Investigating the combined role of the introduced honeybee (Apis mellifera) and magnet plants on the pollination of both native and exotic plants in Australian ecosystems

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Investigating the combined role of the introduced honeybee (Apis mellifera) and magnet plants on the pollination of both native and exotic plants in Australian ecosystems

This thesis is submitted in fulfilment of the requirements of the award of the Degree Doctor of Philosophy from
University of Wollongong

Amy-Marie Gilpin

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Abstract

In Australia, the effects of introduced mass flowering species and their interaction with exotic pollinators, such as the European honeybee (*Apis mellifera*), on the pollination systems of native plants have been largely unstudied. In the northern hemisphere, resource-rich plants have been shown to have the potential to draw pollinators from the surrounding matrix, resulting in them acting as ‘magnet plants’. Such magnets may comprise of an individual plant, a cluster of plants or entire population of plants. Two possible outcomes exist for any co-flowering species that offer contrasting levels of floral reward. Firstly, spill-over effects may lead to increases in visitation and pollination due to proximity to the magnet plant. Alternatively, the co-flowering species may suffer reduced visitation by pollinators that are otherwise drawn to the magnet species. Australian plants have largely evolved without social insect pollinators and some have adapted to pollination by birds and mammals, in contrast to northern hemisphere pollination systems that are frequently pollinated by highly social insect species. However, these interactions are potentially of great ecological significance as pollination systems in Australia are highly disturbed, affected by both the introduction of exotic magnet plants and social pollinators. Therefore, predicting the interaction between introduced magnet plants, native Australian plant species and the introduced honeybee is difficult within the Australian setting.

Despite the potentially large impacts that these factors may have on the pollination biology of Australian plant species, little research has been done in this field. By testing four hypotheses I aimed to examine the impact of magnet plant populations on plant-pollinator interactions within Australian ecosystems.
Firstly, I investigated the impact of pollination and flower visitation by the exotic pollinator, *A. mellifera*, on vertebrate pollinated plant, the Proteaceous shrub *Banksia ericifolia*.

Honeybees are predicted to have foraging patterns that involve far more intra-plant pollen transfer than vertebrate pollinators. I compared the effects upon the plant’s reproductive output and fitness of pollination by *A. mellifera* (and other invertebrates) with that of pollination by all pollinators. To achieve this I had two treatments, open pollination and a vertebrate exclusion, whereby entire plants were covered with exclusion netting to prevent pollination by birds and small mammals, whilst allowing invertebrates to access flowers. Additionally, I compared the frequency of flower visitation and foraging behaviour of birds and insects within populations of *B. ericifolia*. For a subset of the study populations, I compared the quality of seed produced by measuring seed weight, germination rates (*T*$_{50}$), percent germination, seedling height after 14 days since the emergence of the cotyledon and time to emergence of the cotyledon. I found that *A. mellifera* was the only apparent insect pollinator and the most frequent flower visitor (n =146) to the open treatment inflorescences, however, inflorescences were also frequently visited by avian pollinators, with 97% of observed plants visited by avian pollinators (seven honeyeater species). The foraging behaviour of honeybees and honeyeaters showed striking differences that potentially affect patterns of pollen transfer, with honeybees making significantly greater proportions of within *cf.* among plant movements and only 30% (n=48) of honeybees foraged for pollen (nectar foragers carried no pollen) whilst all birds were observed to contact both stigmas and anthers when foraging for nectar. Despite these fundamental differences in behaviour, there was little effect of treatment on seed set or quality. Our data show that
while honeybees appear to alter patterns of pollen transfer within *B. ericifolia* populations, they do not impact reproductive rates or performance of early life-stages.

Secondly, over the course of my PhD I developed procedures and protocols to define the applicability of novel pollinator observation techniques utilising action video cameras. Such technology allowed me to overcome issues faced in regard to the high volume of observational data required with the limited man-power at my disposal. Most importantly I compared the results of direct observation to results obtained using Digital Video Recording (DVR). Two plant taxa, *Lavandula angustifolia* and *Canna* sp. with differing floral morphology, were used, to for the first time, assess the value of DVR compared to direct observations in estimating honeybee (*A. mellifera*) visitation, flower density and number of flowers visited per foraging bout. I found that the two methods yielded identical results when observing the structurally simple *L. angustifolia* at both high and low honeybee density. However, DVR misrepresented the number of flowers scored in the field of view relative to the whole plant, the number of honeybees observed and the number of flowers visited during foraging bouts on the more complex *Canna* sp. I concluded that portable weatherproof DVR devices such as the GoPro Hero are valuable tools for pollination biologists, allowing a single researcher to make simultaneous observations of multiple plants, across one or more sites, whilst also allowing the footage to be reviewed although, the complexity of the plant needs to be factored into the experimental design. As a result of this study I made use of GoPro cameras in the next components of my research, conducting a range of pollinator observations upon small, structurally simple plants.
Thirdly, to determine whether contrasting attractiveness of plants within natural settings, combined with visitation by honeybees, influences visitation and subsequently seed set, I studied the pollination systems of a range of natural ecosystems. I sought to determine the presence of native putative magnet plants within an Australian setting. I also aimed to test for spill-over of pollinators from native magnets to native co-flowering plants. I measured pollinator visitation, diversity, seed set and seed weight in three case studies. For two case studies I also compared visitation rates to co-flowering species in the presence of magnet species with those obtained following magnet removal, and also examined variation between years. In all cases I found the hypothesised magnet plants attracted a greater number but not diversity of flower visitors compared to their respective co-flowering species. Most surprising we found no support for indirect (spill-over effects) between magnet plants and the pollination of co-flowering species exhibited as either variation in pollinator visitation rates, diversity of pollinators or pollinator fidelity with distance from the magnet plants.

Finally, in order to determine the combined effects of introduced ‘magnet’ agricultural species and the exotic European honeybee on the pollination of surrounding co-flowering native Australian species and European honeybee co-evolved species, a large-scale manipulative experiment was undertaken. When introduced agricultural plants are flowering, they provide a short-term, bountiful food resource in an otherwise resource limited landscape to both native and introduced pollinators, with two possible consequences to pollinator visitation; (1) Competition, where pollinators are drawn from the surrounding matrix to forage on the magnet plants, resulting in decreased visitation to co-flowering species or; (2) Increased pollinator visitation or spill-over due to the proximity of magnet plants. The pollinator which often dominates these
landscapes within Australia is the introduced generalist the honeybee, whose social and physiological behaviour may affect both native pollinators and native flowering species in areas adjacent to magnet plants.

In order to achieve such aims, I tested the effects of distance from introduced (northern hemisphere) magnet agricultural species in three agro-ecosystems in Australia (lavender fields, pasture land invaded by the weed *Echium plantagineum* and nectarine orchards) on honeybee and native pollinator visitation to two Australian native plants (*Melaleuca thymifolia* and *Backhousia myrtifolia*) and two European honeybee co-evolved plant species (*Lavandula angustifolia* X and *Thymus citriodorus*) manipulatively placed in the agro-ecosystem along transects at cardinal directions from within the magnet patch out to a maximum distance of 250 meters. Subsequent flower visitors to the test species were documented and collected, while honeybee abundance was manipulated through the introduction of hives to determine if abundance of honeybees influences the pattern or visitation rates to test species. In stark contrast to northern hemisphere studies, I found little evidence of spill-over of flower visitors from magnet plants out to a distance of 250m. Honeybees were found to dominate these pollination systems, although, surprisingly co-flowering plants typically attracted a greater diversity of flower visitors than magnet species. I also found that honeybee abundance did not influence the visitation rate to co-flowering test species even when honeybee numbers were boosted by ~250,000. There also was no clear effect of origin of the study species (either European, honeybee co-evolved or Australian native plants) in relation to the number of honeybees or native flower visitors.
My research has shown that the impact of *A. mellifera* upon native plants is complex and may be reliant upon many factors, including the interaction with co-flowering species and the mating system of the plant. In contrast to many northern hemisphere studies my research has found that these systems are dominated by *A. mellifera* and I found very little evidence of spill-over effects from introduced agricultural magnet species. Although, this may not be surprising given that northern hemisphere studies that have documented spill-over effects within agricultural systems have primarily documented solitary bees and bumblebees as the most numerous flower visitors. Therefore, further research is needed within Australian systems as they have the potential to show striking differences to those found within the northern hemisphere presumably due to the vastly different suite of pollinators that service these areas.

My research has shown that understanding the pattern of visitation and foraging behaviour of honeybees and native plant-pollinator dynamics is important in predicting affects of introduced pollinators on these systems and subsequent plant reproductive output and seed fitness. I have also developed novel sampling techniques for surveying pollinators and guidelines for their application. Most importantly, this research provides a foundation within Australia for assessing the behaviour and interaction between *A. mellifera* and magnet plants both within agricultural systems and native ecosystems.
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Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Amy-Marie Gilpin and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

The author acknowledges that copyright of published works contained within this thesis (as listed below) resides with the copyright holder(s) of those works. I also give permission for the digital version of my thesis to be made available on the web, via the University’s digital research repository, the Library catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

This thesis contains four data chapters, each of which is written as a manuscript for publication, and is therefore intended to stand-alone. With this, some information may be redundant or repeated. While the candidate made substantial contributions to the manuscripts and is fully responsible for the work presented in this thesis, where the first person is used in the manuscripts it is used in the plural (‘we’) to reflect contributions from co-authors. Contributions by co-authors are detailed for each manuscript below. Prof David Ayre and Mr Andrew Denham were involved in designing the project, guided data collection and provided comments on drafts of all chapters. All data used in this thesis was collected during the period of the PhD.


AMG, DJA and MKJO developed the experimental approach, AMG and JCC performed the experiment, AMG performed the data analysis, AMG was the lead author

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of the manuscript, AMG, DJA, AJD and MKJO contributed to the writing and editing of the manuscript.


AMG and DJA developed the experimental approach, AMG performed the experiment, AMG performed the data analysis, AMG was the lead author of the manuscript and DJA and AJD contributed to the writing and editing of the manuscript.

Chapter 4: The combined effects of honeybee preference and magnet species in influencing the pattern of visitation and rate of co-flowering species. Amy-Marie Gilpin, Andrew J. Denham and David J. Ayre.

AMG, DJA and AJD developed the experimental approach, AMG performed the experiment, AMG performed the data analysis, AMG was the lead author of the manuscript and DJA and AJD contributed to the writing and editing of the manuscript.

Chapter 5: Are magnet plants in agricultural areas influencing the pollination of co-flowering plants in close proximity? Amy-Marie Gilpin, Andrew J. Denham and David J. Ayre.

AMG, DJA and AJD developed the experimental approach, AMG performed the experiment, AMG performed the data analysis, AMG was the lead author of the manuscript and DJA and AJD contributed to the writing and editing of the manuscript.

Amy-Marie Gilpin

February 2017
Publications and presentations

Throughout my PhD I have written or contributed to the following journal articles and presented my work at numerous national and international conferences. These activities are listed below.

Journal article

**Gilpin, A-M., Collette, J., Ooi, M., Denham, A.J. and Ayre, D.J. (2016) Do introduced honeybees effect seed set and seed quality in a plant adapted for bird pollination?**  
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Conference presentations

2016  **Gilpin, A-M., Ayre, DJ and Denham, AJ.** Do introduced honeybees affect seed set and seed quality in a plant adapted for bird pollination? Oral presentation at the Ecological Society of Australia meeting in Freemantle, Australia.


2014  **Gilpin, A-M., Ayre, DJ and Denham, AJ.** If you have a rich neighbour will you get robbed? Oral presentation at the Ecological Society of Australia meeting in Alice Springs, Australia.

