nest next to it. Once partially closed, such accidentally closed nests would be fully closed off. Markings on the nesting substrate that signal position to the bees may help to prevent such mistakes.

Furthermore, the brood numbers inside the resin bee nests were low. While the leafcutter bees had filled up their nests and the number of cells seemed to be constrained by the length of the tunnel, the resin bee nests contained mostly one, sometimes two cells and had long, empty stretches. It is possible that placement of brood into different nests is a risk avoidance strategy, aimed at reducing the chances of parasitisation of the brood. One of the main brood parasites of resin bees were evanoid wasps (Hogendoorn 2011, Hogendoorn submitted). These large wasps use their long ovipositor to lay eggs in the cell of the bee, and were seen to enter bee nests, but were not observed to parasitise closed nests. Furthermore, evanoid wasp larvae can in some cases consume two rather than a single larval resin bee within a nest (Hogendoorn pers. obs.). Therefore, the bees may avoid parasitisation and loss of excess brood by closing nests that contain a single cell and starting a new nest, rather than adding cells to an existing nest. This would be a particularly beneficial strategy if, as was the case in our set-up, an excess of nesting substrate available.

In conclusion, native resin and leafcutter bees that are useful pollinators of lucerne have different nesting preferences and partially different requirements when it comes to protection of nests from natural enemies. The efficacy of different nesting substrates and management strategies in meeting these requirements need to be further studied and developed for Australian conditions. Factors that have been specifically identified to affect nesting success of leafcutter and resin bees are high ant densities, and build up of parasitic wasp species. Control of temperature fluctuations in bee shelters is another area that deserved further attention.

Assessment of bee visitors of lucerne

Several native bees were observed foraging on lucerne at both the Waite Campus and Urrbrae High School. The most frequently observed species were ground nesting bees of the tribes Halictini and Nomiini. The visitation by native halictine and megachiline bees on lucerne align with and expand the data available on plant visitation in Walker's Native bees of Australia database (Walker 2012).

The small nomiine bees at the Waite Campus were 32 times as efficient as honeybees in tripping lucerne and, in contrast to the honeybees at this location, they were collecting pollen from the crop. This is a particularly interesting observation, because the bees in question belong to the same tribe as the ground nesting alkali bees used in pollinating lucerne in the USA (Cane 2008).

While it may be tempting to do so, the comparative efficiency of the different native bees and honeybees should not be phrased in terms of a contest in efficacy between the species, or between native bees and introduced honeybees. Pollination can be a numbers game. Thus, although individually practically useless, honeybees can be reasonably efficient pollinators of lucerne simply because they can be moved into the crop in large numbers (Somerville 2002). However, the results reported here do emphasise that some Australian native bees can and do pollinate lucerne. Finding ways to place and enhance, for example, ground-nesting nomiine bees in the proximity of seed lucerne fields, as is done in the USA (Cane 2008), may prove beneficial, particularly so if feral and managed honeybees become scarce.

The complete absence of native bees on the seed crop at Bordertown was unexpected for two reasons. First, C. Hilton is known for his reluctant and considerate use of insecticides. Second, during the 1950s and early 1960s, K. Doull collected various resin and leafcutter bee species form lucerne at roughly the same locality. However, absence of native bees on seed lucerne crops in the Keith region in recent times has been confirmed by J. de Barro (pers. com.).

There are a number of possible explanations for this apparent decrease in the presence of native bees on lucerne in the region. First, border check irrigation (flood irrigation) may preclude the presence of native ground nesting bees within the crop. The validity of this explanation seems doubtful because there would be ample opportunity for native bees to nest in the small dams and the areas surrounding the paddocks, and resin and megachilid bees do not nest in the soil. Second, general habitat degradation due to grazing and land clearing may have reduced the sustenance for native bees outside the flowering time of the crops, as has happened elsewhere in the world (Ricketts et al. 2008). Third, both historically and at present, the lucerne industry has made use of a large range of insecticides that are detrimental to bees. Examples include a range of organophosphates and synthetic pyrethroids(De Barro 2001), and fipronil and neonicotinoids (McManus et al. 2010). In addition to the well-known high acute toxicity of several of these products to bees, which can cause local extinctions (Johansen 1977), some of these insecticides may have long-term residual effects (e.g. Estesen et al. 1992). However, often the sensitivity of solitary bees to insecticides is not clear. A striking demonstration of this is the accidental killing of more than a million released leafcutter bees with a single evening spray of indoxacarb in Australia in 2003 (Anderson 2006). A second example is the recently demonstrated long-term effects of exposing bumble bees to neonicotinoids (Whitehorn et al. 2012). In lucerne, neonicotinoids are used in seed coatings and sprays. Therefore, in conjunction with the development of population maintenance strategies for solitary bees, compatibility of lucerne growing practises with native bee keeping requires investigation, especially the range of pesticides that is used in protecting the crop.

Implications and Recommendations

This proof-of-concept project has identified three Australian native bee species that effectively pollinate lucerne. As these are locally occurring species, it is likely that they are better suited to local Australian conditions than the introduced *Megachile rotundata*.

Two of the native species, the resin bee *Megachile nigrovittata* and the leafcutter bee *M. quinquelineata*, used artificial nesting substrate presented outside. Their densities increased even without protection from parasitoids and predators. Therefore, these species could be amenable for use in the crops. The third species, the wide-spread nomiine bee *Lipotriches flavoviridis* nests in the soil, and further work is needed to find out how to enhance nesting conditions for this species.

To further explore and develop the potential of native bees in crops, the following information is required:

- Assessment of the utility of M. *nigrovittata* and *M. quinquelineata* in open pollinated lucerne crops;
- Exploring options to increase population sizes of *M. nigrovittata* and *M. quinquelineata* in the field. This includes the following work:
    - further development of cheap nesting structures and techniques that allow winter management of parasites and protection from ant raids;
    - investigating the utility of planting native forage plants that flower before and/or after the flowering time of the crop;
    - evaluation and enhancement of the compatibility of current crop management practises with the presence of native bees needs to be evaluated and where necessary improved. Here, the emphasis should be on the use of insecticides.
- Investigating the options for maintaining and increasing ground nesting nomiine bees in the vicinity of the crops, as these are very useful pollinators of lucerne;
- Finally, evaluation of the costs of crop and bee management practises required to maintain native bees in lucerne crops against the current and future economic and environmental benefits of deploying these bees for lucerne pollination.

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Native Australian Bees as Potential Pollinators of Lucerne

by Katja Hogendoom and Mike Keller

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The seed lucerne industry in Australia produces around $AUD 38 million worth of seeds annually. It is generally recognised that improved pollination would increase yield. This report summarises a proof-of-concept study to investigate the efficacy of some common Australian native bee species to pollinate lucerne.

Over the course of two years, the project evaluated the utility of some bees for the pollination of lucerne in captivity and in the field, and investigated methods to enhance these bees in the crop environment.

This report is relevant to the pasture seeds industry in particular and more generally to growers of crops that profit from bee pollination (e.g. lucerne, canola, cotton, and many horticultural crops).

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