Invited presentations


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1 Introduction:
The outcomes of pollinator mediated interactions between co-evolved plants can be difficult to predict and may vary in space and time. Outcomes may be competitive, facilitative or effectively neutral, reflecting many factors including conspecific and overall flower density, the extent of pollen limitation and the nature or similarity of floral displays and floral rewards and, perhaps most critically, pollinator behaviour. However, in the northern hemisphere the outcomes of interactions have proved easiest to predict when they involve typically generalist insect pollinators such as hoverflies, many bombus species, butterflies, *Apis mellifera* and ‘magnet plants’ that are highly attractive to such pollinators (Lázaro *et al.*, 2009; Bartomeus *et al*., 2010; Diekötter *et al*., 2010; Samnegård *et al*., 2011; Holzschuh *et al*., 2012; Montero-Castaño *et al*., 2016). In most cases magnets have been shown to consistently enhance pollinator visitation to neighbouring co-flowering species (Laverty 1992; Ghazoul 2006; Samnegård *et al*., 2011) although this interaction was often dependent on the amount of the magnet species present (Diekötter *et al*., 2010; Holzschuh *et al*., 2012). However, the presence of increased numbers of generalist pollinators can be detrimental if they carry mixed species pollen loads or alter patterns of pollen dispersal (Campbell and Motten 1985; Brown *et al*., 2002).

In Australia, pollinator mediated interactions between co-flowering plants, including the possible role of highly attractive plants which act as pollinator ‘magnets’, are less well studied than their northern hemisphere counterparts. The nature of such interactions between Australian native plants may differ from those seen in the northern hemisphere because many more Australian plant species are dependent upon and have evolved with a range of vertebrate pollinators, including marsupials and birds. Moreover, the pollination systems of many Australian plant species, including those
normally thought to be bird or mammal adapted, are now dominated by the exotic honeybee *Apis mellifera*. *Apis mellifera* has impacted Australian pollination systems through outcompeting native pollinators (Gross and Mackay 1998) and has the capacity to alter plant mating systems because its foraging behaviour differs markedly from native pollinators, especially vertebrates (Whelan *et al.*, 2009; Gilpin *et al.*, 2016). Conceivably, within Australia, the effect of *A. mellifera* will be greatest when examining the interaction between Australian plants and the now abundant exotic plant species, including many crops that are known to act as magnets in northern hemisphere plant communities. However, as in the northern hemisphere, the strength of these interactions may be dependent upon the extent to which native and exotic plants share pollinators and the relative attractiveness of Australian plants to the exotic *A. mellifera*.

Here I review published studies examining: the outcome of northern hemisphere studies investigating pollinator mediated interactions between magnet plant species including agricultural crops and native plants; and the interaction between *A. mellifera*, the most common exotic pollinator within Australia, and native Australian plants.

*Magnet plants and their interaction with co-flowering plants*

Interactions between plants and shared pollinators are usually thought of as competitive (Levin and Anderson 1970; Gross and Werner 1983; Callaway 1995; Palmer *et al.*, 2003; Brown *et al.*, 2002; Flanagan *et al.*, 2009). Studies have typically identified or inferred the presence of competition if co-occurring species receive reduced pollinator visitation (Levin and Anderson 1970; Gross and Werner 1983; Brown *et al.*, 2002; Flanagan *et al.*, 2009). Conversely, studies have shown that co-
flowering plants may receive greater pollinator diversity or rates of visitation when found in the presence of other plant species, although, this need not imply more successful pollination as the pollen being transferred, may be of lower quality or contaminated by other species pollen (Campbell and Motten 1985; Flanagan et al., 2009). Because rates of visitation may well be powerful indicators of reproductive success, all such studies should also quantify features such as pollinator behaviour, identity and fidelity effects on seed set and quality.

A series of largely northern hemisphere studies show that plant-plant interactions are often dynamic, and even for specific species pairs, interactions can range from facilitation to competition (Grabas and Laverty 1999; Ghazoul 2006). However, there are some situations under which we may expect the sign of the interaction to be more pronounced. For example, studies have shown that when the density of conspecifics or flowers increases or when pollen is limited, competition in turn can increase (Grabas and Laverty 1999; Feinsinger and Tiebout 1991; Carusso 2002; Ghazoul 2006; Carmona-Diaz and Garcia-Franco 2009). Studies have found that congeneric species or species with similar flowering phenology, which co-flower simultaneously and have co-evolved with the same pollinators, will experience a greater likelihood of pollinator sharing and thus the potential for competition or facilitation (Gross and Werner 1983; Brown et al., 2002; Moeller 2004). More broadly, these interactions are strongest when pollinators are generalists. Perhaps the most striking effects have been described for interactions of less common or less attractive plant species with neighbouring highly attractive magnet plant species (Chittka and Schürkens 2001; Lopezaraiza-Mikel et al., 2007; Diekötter et al., 2010; Gibson et al., 2013; Montero-Castaño et al., 2016).
Although, Thomson (1978) described a magnet plant as being a species that increases local pollinator abundance in an area to the benefit of other plant species that offer inferior rewards (Laverty 1992; Johnson et al., 2003), throughout this dissertation I will use a broader definition to include highly attractive species with the ability to draw pollinators from co-flowering species. This latter situation has often been described when a highly attractive invasive species or crop plants interact with surrounding native plants and the foraging preference of native pollinators is altered (Lopezaraiza-Mikel et al., 2007; Diekötter et al., 2009; Gibson et al., 2013; Montero-Castaño et al., 2016), although even in these circumstances a spill-over of pollinators from the croplands into adjacent areas can occur (Hagen and Kraemer 2010), potentially facilitating the pollination of native plants.

The competitive interaction of magnet plants with co-flowering neighbours can occur through two key mechanisms: (1) Exploitative competition where plants compete for pollinator visits through investment in more attractive floral traits, specifically nectar and floral display. The competitive effects of magnet plants will be greatest when the magnet species is more attractive to pollinators than co-flowering species and pollination services are limiting, and; (2) Interference competition, whereby pollinators are generalists and the magnet species pollen is deposited on the stigma of co-flowering species and likewise it can also result in the loss of conspecific pollen to the magnet species (Rathcke 1983, Brown et al., 2002, Cariveau and Norton 2009).
Facilitation among co-flowering neighbours, including magnet plants, may occur when attractive neighbours facilitate pollination success by promoting a general increase in abundance of pollinators and thus pollinator visits (Moeller 2004; Moragues and Traveset 2005; Peter and Johnson 2008; Seifen et al. 2014). Pollinators may be attracted to or remain in an area because of the presence of an attractive species and increase visitation to not only co-flowering species but species that flower at alternate times, thus allowing the pollinator community to be sustained throughout the year (Kennedy et al. 2013). This increase in visitation may lead to a subsequent increase in seed set (Ghazoul 2006; Peter and Johnson 2008; Carmona-Diaz and García-Franco 2009).

Several overseas studies have examined the effect of introduced magnet species on native plants and pollinators and found a range of contrasting effects.

Increasingly, pollination research has focused on the inter-play between crops and the role of native remnant vegetation in supporting pollinator networks (Walther-Hellwig and Frankl 2000; Hagen and Kraemer 2010; Hanley and Wilkins 2015; Kremen and M’Gonigle 2015; M’Gonigle et al., 2015; Montero-Castaño et al., 2016). Several reports indicate increased pollinator abundance, diversity and pollination of crop plants in areas adjacent to (within 750m of field edge) native remnant vegetation (Morandin and Winston 2006; Diekötter et al., 2010) or where weed species are allowed to grow providing a heterogenous suite of flowering plants (Carvalheiro et al., 2011). Although, very few studies have explored the effect these large magnet crops are having on the pollination of co-flowering native species surrounding such areas (Kremen and M’Gonigle 2015; M’Gonigle et al., 2015; Montero-Castaño et al., 2016).
Although specific pollination outcomes were not assessed, in western Kenya, Hagen and Kraemer (2010) surveyed three different habitat types (farmland, forest edge and forest understorey) to determine the relative contribution of each habitat to native bee diversity and overlap in plant-bee community interactions. Interestingly they found that the farmland and forest edge which contained a higher number of flowering species than the forest understorey also contained the highest diversity of bee species, pollinator abundance and largest pollination network. The authors attributed this to the structurally diverse farmland which they describe as supporting and maintaining bee communities in the natural forest remnant.

In order to clearly determine the impacts of plant-plant interactions, studies must not only compare differences in visitation rates, but also subsequent effects upon reproductive output. In Great Britain, Lopezaraiza-Mikel et al., (2007) carried out a manipulative field experiment to assess the impact of a showy and highly rewarding invasive species Impatiens glandulifera on a suite of native flowering species by comparing invaded and removal plots. Plots containing I. glandulifera had significantly higher visitor species richness, abundance and visitation. However, the pollen transport web was completely dominated by I. glandulifera pollen and did not result in pollination of native species. A similar experiment was undertaken in South Africa where a prolifically flowering invasive Australian species, Acacia saligna, was found to be an effective magnet and significantly reduced visitation rates of honeybees and insects to the native species Roepera fulva which had the highest flower visitor overlap (Gibson et al. 2013). Such studies highlight the need to examine the entire sequence of plant-pollinator interactions to accurately determine effects upon reproductive output.
Magnet plants in agricultural settings.

Large-scale agriculture has produced vast monocultures of florally resource rich, crop species, which often act as “magnets”, drawing pollinators from existing plant communities (Holzschuh et al., 2011; Montero-Castaño et al., 2016). To date, research has focussed on the effect of magnet plants upon northern hemisphere plant communities, with two broad outcomes reported. Firstly, magnets have been shown to enhance the pollination success of neighbouring co-flowering plants (Samnegård et al., 2011), due to the magnet plant species either supporting a greater abundance or diversity of pollinators, which in turn may result in spill-over effects from the magnet and onto co-flowering neighbours (Hagen and Kraemer 2010). Native plants may be alternatively overlooked as existing pollinators could be drawn to magnet species, which have higher floral density and reward (Holzschuh et al., 2011; Montero-Castaño et al., 2016).

Interestingly, within disturbed agricultural landscapes, (e.g. when fields are ploughed or crops are not in flower), less abundant but highly attractive garden plants may function as magnets with spillover of pollinators to native species (Samnegård et al., 2011). Such as in a study in Sweden, where gardens were found to enhance pollination of a native out-crossing plant Campanula persicfolia in intensively managed agricultural landscapes (Samnegård et al., 2011). Campanula persicfolia plants that were close to gardens (<15m) showed an increase in seed set, higher bee abundance and species richness compared to those far away (>140m). Thus gardens under some circumstances may facilitate the pollination of surrounding co-flowering native plants. In contrast, Montero-Castaño et al., (2016) found that when Hedysarum coronarium, a
florally resource rich mass flowering legume, was in flower, honeybee and other native bee abundance and visitation to native plants in adjacent shrublands decreased.

In Australia, very little research has focussed on the impact of magnet plants and in particular magnet crops and their interaction with co-flowering species. Here the effects of natural and introduced magnet species are likely to be complicated by the domination of many pollen webs by the exotic *A. mellifera*. Many pollination webs are highly disturbed with the honeybee successfully utilizing over 200 Australian plant genera (Paton 1995) and is now considered the only effective pollinator of several native species (Hermansen *et al*., 2014; Gilpin *et al*., 2014). Magnet plants may have great but unknown ecological significance in Australia. Australian crops are almost entirely northern hemisphere species of which a large proportion are dependent on the pollination services provided by honeybees (Cunningham *et al*., 2002). These honeybees are either brought in to provide pollination in managed hives or are present as wild colonies.

*Apis mellifera and its interaction with native plants and pollinators*

Worldwide, when *A. mellifera* has been introduced to ecosystems, its interaction with native pollinators and utilization of native plants has been highly variable (Roubik 1996; Kato *et al*., 1999; Thomson 2004; Dupont *et al*., 2004).

A study in Mauritius found competitive interactions between introduced honeybees and native bird pollinators (Hansen *et al*., 2002). In their study the visitation rates and behaviour of two endemic birds when visiting *Sideroxylon* trees was altered by
the presence of honeybees. They found that neither species foraged on either *Sideroxylon* species once honeybee activity lowered the standing crop of nectar. They also found that bagged branches of both *Sideroxylon* to which honeybees only had access had lower fruit set than branches that both birds and bees had access to. This highlights the need for manipulative field studies to investigate the effect of introduced honeybees on the pollination of native plants with insect and especially native vertebrate pollinators. Within Australia, honeybees have been found to be frequent visitors to bird-adapted plants, such as many Banksia and *Grevillea* spp, often exploiting both nectar and pollen resources (Paton and Turner 1985; Cellebreeze and Paton 2004; Whelan et al., 2009). To date no study has investigated the effect of honeybees on a vertebrate co-evolved plant species and the effect on reproductive output and fitness of seed sired by these pollinators.

Australia provides an opportunity to study the possible effects of honeybees on pollination systems that have evolved with a different suite of pollinators and it is perhaps surprising that few studies have been conducted. Here I will describe the outcomes of an array of existing studies that attempt to document the interaction of honeybees with Australian native plants and their pollinators and then from this make predictions about the possible interaction honeybees may have with magnet plants within Australia. The impact of the introduced *A. mellifera* on Australian native plants and pollinators has been documented for some well-studied communities; however, much of the research has shown conflicting results and is often species and site specific (Gross and Mackay 1998; Gilpin *et al.*, 2014; Hermansen *et al.*, 2014).
Paton (1993) lists over 200 Australian plant genera known to be visited by honeybees. This is likely to be a vast underestimation of the number of genera and species of plants that interact with honeybees. Although a substantial number of plant species are utilized by honeybees, I found an under-representation of studies examining the impact of honeybees on these plants, with only 5 families represented (Table 1.1). The honeybee has also been documented as the dominant flower visitor and only effective pollinator of *Acacia ligulata* (Gilpin *et al.*, 2014), *Avicennia marina* (Hermanssen *et al.*, 2014), *Persoonia bargoensis* (Field *et al.*, 2005). Australia lacks data on pollination systems before the introduction of honeybees and thus it is impossible to quantify the true impact of honeybees on native plant mating systems and natural pollinators.
### Table 1 - Australian studies that investigate the interaction between introduced *A. mellifera* and Australian native plant species.

<table>
<thead>
<tr>
<th>Family</th>
<th>Plant sp</th>
<th>Mating system</th>
<th>Honeybee behaviour</th>
<th>Effect on fruit set</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proteaceae</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Banksia</strong></td>
<td><em>spinulosa</em></td>
<td>Partially self-compatible</td>
<td>Honeybees made more visits per day than honeyeaters.</td>
<td>Honeybees can pollinate <em>B. spinulosa.</em></td>
<td>Vaughton, G. (1992)</td>
</tr>
<tr>
<td></td>
<td><em>ornata</em></td>
<td>NA</td>
<td>No difference in the number of honeyeaters within a 500m radius of commercial hives and sites with no honeybees.</td>
<td>Honeybee stocked sites had significantly more seed set.</td>
<td>Paton 1995</td>
</tr>
<tr>
<td></td>
<td><em>menziesii</em></td>
<td>NA</td>
<td>Nectar foraging honeybees did not contact the stigma. Honeybees visited inflorescences 10 times more frequently than birds although they deposited only 25% of the pollen that birds did on stigmata.</td>
<td>Honeybees can pollinate <em>B.menziesii</em> however birds are far more effective.</td>
<td>Ramsey (1988)</td>
</tr>
<tr>
<td><strong>Persoonia</strong></td>
<td><em>bargoensis</em></td>
<td>Self-compatible with preference for outcross pollen</td>
<td>Pollen collecting honeybees visited many flowers on each inflorescence and were seen to make inter and intra plant movements.</td>
<td>No significant difference between the proportion of inflorescences that set fruit in the presence of birds compared to that by diurnal insects.</td>
<td>Paton and Turner 1985</td>
</tr>
<tr>
<td></td>
<td><em>mollis subsp.</em></td>
<td>Self-compatible with preference for outcross pollen</td>
<td>Honeybees were by far the most common visitor</td>
<td>Honeybees can pollinate <em>P. bargoensis</em>.</td>
<td>Field <em>et al.</em>, (2005)</td>
</tr>
<tr>
<td></td>
<td><em>Nectens</em></td>
<td>All self-incompatible</td>
<td>Honeybees were the most frequent visitor to flowers of all spp. Honeybees</td>
<td>NA</td>
<td>Rymer <em>et al.</em>, (2005)</td>
</tr>
<tr>
<td>Family</td>
<td>Plant sp</td>
<td>Mating system</td>
<td>Honeybee behaviour</td>
<td>Effect on fruit set</td>
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<tr>
<td>Persoonia</td>
<td>mollis subsp. Maxima, Persoonia lanceolata, Persoonia glaucescens</td>
<td></td>
<td>made more intra plant movements and moved less distance between plants than native bees. There is a difference between honeybees and native bees and the appendage that they use to contact the stigma.</td>
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<td></td>
</tr>
<tr>
<td>Grevillea</td>
<td>sphacelata and Grevillea</td>
<td>G. mucronulata self-compatible with a preference for outcross pollination, G. sphacelata self-incompatible</td>
<td>Honeybees were the only observed flower visitors to G. sphacelata</td>
<td>Honeybees were poor pollinators of G. mucronulata, rarely contacting the stigma. Honeybees made almost universal contact with stigmas on G sphacelata (99.5% of 964 observed visits) however, there was comparatively very low fruit set.</td>
<td>Richardson et al., (2000)</td>
</tr>
<tr>
<td>Grevillea</td>
<td>macleayana</td>
<td>NA</td>
<td>Bird and honeybee foraging behaviour differed with honeybees never seen to make inter-plant movements compared to birds that made frequent inter plant movements.</td>
<td>NA</td>
<td>Whelan et al., (2009)</td>
</tr>
<tr>
<td>Fabaceae</td>
<td>Dillwynia juniperina</td>
<td>Obligately outcrossing</td>
<td>Honeybee make more intra plant movements than Leioproctus sp. 1 and Lasioglossum sp. Native bees spent</td>
<td>No difference in fruit set by honeybees compared to native bees.</td>
<td>Gross (2001)</td>
</tr>
<tr>
<td>Family</td>
<td>Plant sp</td>
<td>Mating system</td>
<td>Honeybee behaviour</td>
<td>Effect on fruit set</td>
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<tr>
<td>Acacia ligulata</td>
<td>Self-compatible</td>
<td>Honeybees made far more intra plant movements than inter plant movements</td>
<td>NA</td>
<td>Gilpin et al., (2014)</td>
<td></td>
</tr>
<tr>
<td>Melastomataceae</td>
<td>Melastoma affine</td>
<td>NA</td>
<td>Honeybees deposited significantly less pollen on stigmas than native bees, honeybees actively removed deposited pollen and thus fruit set and seed set were significantly lower in flowers to which honeybees were the last visitor. Aggressive behaviour between honeybees and native bees.</td>
<td>Honeybees reduced fitness and had a lower rate of seed set compared to native bees.</td>
<td>Gross and Mackay (1998)</td>
</tr>
<tr>
<td>Eucalyptus</td>
<td>Eucalyptus camaldulensis</td>
<td>Mixed mating system with a preference for outcrossing</td>
<td>NA</td>
<td>Honeybees contributed to the pollination of isolated trees due to their greater potential foraging range than native pollinators. The only effective pollinator</td>
<td>Ottewell et al., (2009)</td>
</tr>
<tr>
<td>Avicenniaceae</td>
<td>Avicennia marina</td>
<td></td>
<td></td>
<td></td>
<td>Hermanssen et al., (2014)</td>
</tr>
</tbody>
</table>
Pollination by honeybees could reduce or enhance the fitness of native plants by altering the quality and quantity of pollen deposited. Honeybees have also been found to reduce the fecundity (Gross and Mackay 1998) of some native plant species, though limited data is available because honeybees are difficult to selectively exclude, (Whelan et al., 2009). Honeybees are able to reduce the fecundity of some species as a result of their ability to effectively sequester pollen and avoid the reproductive parts of certain flowers (Richardson et al., 2000; Paton 1993). In Gross and Mackays’ (1998) study, honeybees were also found to deposit significantly less pollen on stigmas of *Melastoma affine* compared to native bees (Gross and Mackay 1998). The study by Gross and Mackay (1998) is the only study to document direct interactions between honeybees and native bees. Honeybees were observed to physically remove native bees foraging on flowers and in 91% of interactions, native bees were disturbed from foraging on flowers by honeybees.

Effects of honeybees on plant mating systems are less easily documented but a range of studies show that rates and patterns of pollinator visitation and pollen deposition by honeybees differs form those of native pollinators (Gross and Mackay 1998; Gross 2001; Whelan et al., 2009) and at least one study has shown increased inbreeding when honeybees replace native pollinators (England et al., 2001; Whelan et al., 2009).

Further compounding effects of honeybees upon plant reproductive success and their interaction between native pollinators stems from the honeybees’ ability to effectively steal floral resources by taking pollen and or nectar from flowers and not reciprocating by pollinating the flower. Although this has been observed in flowers of
all sizes (Gross and Mackay 1998; Paton 1993), in particular it appears to occur more often in larger flowers which are often bird-pollinated, where the reproductive parts are configured in a way that permits honeybees to avoid contacting the stigma, while accessing floral resources (Gilpin et al., 2016). Due to the depletion of floral resources by honeybees, native pollinators may be displaced from flowers and potentially entire areas. In a study by Paton (1993), the population density of New Holland honeyeaters was reduced when the number of honeybees was high due to a commercial apiary being located nearby. Paton (1993) also documented that in Callistemon rugulosus, honeybees were observed to displace honeyeaters from flowers, leading to reduced seed set due to differences in pollination services. In Banksia spinulosa (Vaughton 1992) and Banksia menziesii (Ramsey 1988), two primarily bird-pollinated species, honeybees were found to be effective pollinators as their foraging behaviour led them to make contact with both species stigma, however, they were not as important as nectarivorous birds, (Vaughton 1992). Although honeybees visited inflorescences ten times more frequently than birds, they only deposited 25% of pollen on stigma, compared to the 75% deposited by birds (Ramsey 1988).

The effects of honeybees on Australian plants are not always thought to be negative. Honeybees have been shown to facilitate gene flow and reproductive success in fragmented ecosystems. A study of Eucalyptus wandoo in Western Australia (Byrne et al. 2008) and study of Eucalyptus camaldulensis in South Australia (Ottewell et al. 2009) found ameliorating effects of honeybees upon gene flow and seed set. Within a highly fragmented ecosystem Both eucalypt species are found often in isolation from conspecifics by distances of 335m (E. camaldulensis) and populations ranging from 15 trees within a 1km radius (E. wandoo). Maintenance of high gene flow through pollen
dispersal in *E. wandoa* despite the species occurring in a fragmented landscape was attributed to the larger foraging range of the honeybee (Goulson 2003) compared to native bees and pollinating insects (Michener 1970; Schwarz and Hurst 1997). In *E. camaldulensis* honeybees made up 90% of floral visits (Ottewell *et al.*, 2005) they also did not detect a decline in reproductive success with distance from conspecifics in this species and this was also attributed to increased foraging range of this introduced pollinator compared to native counterparts.

*Thesis outline*

My overarching aims of this thesis are to (1) determine whether Australian native species act as magnets and to test whether potentially competition or facilitation effects decrease with distance from magnet plants. (2) To determine whether northern hemisphere plant species can function as magnets within an Australian setting (3) To determine any possible threshold at which pollination service to co-flowering species is increased through *A. mellifera* abundance. (4) To determine if the effect of northern hemisphere species that are highly attractive to pollinators on pollination systems varies for co-flowering native and northern hemisphere species. (5) I sought to determine the difference in reproductive output and early life history stages of seed sired by introduced honeybees compared to seed produced by vertebrate pollinators in a plant typically vertebrate pollinated.
References


Hermansen TD, Britton DR, Ayre DJ, Minchinton TE (2014) Indentifying the real pollinators? Exotic honeybees are the dominant flower visitors and only effective pollinators of *Avicennia marina* in Australian temperate mangroves. *Estuaries and Coasts*, 37: 621-635.


Do introduced honeybees affect seed set and seed quality in a plant adapted for bird pollination?

Published as

2.1 Abstract

Worldwide, evidence suggests that exotic pollinators can disrupt plant mating patterns. However, few studies have determined if pollination by the honeybee *Apis mellifera* (the world’s most widely introduced pollinator) reduces offspring quality when compared with pollination by native birds. The Australian Proteaceae provide an excellent opportunity to test the impact of honeybees in pollination systems that are adapted to birds and non-flying mammals.

We compared the frequency of flower visitation and foraging behaviour of birds and insects within seven populations of *Banksia ericifolia*. *Banksia ericifolia* is hermaphroditic and has large nectar-rich, orange inflorescences typical of bird and mammal pollinated species. For a subset of the study populations, we compared the quality of seed produced via an exclusion treatment (that only allowed invertebrates to access flowers) with an open-pollination treatment (potentially visited by mammals, birds and invertebrates), by measuring seed weight, germination rates (*T*₅₀), percent germination, seedling height after 14 days since the emergence of the cotyledon and time to emergence of the cotyledon.

*Apis mellifera* was the only apparent insect pollinator and the most frequent flower visitor, while the open treatment inflorescences were also frequently visited by avian pollinators, primarily honeyeater species. The foraging behaviour of honeybees and honeyeaters showed striking differences that potentially affect patterns of pollen transfer. Honeybees made significantly greater proportions of within *cf.* among plant movements and only 30% (n=48) of honeybees foraged for pollen (nectar foragers carried no pollen) whilst all birds were observed to contact both stigmas and anthers when foraging for nectar. Despite these fundamental differences in behaviour, there was little effect of treatment on seed set or quality. Our data show that while honeybees appear to alter patterns of pollen transfer within *B. ericifolia* populations, they do not impact reproductive rates or performance of early life-stages.
2.2 Introduction

The European honeybee (Apis mellifera) has successfully invaded ecosystems worldwide and many studies have found evidence of consequent competition between honeybees and native pollinators (e.g. Kato et al. 1999; Hansen et al. 2002; Thomson 2004). However, relatively few studies have investigated whether seedling fitness is altered by consequent changes to patterns of pollen dispersal (Paton1993). Taken collectively, these papers suggest that when larger native vertebrate pollinators are excluded, seed production or rates of outcrossing may be decreased (e.g. Ramsey 1988; Richardson et al. 2000; England et al. 2001; Celebrezze and Paton 2004) but to our knowledge none has investigated the effects of A. mellifera on the quality of seeds produced.

The Proteaceae is a cosmopolitan family, represented across South America, Africa and Australasia (Johnson and Briggs 1975). The greatest diversity of species occurs in South Africa and Australia, and vertebrate-pollination is a feature of many genera (Collins and Rebelo 1987; Myerscough et al. 2001; Johnson and Pauw 2014). In the temperate regions of Australia, pollination systems have evolved without social bees (Michener 1979). Thus in this region, the flora is often dominated by species such as those within the Proteaceae, that have evolved with birds and mammals as their primary pollinators. Currently, however, the relatively recently introduced A. mellifera (present for ~200 years) (Doull 1973) is the numerically dominant insect pollinator of many plant species (Gilpin et al. 2014; Hermansen et al. 2014) including many that would previously have been expected to be almost exclusively pollinated by birds (e.g. Whelan et al 2009).

The impact of honeybees is especially evident in the pollinator assemblages of the Proteaceae (Ramsey 1988; Vaughton 1992; Richardson et al. 2000; Celebrezze and Paton 2004; Whelan et al. 2009). This family features large, showy, nectar-rich inflorescences that are typically considered adapted to pollination by both birds and marsupials (Ayre and Whelan 1989) but are also highly attractive to both nectar and pollen-foraging honeybees (Paton and Turner 1985; Myerscough et al. 2001). Honeybees have been reported to be effective pollinators of a range of Proteaceae (e.g. Vaughton 1992; Whelan et al. 2009) but frequently, in foraging for both nectar and
pollen, they are considered to deplete the resources available to native pollinators (Vaughton 1996) and are typically observed to make more foraging movements within plants or among sets of near neighbours than is the case for native birds or insects (Richardson et al. 2000; Celebrezze 2002; Rymer et al. 2005; Whelan et al. 2009).

The effect of different patterns of pollen transfer will almost certainly vary with each species’ level of self-compatibility, the quantity and quality of pollen transferred and underlying spatial genetic variation. Evidence from a number of studies shows that self-compatibility and realised mating systems can vary sharply within and among species of Proteaceae (Carthew et al. 1988; Ayre et al. 1994; Hoebbe and Young 2001; Llorens 2004). Moreover, seedling fitness can be affected by pollen dispersal distance, in some but not all cases, where pollen has been experimentally transferred within and among populations (e.g. Heliyanto et al. 2005; Holmes et al. 2008; Forrest et al. 2011; O’Brien et al. 2013). However, the consequences of honeybee cf. vertebrate pollination for both outcrossing rates and offspring fitness have been largely ignored and no study has tested for changes in seed quality as a consequence of honeybee pollination. England et al. (2001) demonstrated that for Grevillea macleayana, pollination by honeybees in a vertebrate exclusion experiment produced a small but significant decrease in outcrossing rates. Vaughton (1996) found decreased seed set (50%) in inflorescences of the same species when birds were excluded compared to inflorescences where both birds and honeybees had access, while similar vertebrate exclusion experiments by Paton and Turner (1985) and Vaughton (1992) detected no clear effect on seed production in Banksia ericifolia or Banksia spinulosa respectively. However, none of these studies comment on seed or seedling quality. Studies of vertebrate pollinator-adapted Protea in Africa have reported reduced seed set following experimental exclusion of vertebrates (Wiens et al. 1983; Hargreaves et al. 2004) but it is unclear whether this simply reflects decreased pollen transfer.

In this study we tested the prediction that vertebrate exclusion and consequent pollination by honeybees would reduce seed set and seedling vigour for the vertebrate pollinator-adapted B. ericifolia (Carpenter 1978) which is also known to be frequently visited by honeybees (Paton and Turner 1985). We focused on three questions: (1) What proportion of inflorescence visits are made by honeybees as compared with birds and
mammals? (2) Are honeybees more likely than vertebrates to transfer self or outcross pollen? (3) Is there a difference in seed production, seed weight and seedling vigour between seeds produced when vertebrate pollinators are excluded and those produced under open-pollination?

2.3 Materials and Methods

Study area and study species

The study was conducted in seven sites, at four locations, six within National Parks: Royal - two sites, (34°09’06.7”S 151°03’34.0”E); Dharawal - two sites, (34°14’30.5”S 150°50’27.2”E) and Budderoo - two sites (34°38’45.3”S 150°41’58.2”E); and one on private land in Helensburgh -(34°10’28.9”S, 150°58’39.2”E), all located south of Sydney, New South Wales, Australia. Sites were similarly sized (~ 6 ha) and chosen due to their similarity in density and size of *B. ericifolia* plants. *Banksia ericifolia* is a shrub or small tree which produces inflorescence spikes in Autumn/Winter each year. Inflorescences range in length from ~10-25 cm, are red-orange in colour and produce copious amounts of nectar and pollen (Lloyd *et al.* 2002). Flowers open sequentially and the inflorescences produce nectar over two to three weeks (Lloyd *et al.* 2002). After nectar production had ceased and all flowers began to brown, we judged the inflorescence to be senescent. Fertilised seed are retained within woody follicles and form an infructescence or cone. The number of seeds per infructescence is limited by the space available for seed development (George 1984). The winged seeds are released after fire stimulates opening of follicles. The mating system of *B. ericifolia* is partially self-compatible (Goldingay *et al.* 1991a; Carthew *et al.* 1996).

Exclusion experiment

In order to compare the frequency of visitation and the pattern of foraging behaviour by vertebrates and insects on inflorescences of *B. ericifolia* and to test the subsequent effectiveness of insects as pollen vectors, we randomly allocated 15 *B. ericifolia* plants at each of seven study sites (Royal site 1 and 2, Dharawal site 1 and 2,
Budderoo site 1 and 2 and Helensburgh) to one of three pollination treatments. The treatments were; a spontaneous autogamy treatment, an open-pollination and a vertebrate exclusion treatment. For the latter two treatments we selected all inflorescences on which the process of flower opening was clearly about to begin, providing 10 to 11 inflorescences per plant. We removed a small number of inflorescences that had open flowers from most plants ensuring that each treatment featured an identical number of similarly mature inflorescences. Older inflorescences with senescent flowers or early stage immature inflorescences were not removed.

Plants allocated to the autogamy treatment each had five tagged inflorescences. These inflorescences were then bagged using a hard plastic inner layer of coarse mesh (Gutter Guard™) covered with a fine organza cloth to exclude all potential flower visitors. We included an autogamy treatment in order to determine whether pollen vectors were necessary, but because there was no seed set within the autogamy treatment at any of the seven sites, these results were excluded from later analysis. Plants allocated to the open pollinated and vertebrate-exclusion treatments each had 10 to 11 inflorescences tagged or caged per plant with identical numbers (10 or 11) of inflorescences allocated to each of these two treatments at each site. Plants allocated to the vertebrate-exclusion treatment were completely covered in netting with a 25 mm x 25 mm aperture which was small enough to ensure no bird or mammal could enter but large enough to allow easy access by honeybees. Trees allocated to the vertebrate-exclusion treatment were also fitted with a plastic guard around their trunk to prevent small mammal access.

Diurnal flower visitor surveys

To determine and compare the assemblage and behaviour of bird and insect flower visitors as well as compare insect visitors to open and vertebrate-excluded treatments, surveys were undertaken during the peak diurnal foraging time of these species within the peak of the flowering season at each study site (May to August). Through preliminary observations, we found that no honeybees visited *B. ericifolia* before 1000 or after 1500, most likely due to air temperatures being low (always below
Bird visitors were observed to visit plants primarily early in the morning (before 1000) or later in the afternoon (after 1430), with far fewer visits outside these observation times. As such, bird surveys were undertaken between either 0630 and 1000 or 1430 and 1800, and insect visitor surveys between 1000 and 1500.

Birds were surveyed on all trees in the open-pollination treatment for a total of seven days at each site, spread throughout the peak flowering period. All inflorescences on each plant were observed simultaneously for 10 minutes from a distance of more than 20 m to minimise disturbance. Insect visitor surveys were carried out on all plants within the open-pollination and vertebrate exclusion treatments on the same seven days as bird observations. The specific time of observation for each plant was chosen at random to avoid any temporal bias. Honeybee movements and behaviour were recorded for 10 minutes on both the open-pollination and vertebrate-excluded treatment plants (see below), with each plant simultaneously observed by two observers to ensure that visits to all inflorescences could be recorded. For both bird and insect visitors we recorded the length of time spent on each inflorescence on the study plant, as well as the number of inflorescences visited within the study plant, and the number of cases where the visitor flew to an inflorescence on a neighbouring plant or alternatively left the observation area. Each flower visitor was observed to determine whether it was foraging in a manner that would facilitate pollen transfer among inflorescences. Subsequently, it was noted that all birds foraged in a manner that would lead to pollen transfer, whilst honeybees were split into those foraging for nectar (no contact made with pollen presenters) and those foraging for pollen (pollen sequestered within their corbiculae) (Thorp 2000) which frequently contacted both pollen presenters and the stigmatic region.

**Nocturnal flower visitor surveys**

To determine whether *B. ericifolia* received nocturnal flower visitors we first undertook direct observations at night using torches at each site on all of the open-pollination treatment plants for three nights (spread throughout the flowering season), and failed to detect any nocturnal visitors. Subsequently, we deployed a set of four
infrared cameras (Faunatech) to conduct observations, at each site on each of three days, spread throughout the flowering season. Cameras were set with infrared trips that triggered the filming of two minute digital video sequences. Preliminary work showed that they were able to detect both nocturnal vertebrate and invertebrate visitors. In contrast to the diurnal surveys, observations were possible for only a subset of the target inflorescences on each of the open-pollination treatment plants (typically 2 to 3), with the number limited by the field of view of the cameras.

*Seed weight*

After inflorescences had been pollinated, bags and bird netting were removed (approximately one month after bagging) and seeds left to develop. All infructescences from the study plants were harvested once they reached maturity. In the laboratory, infructescences were then subjected to a heat treatment of 200°C for 20 minutes to open follicles and allow seeds to be extracted. The seed wing, septum and false seed were separated and the seed subsequently weighed to the nearest 0.1 mg. Thirty seeds per plant were randomly selected and weighed. In two cases where 30 were not produced, all available seeds were weighed (n = 12 for the vertebrate exclusion treatment and 28 for the open pollination treatment). Seed weight data for all plants from within the same site and treatment groups were pooled to compare among treatments.

*Seed germination trials*

Seeds from five plants within each treatment (vertebrate excluded and open pollination) at four sites were used to assess the effects of different pollinator types on germinability (Helensburgh, Royal site 1, Dharawal site 1 and Budderoo site 1). From each plant 75 seeds were randomly selected, giving a total of 375 seeds per treatment at each site. In order to discern the number of germinable seeds and the rate of germination, seeds were placed in petri dishes on moistened filter paper, sealed and then placed in an incubator on a 12 hour light/dark and 25°C/18°C temperature cycle to
simulate mean day/night summer temperatures of the region (Ooi et al. 2014). Dishes were checked every two days for a total of 25 days, and germination scored based on emergence of the radicle. At the end of the trial period, any seeds that failed to germinate were tested for viability using tweezers to discern the hardness of the seed, followed by a cut test. Any soft or mouldy seeds as determined from the cut test (Ooi et al. 2004) were discarded and scored as inviable. Total germination at the end of the trial was then calculated as the percentage of viable seeds. The time to reach 50% germination ($T_{50}$) was also calculated by plotting cumulative germination against time and fitting either a linear or quadratic model to the data, and solving the equation for $x = 0.5$.

**Growth rate of seedlings and emergence of the cotyledons**

In order to measure seedling growth rates, seeds from each of four sites (Helensburgh, Royal site 2, Dharawal site 1 and Dharawal site 2) were used to assess the effects of different pollinator types on the growth rate of seedlings and the time till emergence of the cotyledon. Twenty seeds in total from each of the two treatments at each site were randomly selected. The twenty seeds were then divided into two groups of ten, with one group from each treatment sown in each of two pots to account for potential pot effects. The timing of emergence of the cotyledon was recorded and seedling height was compared two weeks after germination.

**Data analysis**

Pollinator observation data were analysed using t-tests and chi square tests. To test for significant effects of site and treatment for all other experiments, we used Generalized Linear Models or ANOVA. Seed set was analysed using a 2-factor GLM with quasi-Poisson distribution and log-link function, to account for overdispersion of the data. Seedling height data were normally distributed and were therefore analysed using a GLM with a Gaussian distribution. Time to emergence of the cotyledons was analysed using a GLM with a Poisson distribution with a log-link function. Seed
viability and germination data were analysed using a 2-factor GLM with binomial
distribution and logit link function. Seed weight data fitted the assumptions of normality
and homogeneity of variances and were analysed using a 2-factor ANOVA. Results are
presented as means ± 1 SE unless otherwise noted.

2.4 Results

Identification and frequency of diurnal flower visitors

The most common flower visitor observed on open inflorescences of B. ericifolia was the European honeybee A. mellifera (n =146) (both nectar and pollen
gatherers) which was observed to visit all of the study plants and 344 study
inflorescences. Honeybees were found to make similar numbers of visits to that of all
other flower visitors combined, with little variation among sites (range = 1 to 3 plants
and range = 1 to 7 inflorescences visited per site). The only other insect visitors that we
observed were ants (Formicidae species) (n = 31) and flies that appeared to be
Muscidae species (n = 8). The diversity and number of insects visiting the vertebrate-
exclusion treatment was almost identical, with no additional species recorded.

Open inflorescences were frequently visited by bird species with 97% of
observed plants visited by avian pollinators (seven honeyeater species). During the
study period, birds were observed to make 161 visits to study plants and made 339 visits
to study inflorescences. The bird species observed were; New Holland Honeyeater
(Phylidonyris novaehollandiae) (n = 21), Silvereye (Zosterops lateralis) (n = 82),
Eastern Spinebill (Acanthorhynchus tenuirostris) (n = 2), Whistler (Pachycephala sp) (n
= 5), Brush Wattlebird (Anthochaera chrysoptera) (n = 9), Superb Blue Wren (Malurus
cyaneus) (n = 7) and Yellow Faced Honeyeater (Lichenostomus chrysops) (n = 9). Total
numbers of bird visits varied across sites, ranging from 8 to 61 and 8 to 178 for plants
and inflorescences respectively.
Identification and frequency of nocturnal flower visitors

During a total of 144 hours of observations at each site in which an average of $57 \pm 8.5$ newly opened inflorescences were observed, we detected no nocturnal flower visitors at any of the study sites.

Effect of treatment on the frequency of flower visitation by insects and the foraging behaviour of pollinators

*Apis mellifera* was by far the most frequent invertebrate visitor and the only one foraging in a manner likely to affect pollination (but only when pollen gathering – see below). Broadly similar visitation rates were made by *A. mellifera* (both nectar and pollen gatherers) to plants with vertebrate exclusion ($15.6 \pm 5.7$) and to the open treatment ($20.9 \pm 4.4$) ($t_{(6)} = 1.25, p = 0.25$) (Table 2.1, below). Moreover, the average number of honeybees foraging for pollen and hence acting as pollinators also did not vary significantly among treatments (vertebrate-exclusion treatment, $4 \pm 0.90$, open treatment $6.86 \pm 1.71$) ($t_{(6)} = 1.32, p = 0.23$). Across all seven sites, foraging individuals of *A. mellifera* (both nectar and pollen gatherers combined) made similar numbers of within plant movements among inflorescences irrespective of treatment (vertebrate-exclusion $2.2 \pm 0.1$, $n = 93$; open treatment $1.6 \pm 0.1$, $n = 75$; $t_{(6)} = 0.24, p = 0.81$)
Table 2-1 Results from observations of flower visitation and behaviour for the open and vertebrate-excluded treatments. Data are means (± 1 standard error) per 10 minute observation period (N = 7)

<table>
<thead>
<tr>
<th>Description</th>
<th>Vertebrate exclusion</th>
<th>Open</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. mellifera</em> total visits per plant (nectar and pollen gathering)</td>
<td>15.6 ± 5.7</td>
<td>20.9 ± 4.4, n=146</td>
<td><em>t</em>(6) = 1.25, <em>p</em> = 0.25</td>
</tr>
<tr>
<td><em>A. mellifera</em> visits per plant (pollen gathering only)</td>
<td>4 ± 0.90</td>
<td>6.86 ± 1.71</td>
<td><em>t</em>(6) = 1.32, <em>p</em> = 0.23</td>
</tr>
<tr>
<td><em>A. mellifera</em> intra plant movements</td>
<td>2.2 ± 0.1, n = 93</td>
<td>1.6 ± 0.1, n = 75</td>
<td><em>t</em>(6) = 0.24, <em>p</em> = 0.81</td>
</tr>
<tr>
<td>Bird visits per plant</td>
<td>N/A</td>
<td>19.3 ± 6, n=135</td>
<td>N/A</td>
</tr>
<tr>
<td>Bird intra plant movements</td>
<td>N/A</td>
<td>1.9 ± 0.1, n=109</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**Bird species**

During 40.8 hrs of observations conducted on bird species, 0.55 birds per 10 minute observation period were recorded foraging on *B. ericifolia* (n = 35). Birds on average made 1.9 ± 0.1 intra-plant movements (Table 1).

**Comparison of foraging behaviour of birds and honeybees**

Overall we observed more honeybees (both nectar and pollen gatherers) visiting inflorescences (146) than birds (135) in the open-pollination treatment. However, birds were clearly more common pollinators than honeybees. All 135 birds that we observed foraged in a manner in which it was likely that they contacted the pollen presenter (which surrounds the stigma) while only 48 honeybees foraged for pollen and in a manner likely to affect pollination. Nectar foraging honeybees avoided contacting reproductive parts by collecting nectar at the base of the flowers. Ants were never observed touching pollen presenters and both ants and flies were only observed gathering nectar. As a result, it is likely that *A. mellifera* is the only observed invertebrate pollinator as both ants and flies did not have a foraging behaviour likely to
induce pollination. Moreover, slightly more birds made intra-plant movements among inflorescences than pollen collecting honeybees (75 vs. 36) within the open treatment. These honeybees, however, moved 10% more frequently among inflorescences within plants than birds ($t_{(6)} = 2.76$, $p = 0.03$). Importantly, the movements of birds would be expected to produce more outcrossing as they displayed a significantly greater proportion of inter-plant movements (26 of 135 birds observed moved among plants cf. 4 of 48 for pollen collecting bees) ($\chi^2_{(1)} = 4.23$, $p = 0.039$). The time spent foraging on inflorescences differed between nectar and pollen collecting honeybees and birds. Nectar gathering honeybees on average spent 104 seconds $\pm 11.5$, pollen gathering honeybees spent 54 seconds $\pm 6.5$, compared to birds that spent 5 seconds $\pm 0.2$.

**Effect of treatment on seed set and seed weight**

We found no consistent relationship for effects of treatment on the percentage of inflorescences that set seed (Fig. 2.1). The GLM analysis revealed no significant interaction between site and treatment ($\chi^2_{(6)} = 6.08$, $p = 0.41$) and no main effects. Our experiment also revealed no consistent effect of vertebrate exclusion on the number of seeds set (Fig 2.2). On average the open pollinated inflorescences produced more seed at five of seven sites but this difference was significant only at Budderoo site 1. GLM analysis revealed no significant interaction between treatment and site ($\chi^2_{(6)} = 151$, $p = 0.48$).

Mean ($n = 30$) seed weight did not vary markedly with site or treatment (range 21.5-27.2 mg across all sites for both the vertebrate-exclusion and open treatments). The interaction between site and treatment was significant ($F_{7, 430} = 10.102$, $p = <0.0001$) with the seeds in the open treatment significantly heavier than those in the vertebrate-exclusion treatment at the Helensburgh site (Fig. 2.3). They were also heavier than seeds from all other sites.
Figure 2-1. The percentage (%) of inflorescences that set seed for the two treatments (open and vertebrate-exclusion) at each site.

Figure 2-2. The mean number of seeds (± 1 standard error) produced per plant in each of two treatments, open (■) and vertebrate-exclusion (□), at each of the seven study sites.
Figure 2.3. The average seed weight (mg) (□ 1 standard error) for the open treatment (■) and vertebrate-exclusion treatment (□) at each site. The number of seeds weighed per treatment were: Buderoo S1 open n=150, vertebrate excluded n=94; Dharawal S1 open n=117, vertebrate excluded n=143; Royal S1 open n=118, vertebrate excluded n=141; Helensburgh open n=90, vertebrate excluded n=128. Different letters above bars denote significant differences (p > 0.05; Tukey’s HSD test).

Effect of treatment on germination

Across all sites and treatments, seed viability (93% - 99.5%) and germinability (98.2% -100%) were high and there was no significant effect of site or treatment on either (viability \( \chi^2(7) = 4.356, p = 0.738 \); germinability \( \chi^2(7) = 3.89, p = 0.792 \)). For germination rate, the mean time taken to reach 50% germination (T\(_{50}\)) ranged from 8 ± 0.5 days at Buderoo site 1 for both treatments to 12 ±1 days for the open treatment at Royal site 1 (Fig. 2.4). Site had a significant effect on T\(_{50}\) (F\(_{3,101} = 15.17, p<0.0001\) but there was no significant difference in T\(_{50}\) between treatments at each site.
Figure 2-4. The mean $T_{50}$ (the time required to reach 50% germination) in days (± 1 standard error), for Banksia ericifolia seeds at four study sites, comparing the open (■) and vertebrate-excluded (□) treatments. Different letters above bars denote significant differences.

Effect of treatment on growth rate of seedlings and emergence of the cotyledon

GLM analysis revealed that there were no significant differences between treatments or sites for seedling growth. Average height in the open treatment was 25.9mm ± 1.6 (n = 39) compared to 24.6mm ± 1.01 (n = 46) for the vertebrate-exclusion treatment. There was also no significant effect of site or treatment on the numbers of days to emergence of the cotyledons (open treatment, 26.1 ± 0.5 (n = 31); vertebrate-exclusion treatment, 26.4 ± 0.4 (n = 29)).

2.5 Discussion

Plants that are considered to be adapted to vertebrate pollination are now increasingly visited by the invasive pollinator A. mellifera (Paton and Turner 1985; Vaughton 1992; Hansen et al. 2002). Nevertheless the consequences of this phenomenon are poorly understood (Traveset and Richardson 2006). Our findings
support those of other studies that have found that the foraging behaviour of birds and honeybees differ in regard to length of foraging bouts, exploitation of floral rewards (Hansen et al. 2002) and, importantly, the proportion of intra and inter-plant movements (Paton 1993). Our data also support earlier studies showing that honeybees make fewer inter-plant movements and more intra-plant movements as compared to avian pollinators (Richardson et al. 2000; Whelan et al. 2009). The difference in foraging behaviour of bird and honeybee pollinators might be expected to influence plant fitness by reducing seed set and altering the genotypic composition of seed produced. Most significantly, our study, which is the first to experimentally evaluate these predictions by using a vertebrate exclusion experiment, found no clear evidence that either seed set or seed quality were reduced when inflorescences were pollinated by honeybees.

Flower visitation and foraging behaviour

As might be expected for a ‘vertebrate-adapted’ species, birds were the most common and presumably most effective pollinators of B. ericifolia due to the way they foraged on inflorescences, contacting the reproductive parts of the plant. Earlier studies of the pollination of Australian plants suggested that the importance of honeybees as pollinators is hard to evaluate and may frequently be overestimated because the foraging behaviour and morphology (body size relative to flower size and shape) of honeybees typically leads to them removing both nectar and pollen, without pollen transfer (Gross and Mackay 1998; Richardson et al. 2000). In Grevillea macleayana which shares a similar floral morphology to B. ericifolia it was found that nectar gathering honeybees were able to actively avoid touching the reproductive parts of the plant and therefore were thought to contribute less to the pollination than pollen gathering honeybees (Whelan et al. 2009). In this study, the majority of bees foraged only for nectar and this behaviour may make inflorescences less attractive to all other effective pollinators. When foraging for pollen on B. ericifolia, honeybees inevitably contact the stigma because, before flowers open, the pollen is deposited onto the stigmatic surface as a pollen presenter (Ayre and Whelan 1989). However, as is typical of foraging honeybees, most pollen is gleaned from their bodies and deposited in corbiculae where it is not available for pollination (Hargreaves et al. 2009).
In all *B. ericifolia* populations examined in this study, honeybees were found to be the most common flower visiting species (although less numerous than the total set of flower visiting birds). However, this clearly overestimated their importance as pollinators as only 30% of honeybees were foraging for pollen. Nectar gathering honeybees that visited *B. ericifolia* avoided contacting pollen presenters by accessing flowers at their base and gathering nectar that ran down the core of the inflorescence. Similarly, Paton (1993) found that for the South Australian *Callistemon rugulosus*, honeybees harvesting nectar only contacted the stigma in 4.4% of 8000 visits compared to pollen harvesting honeybees which contacted the stigma in 16.7% of 1649 visits. In contrast, nectar-foraging birds contacted the stigma more than 50% of the time.

The contribution of non-flying mammals to pollination in our open-pollination treatment is difficult to assess. Although earlier work identifies both birds and non-flying mammals, especially *Antechinus flavipes*, *Melomys burtoni* and *Rattus tunneyi* as pollinators of *B. ericifolia* (Hackett and Goldingay 2001), our study did not detect any inflorescence visitation by non-flying mammals or moths despite the use of both human observations and infrared cameras. While our failure to capture images of marsupial pollinators could reflect insufficient trapping effort (e.g. Goldingay et al. 1991b), it is likely that their local densities are low (*M. burtoni* and *R. tunneyi* do not occur in the study area) and hence they would not significantly influence pollination of the inflorescences in our study.

For many plant species, pollinator effectiveness will be determined by both the quantity and quality of pollen transferred and this will in turn vary with the degree of self-compatibility and the spatial genetic structure of the plant populations (Burley and Willson 1983; Waser 1993; Holmes et al. 2008). For *B. ericifolia*, birds and honeybees were observed to make a majority of intra-plant movements, with both likely to transfer pollen within and among inflorescences on each plant visited. This pattern of self-pollen transfer is likely to produce less seed set than among plant movements since this species is at least partially self-incompatible (Carthew et al. 1996). However, in common with observations for many other Australian Proteaceae, the foraging behaviour of birds and honeybees differed, with birds making greater numbers of inter-plant movements and
hence expected to transfer more outcross pollen (England et al. 2001; Whelan et al. 2009). Moreover birds are more likely than bees to move pollen among more distantly separated plants within populations or among neighbouring populations and hence may deliver more suitable pollen than honeybees.

There appear to be few, if any, comparable observations of the contrasting effects of bird and insect pollination in predominantly bird pollinated African Proteaceae, although Steenhuisen et al. (2012) report that for the largely insect pollinated and autogamous *Protea caffra*, outcrossing rates do not vary when vertebrates are excluded.

**Effects of vertebrate exclusion on seed set and performance**

The results of this study confirm that honeybees can be effective pollinators of *B. ericifolia* as has been reported for a range of other Proteaceae (Vaughton 1992; Richardson et al. 2000; Whelan et al. 2009). For six of seven sites, similar levels of infructescence production and seed set were produced on open pollinated plants that received similar visitation by birds and honeybees, and on vertebrate-excluded plants that were almost exclusively visited by honeybees. The one exception was found at Buderoo site 1, where open treatments produced greater seed set than exclusion treatments. This was likely to be a result of comparatively few honeybees present at this site.

Importantly, across all seven sites, inflorescences assigned to an autogamy treatment did not set seed, demonstrating the need for a pollen vector. We detected low overall seed set, with many inflorescence setting no seed, which is consistent with other studies of *B. ericifolia* (Paton and Turner 1985; Carthew et al. 1996) and Proteaceae in general (Ayre and Whelan, 1989). This pattern has been used to argue that *Banksia* may display high levels of mate choice to compensate for variation in the quality of pollen transferred by different pollinators (Ayre and Whelan 1989; Goldingay and Carthew et al. 1988). Nevertheless, our finding that the vertebrate-excluded inflorescences did not produce fewer seeds is surprising since Carthew et al. (1996) provide experimental
evidence that inflorescences given both self and outcross pollen predominantly set seed from outcross pollen.

Perhaps the most surprising outcome of our study, given the reduced pollen diversity expected within the vertebrate exclusion treatment, was that seed quality and early seedling performance were again little affected by treatment. Pollen transfer between neighbouring subpopulations has been shown to increase seedling performance in some other self-incompatible Proteaceae (Holmes et al. 2008; Forrest et al. 2011; but see Ayre and O’Brien 2013) and again we expected this to be facilitated by bird but not honeybee visitation. We detected similarly high levels of germination success and viability and similar time to germination, emergence from the cotyledon and seedling growth. Overall the similar reproductive success and early performance of seed from plants visited only by honeybees, as compared with those visited by both birds and bees, implies that within most sites seed set is limited by resource availability (Ayre and Whelan 1989) rather than pollen quantity or quality.

Contrary to our expectations, we found no evidence that pollination by honeybees has a detrimental effect on the fitness of *B. ericifolia*. Our study is the first to test experimentally whether introduced honeybees are having an impact on seed or seedling fitness in a bird-adapted plant species. Without such studies, understanding the true impact of honeybees remains speculative. Although we acknowledge that the effects of our pollination treatment on seedling vigour may not be detectable until later stages of the life-history, we predict that most seeds set are outcrossed as observed for this species by Carthew *et al.* (1996). Further studies are needed to determine whether the effect of honeybee pollination appears equally benign when plants are pollen rather than resource limited, or for species that display higher levels of self-incompatibility.
References


Hermansen TD, Britton DR, Ayre DJ, Minchinton TE (2014) Identifying the real pollinators? Exotic honeybees are the dominant flower visitors and only effective pollinators of *Avicennia marina* in Australian temperate mangroves. *Estuaries and Coasts* **37**, 621-635.


3 The use of Digital Video Recorders in pollination biology

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3.1 Abstract

1. Digital Video Recording (DVR) devices, such as the GoPro Hero, have the potential to greatly benefit pollination ecology, but the advantages of digitally recording pollinator activity over direct human observation has not been formally assessed.

2. We used two plant taxa, *Lavandula angustifolia* and *Canna* sp. with differing floral morphology, to compare the value of DVR and direct observations in estimating honeybee (*Apis mellifera*) visitation, flower density and number of flowers visited per foraging bout.

3. We found that the two methods yielded identical results when observing the structurally simple *L. angustifolia* at both high (10.54 ±0.52 per plant) and low honeybee density (2.24 ±0.20 per plant). However, DVR underestimated the number of flowers scored in the field of view (28.7 ±1.8 direct vs. 22.7 ±0.9 DVR), the number of honeybees observed (5.3 ±0.8 direct vs. 3.7 ±0.7 DVR) and the number of flowers visited during foraging bouts (8.3 ±1.2 direct vs. 5.5 ±1.0 DVR) on the more complex *Canna* sp.

4. We conclude that portable weatherproof DVR devices such as the GoPro Hero are valuable tools for pollination biologists allowing a single researcher to make simultaneous observations of multiple plants, in one or more sites, whilst also allowing the footage to be reviewed. However, DVR devices are limited by their depth and field of view when target plants are large or structurally complex.

Key words: GoPro, action camera, remote monitoring, pollinator observations, *Apis mellifera*, pollination biology.
3.2 Introduction

In recent years the volume of research on pollination has increased substantially in response to widespread evidence of pollinator declines (Kearns et al., 1998; Potts et al., 2010; Carvalheiro et al., 2013; Gonzalez-Varo et al., 2013), understanding pollinator response to anthropogenic land-use (Winfree et al., 2009) and invasions of pollination systems by exotic generalist pollinators such as *Apis mellifera* (Hermansen et al., 2014; Gilpin et al., 2014) and *Bombus* spp (Goulson 2003; Nagamitsu et al., 2010). In an effort to determine the effects of such changes, a range of large-scale pollinator observation studies have been conducted, which require significant outlay and investment of research time spent in the field (e.g., Winfree et al., 2008; Holzschuh et al., 2016). Coincidentally, in recent times there has been widespread and growing development and availability of cost effective, weather resistant action video cameras that may provide improved opportunities for researchers to make valid and accurate assessments of pollinator assemblages, abundance or behaviour, with less investment of time, allowing greater replication over a broader scale.

To ensure that a sampling technique is appropriate for ecological study, researchers must demonstrate that the sampling method provides an accurate representation of the patterns in nature and generate sufficient data to assess treatment effects whilst minimising associated biases. Direct observation techniques have long been considered an effective sampling technique and continue to be an effective tool in the monitoring of pollinator behaviour (e.g. Kaiser-Bunbury et al., 2010; Hermansen et al., 2014; Gilpin et al., 2016). However, this approach is highly labour intensive and is also limited by observer bias (Westphal et al., 2008; Popic et al., 2013). Digital video
recording (DVR) has the potential to allow more efficient observation, with a single researcher able to conduct multiple simultaneous observations over a range of temporal and spatial scales. In recent times, cameras and video recordings have been used in pollination biology (Bumrungsri et al., 2009; Steen and Thorsdatter Orvedal Aase 2011; Lihoreau et al., 2012; Lortie et al., 2012; Nakase and Suetsugu 2016; Steen 2016), with the optimum technique dependent upon the type of flower visitor expected and the study plant morphology. Steen (2016) demonstrated the applicability of automatic camera monitoring of pollinators, utilising a motion detection script in a small scale (single inflorescence) survey. Our study aims to extend such research by establishing the spatial constraints and capabilities of action video cameras by explicitly comparing the effectiveness of DVR devices to human observations.

Simultaneous observation of multiple plants or populations is often critical in pollination biology, as both the behaviour of pollinators and the intensity and nature of floral display undergo daily or even hourly variation (Herrera 1995). Simultaneous recording by multiple human observers requires significant labour and may introduce ‘noise’ through observer bias (Westphal et al., 2008). Using modern DVRs it is possible for a single researcher to conduct multiple, simultaneous surveys across a range of target plants at one or more sites. Digital data can be stored and examined at a later date with the observer having the ability to fast forward through periods without pollinator activity or pause and replay important sequences when observing the detailed behaviour of one or more individual pollinators.

The three dimensional nature of flowers and plants together with the often rapid movement of floral visitors can reduce the accuracy of estimates of pollinator activity
both by direct observation and DVR methods. In other fields of ecology, studies have utilised action video cameras to document rare (Kadaba 2014) and elusive animal species (Murphy et al., 2016), and the ongoing development of action video cameras in conjunction with portable user-friendly drones provides opportunities for “near real-time mapping of local land cover, monitoring of illegal forest activities, and surveying of large animal species” (Koh and Wich (2012).

To test whether DVR is an appropriate sampling method for pollination biology we tested the hypotheses: (a) that under high levels of floral visitation, direct observation will be less accurate and will both underestimate the number of visitors present and allow observation of fewer foraging bouts than DVR methods; and (b) as the size and complexity of floral structures increase, the accuracy of DVR is reduced. These predictions are a result of the more limited field of view and depth of focus of the camera compared to human eyes.

3.3 Materials and Methods

Study species

We studied pollinator visitation to *Lavandula angustifolia* X, and *Canna* sp. These taxa were selected because they differ in size and structural complexity. *Lavandula angustifolia* plants were much smaller and characteristically simpler. Their inflorescences typically protrude above the leaves allowing for better visibility of flowers. Insects only gather resources on the outside of inflorescences and thus remain visible at all times. In contrast, *Canna* plants have more complex flower morphology and floral visitors typically have to burrow among the flower petals to secure resources.
thus becoming hidden from view. Although they were not specifically targeted by us, *Apis mellifera* were the only floral visitors to either plant taxon during our experiments. Since honeybees are medium sized invertebrate floral visitors and commonly visit many plant species, they should represent a suitable ‘model’ organism for comparison.

**DVR - GoPro™ apparatus**

The GoPro Hero 3 Silver Edition (2013, gopro.com) DVR with waterproof housing was used throughout. The camera was fitted with a 16GB micro SD card. Cameras were set to record in full HD (1920 x 1080 pixels). This camera was selected due to the high popularity and widespread availability of the device worldwide. A range of similar cameras exist, however, the GoPro line of cameras provide a benchmark for weatherproof, portable and affordable digital recording devices. Analysis of recording was undertaken using Windows Media Player™.

**Experimental design:**

*Comparison of DVR and direct human observations under low and high honeybee densities.*

A pilot determined that a site at Blackheath, N.S.W, Australia (33°37’44.6”S 150°17’05.8”E) had relatively more honeybees than at the Environmental Research Centre, University of Wollongong, N.S.W, Australia (34°24’17.3” S 150°52’18.3”E). Hence we used these sites to investigate the effect of honeybee density upon the accuracy of each observation method under high and low honeybee densities during spring 2015. Forty *L. angustifolia* plants were placed in four rows of 10 plants spaced at
one meter intervals. The number of sexually mature inflorescences (as determined by the presence of open, vibrantly coloured flowers) on each plant was recorded, as was the width, depth and height of the entire test plants. Inflorescence number was not manipulated in order to expose possible limitations of the camera’s depth of field compared to human observations (three observers at Blackheath, but only one of these at Wollongong). This allowed the results obtained in the low honeybee density experiment to be compared against the more structurally complex *Canna* sp. as outlined in the experiment below. Of the 40 plants present at each site, 25 were randomly selected for 20 minute simultaneous observations using both human and DVR techniques. Observations were conducted daily for four concurrent days and totalled 33.3 hours of observation time, at each site, for each observation technique. The number of honeybees at commencement of observations, the number of honeybees present each minute up to 20 minutes, the number of inflorescences visited during a foraging bout and the number of sexually mature inflorescences that could be observed was recorded using each observation technique. A single foraging bout was defined as the interval between a pollinator arriving at and leaving an individual plant or study area.

*Comparison of DVR and simultaneous human observations upon simple and complex plant morphologies.*

To establish the manner in which complexity of flower and plant morphology impacts the accuracy of documenting pollinator behaviour made through DVR and human observations, the following experiment was conducted.
Canna sp were selected as a target plant due to their large size and complex floral array compared to the reduced size and floral complexity of L. angustifolia. Ten plants were randomly selected per day from a population of hundreds within the Wollongong Botanic Gardens, N.S.W, Australia (34°24′35.2″ S 150°52′29.4″ E) during early summer 2016. The number of sexually mature flowers (as determined by the presence of open, vibrantly coloured flowers) on each plant was recorded, as was the width, depth and height of the test plants to allow the results to be compared against plant volume. Simultaneous human and DVR observations were made for 20 minute periods. Visitation data were scored as per the previous experiment. Ten observations were conducted each day across two concurrent days, amounting to 6.66 hours of observation time for both human and DVR techniques.

The results obtained in the previously described L. angustifolia experiment were compared and contrasted against those obtained through the observation of Canna sp. to determine the effect of plant structure upon the success of DVR.

Data analysis

Comparison of DVR and direct human observations under low and high honeybee densities.

Generalised Linear Models (GLM’s) (developed using R software) with a quasi-Poisson distribution to account for overdispersion and log link function were used to test for differences in pollinator behaviour between the two observation methods, in relation to the number of honeybees at commencement of observations and number of flowers observed. GLM with Poisson distribution and log link function were used to test
for differences in the mean number of honeybees observed, and the number of inflorescences visited during a foraging bout. All data were analysed using the open source R 3.0.3 statistical platform (R Core Development Team., 2014) and the package Car (Fox and Weisberg 2011). All GLMs had fixed effects of observation method (human or DVR), treatment (high or low honeybee density) and plant ID.

Comparison of DVR and simultaneous human observations between differing plant morphological complexity.

We used paired t-tests (using Microsoft Excel) to determine whether the two observation methods differed for each plant species in relation to the mean number of honeybees at commencement of observations, the mean number of flowers observed, the mean number of flowers visited per foraging bout and the mean total number of honeybees. Assumptions of normality were tested by examining plots and a Shapiro-Wilks test. To verify homogeneity of variances a Levene’s test were performed. The mean number of flowers visited per foraging bout and the mean total number of honeybees were normally distributed after a square root transformation.

3.4 Results

Comparison of DVR and direct human observations under low and high honeybee densities.

The mean honeybee visitation rates recorded through playback of DVRs and direct observation of L. angustifolia plants (n= 50 for each technique and for each treatment) were identical at both high and low density. There was no variation among
observers. GLM revealed no significant interaction between observation method and density treatment although there was a significant difference between the two honeybee density treatments ($\chi^2_{(1)} = 293, p < 0.001$) and plant ID ($\chi^2_{(1)} = 14.8, p = 0.0001$).

There was no significant difference between the number of flowers that were directly observed and DVR recorded for both low and high honeybee density treatments. There was also no significant interaction between observation method and density treatment in respect to the number of flowers observed (Table 3.1). Similarly, the two methods revealed identical numbers of honeybees on commencement of observation at each of the test plants, regardless of honeybee density (Table 3.1).

We found that playback of DVR, in contrast with direct observation, allowed detailed observation of multiple foraging bouts under the high honeybee density with slightly more foraging bouts recorded by DVR compared to directly observed (Table 3.1). We also found a significant difference in honeybee density ($\chi^2_{(1)} = 293, p < 0.0001$) and plant ID ($\chi^2_{(1)} = 15.5, p < 0.0001$) in respect to the number of inflorescences visited.
Table 3-3 A comparison of human observations and Digital Video Recordings of the study species *L. angustifolia* X in relation to honeybee visitation and foraging behaviour when observations are undertaken simultaneously under high and low honeybee density.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Direct</th>
<th>DVR</th>
<th>Test and significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Method</td>
<td>Density</td>
<td>Plant ID</td>
</tr>
<tr>
<td>Number of honeybees on commencement (L)</td>
<td>0.12</td>
<td>0.12</td>
<td>$\chi^2_{(1)} = 0$, p = 1</td>
</tr>
<tr>
<td></td>
<td>±0.054</td>
<td>±0.054</td>
<td></td>
</tr>
<tr>
<td>Number of honeybees on commencement (H)</td>
<td>0.18</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>±0.054</td>
<td>±0.054</td>
<td></td>
</tr>
<tr>
<td>Number of flowers observed (L)</td>
<td>32 ±1</td>
<td>32 ±1</td>
<td>$\chi^2_{(1)} = 0.34$, p = 0.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of flowers observed (H)</td>
<td>36 ±1</td>
<td>34 ±1</td>
<td></td>
</tr>
<tr>
<td>Mean number of honeybees (L)</td>
<td>2.24</td>
<td>2.24</td>
<td>$\chi^2_{(1)} = 0.00$, p = 1</td>
</tr>
<tr>
<td></td>
<td>±0.20</td>
<td>±0.20</td>
<td></td>
</tr>
<tr>
<td>Mean number of honeybees (H)</td>
<td>10.54</td>
<td>10.54</td>
<td>$\chi^2_{(1)} = 0.00$, p = 1</td>
</tr>
<tr>
<td></td>
<td>±0.52</td>
<td>±0.52</td>
<td></td>
</tr>
<tr>
<td>Number of inflorescences visited (L)</td>
<td>2.24</td>
<td>2.24</td>
<td>$\chi^2_{(1)} = 0.16$, p = 0.69</td>
</tr>
<tr>
<td></td>
<td>±0.20</td>
<td>±0.20</td>
<td></td>
</tr>
<tr>
<td>Number of inflorescences visited (H)</td>
<td>9.94</td>
<td>10.54</td>
<td></td>
</tr>
<tr>
<td></td>
<td>±0.48</td>
<td>±0.52</td>
<td></td>
</tr>
</tbody>
</table>

(L) = low honeybee density. (H) = high honeybee density.
Comparison of DVR and simultaneous human observations between differing plant morphological complexity.

When comparing the number of honeybees on commencement of observations, the number of flowers observed, the total number of honeybees and the number of flowers visited through digital recording and human observations, we found the results to be identical for the less morphologically complex *L. angustifolia*. There was no variation among observers. When plant size and complexity was increased, using the study plant *Canna* sp, we found significant differences between DVR and direct observations in relation to the number of flowers that were observable (*t* (19) = 4.46, *p* = 0.0003), the total number of honeybees recorded during the observation period (*t* (19) = 4.19, *p* = <0.001) and the number of flowers visited by a foraging honeybee (*t* (19) = 5.38, *p* = 0.0001) (Table 3.2). The average volume of the larger and more complex *Canna* sp. was 0.73 m³ ±0.07 m³ compared to the small and less complex *L. angustifolia* 0.049 m³ ±0.002 m³ used in the other experiments. Additionally, we found that direct observations and DVR methods produced identical results in relation to the number of honeybees that were foraging on the focal plant at the commencement of recording (Table 3.2).
Table 3-2 A comparison of digital video recording (DVR) and simultaneous human (Direct) observations in relation to honeybee visitation and foraging behaviour for different plant morphologies.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Direct</th>
<th>DVR</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of honeybees on commencement (c)</td>
<td>0.1 ±0.07</td>
<td>0.1 ±0.07</td>
<td>ns</td>
</tr>
<tr>
<td>Number of honeybees on commencement (s)</td>
<td>0.12 ±0.05</td>
<td>0.12 ±0.05</td>
<td>ns</td>
</tr>
<tr>
<td>Number of flowers observed (c)</td>
<td>28.7 ±1.8</td>
<td>22.7 ±0.9</td>
<td>$t_{(19)} = 4.46$, $p = 0.0003$</td>
</tr>
<tr>
<td>Number of flowers observed (s)</td>
<td>32.3 ±1.1</td>
<td>32.2 ±1.1</td>
<td>ns</td>
</tr>
<tr>
<td>Number of honeybees (c)</td>
<td>5.3 ±0.8</td>
<td>3.7 ±0.7</td>
<td>$t_{(19)} = 4.19$, $p = &lt;0.001$</td>
</tr>
<tr>
<td>Number of honeybees (s)</td>
<td>2.24 ±0.20</td>
<td>2.24 ±0.20</td>
<td>ns</td>
</tr>
<tr>
<td>Number of flowers visited (c)</td>
<td>8.3 ±1.2</td>
<td>5.5 ±1.0</td>
<td>$t_{(19)} = 5.38$, $p = 0.0001$</td>
</tr>
<tr>
<td>Number of flowers visited (s)</td>
<td>2.24 ±0.20</td>
<td>2.24 ±0.20</td>
<td>ns</td>
</tr>
</tbody>
</table>

(c) = florally complex and larger plant species (Canna sp.). (s) = less florally complex and relatively small species (L. angustifolia X ). ns = not significant.
3.5 Discussion

The surging global popularity of action cameras such as GoPros has resulted in low cost units with powerful capabilities. Such features as durability, high waterproof rating, high definition resolution, ease of use and long battery life has led to up-take of the technology across many disciplines such as videography, action sports and growing use in biological applications. When compared to video units available only a decade ago, GoPros are far more powerful and substantially cheaper than equivalent older model waterproof cameras. As such they have gained wide-spread use in marine research documenting trawl net interactions with wildlife (Ferrari et al., 2015), monitoring fish assemblages (Letessier et al., 2013) and even documenting seagrass dispersal (Darnell et al., 2015). Terrestrial uses for the technology are growing with documentation of prey cue preferences in the dusky pygmy rattlesnake (Sistrurus miliarius barbouri) (Holding et al., 2016), the feeding behaviour of large mammals (Owen et al., 2015) and some use in pollination biology (Steen and Thorsdatter Orvedal Aase 2011; Lortie et al., 2012; Edwards et al., 2015; Nakase and Suetsugu 2015). The nature of the technology and the ongoing development of the cameras capabilities leads to the application of action video cameras being limited only by the researcher’s imagination.

This study highlights the capacity of DVR devices, such as, the GoPro to enhance studies of pollination and pollinator behaviour. As expected, we found that DVRs provided the opportunity for a single researcher to simultaneously gather data for multiple plants that were identical to data gathered through direct observation. Most critically, the ability to pause and re-play images of pollinator visitation meant that DVRs, in contrast to data gathered through direct observation, could be used to
document the detail of foraging behaviour for multiple simultaneously active floral visitors. The advantages of DVR therefore increase with pollinator density although the amount of time spent processing and analysing video recordings may increase according to the complexity of the pollinator network. The applicability of our methods may be less useful for smaller floral visitors since smaller bee and fly species are more difficult to distinguish and their behaviour is more challenging to ascertain.

Our study also shows that the use of DVR’s is limited when study plants are large and structurally complex. When compared to the results obtained upon the structurally simple *L. angustifolia*, the more morphologically complex *Canna* sp. was more effectively recorded through direct observation than DVR, with respect to the number of observable flowers, the number of flowers visited and the total number of honeybees. Although, there was a greater observation time on simple compared to complex target plant species in our experiment, given that observation conditions were ideal during both experiments, we expect no changes to the patterns shown with an extension of the observation period on the complex target plants. Rather, these limitations reflect the limited depth and field of view of the GoPro. Honeybees that visited *Canna* sp often foraged deep within the plant and were often concealed from view. The limited depth of focus provided by the GoPro’s meant that direct observation was more reliable in determining whether the honeybee was indeed contacting the reproductive parts of the flower, due to the ability of the observer to move, change perspective or angle during an observation to gain an accurate measure of pollinator behaviour. Researchers intending to use DVR techniques on an entire structurally complex plant, rather than measure a subset of flowers, should use a number of cameras
per plant to ensure all flowers are captured in focus. In this case, we recommend labelling individual flowers in order to differentiate them during playback.

Whilst providing the opportunity to conduct multiple simultaneous observations of foraging bouts, the duration of observation achievable using GoPro is arguably limited by relatively short battery life (1.5-2 hours) and the storage space available on micro SD cards. Hence recording for longer periods requires intervention at frequent intervals. Additional battery packs and larger SD cards can extend the run time of the GoPro (we recommend using 16GB micro SD card or larger in order to allow for a recording period that exceeds the battery life depending on the resolution settings). However, the process of exchanging a full SD card and drained battery with an empty SD card and charged battery takes only approximately 30 seconds. We recommend using action cameras for experiments where highly detailed observations are required or where data needs to be collected simultaneously across multiple sites or plants.

The main advantage of the GoPro or similar devices is that it allows multiple samples to be simultaneously recorded remotely under a range of weather conditions (due to the waterproof housing) by a single researcher. The footage is reviewable, allowing a range of behavioural and physiological data to be obtained from the one sample event and may aid in the identification process of flower visiting species. Such benefits have been demonstrated to come at no sacrifice to data quality compared to human observations, provided appropriate and realistic field of view is used. DVR devices are a highly useful tool for pollination biologists as they provide a means to gather large volumes of data with minimal time in the field. Critically, no time frames
exist in regard to the valid review of collected data and can subsequently occur at the most appropriate time for the researcher. Despite the application of DVR for pollination research being only in its infancy, this study demonstrates that through the powerful capabilities of the progressing technology, DVR provides the opportunity for widespread, long term and large scale surveys to be conducted by a small team of researchers.
References


Steen, R. (2016) Diel activity, frequency and visit duration of pollinators in focal plants: 


4 The effect of natural magnet plants on pollinator visitation and seed set of less attractive native co-flowering species.

This chapter has been formatted for submission to Oecologia.
4.1 Abstract
In all plant communities, the contrasting levels of floral resources provided by resource-rich species and those that offer fewer floral rewards leads to variation in attractiveness to pollinators. Resource-rich plants potentially act as “magnet plants” drawing pollinators from the surrounding floral matrix. Research to date has focussed on plant communities within the northern hemisphere, therefore, we aimed to determine whether the same interactions are found within an Australian context. Investigations were conducted within three plant communities contrasting a florally resource rich flowering species (putative magnet species) with a co-flowering species that overlapped in flowering season and was present across a range of distances from a defined putative magnet patch. Here we used a comparative approach to ask the following questions; (1) Do putative magnets attract more pollinators and more diverse suites of pollinators? (2) Does seed set and seed weight vary with distance from magnet plants? And used a manipulative experiment to ask (3) does the quantity, diversity and fidelity of pollinators vary with distance from magnet plants? We found only limited evidence that resource rich Australian native plants are acting as magnets. For each of three species we found that they were attracting relatively large numbers of pollinators’ cf adjacent co-flowering species but flower visitation was dominated by *A. mellifera*. In further contrast to northern hemisphere systems we found little evidence of biological interactions/ spill-over effects between plant species. Pollinator abundance and diversity on co-flowering species did not vary with distance from 'magnets' or after magnets were removed. We found no significant relationship between seed set and distance and only one case study where there was a significant negative effect of seed weight and distance.

Key words: *Apis mellifera*, magnet plants, Southern hemisphere, Australia.
4.2 Introduction

Competition between plants for pollination has been explored in numerous systems and has been found to be influenced by factors including the amount and type of floral reward, flower number and flower density (Grabas and Laverty 1999; Feinsinger and Tiebout 1991; Carusso 2002; Ghazoul 2006; Carmona-Diaz and Garcia-Franco 2009). In northern hemisphere studies the term magnet plant has been used to describe plants that offer large floral rewards, increasing localised pollinator abundance and interacting with co-flowering species by altering the behaviour of their pollinators (Thompson 1978; Laverty 1992; Johnson et al., 2003). In order for magnets to influence co-flowering plants, both plants must be visited by the same pollinator, which is almost always a recognised generalist (Lázaro et al. 2009; Bartomeus et al. 2010; Diekötter et al. 2010; Samnegård et al. 2011; Holzschuh et al. 2012; Montero-Castaño et al. 2016).

To date, research has focussed on the effect of magnet plants within northern hemisphere plant communities, with two broad outcomes reported in regard to effects on pollinator visitation. Firstly, magnets have been shown to enhance the pollination success of neighbouring co-flowering plants, due to the magnet plant species either supporting a greater abundance or diversity of pollinators (Samnegård et al. 2011). This can result in spill-over effects from the magnet onto co-flowering neighbours (Hagen and Kraemer 2010). Alternatively, native plants may be overlooked as existing pollinators are drawn to magnet species, which have higher floral density and reward (Holzschuh et al. 2011). Another mechanism underlying competition between plants is interspecific pollen transfer which has been shown to occur between plants that compete
and that are visited by the same pollinators (Campbell and Motten 1985; Morales and Traveset 2008).

The interaction between competing plants is dependent on numerous factors including plant density, spatial distribution and distance with interactions ranging from positive to negative within a single study system (Seifen et al. 2014; Bruckman and Campbell 2016). The distance of conspecifics from magnet plants is also an important variable that can affect the interaction between plant species (Cariveau and Norton 2009; Bruckman and Campbell 2016). A study in North America which simulated the invasion of *Brassica nigra* and its subsequent effect on a native plant species *Phacelia parryi* found that *P. parryi* located near invasive patches and within low invasive density areas showed the highest reproductive output due to facilitation of pollinator visits and lower heterospecific deposition than plants within high invasive density areas (Bruckman and Campbell 2016). A study which investigated the interaction of the exotic plant *Cardus nutans* and distance to a native plant *Monarda fistulosa* found that visitation rate did not decrease in the presence of the exotic *C. nutans* when it was 15m away (Cariveau and Norton 2009). However, when *M. fistulosa* was one and five meters from *C. nutans* flower visitation decreased.

There are several reasons why interactions between Australian native plants may differ from those seen in many northern hemisphere systems. Australia has vastly different pollination networks to those found in the northern hemisphere with a higher proportion of Australian plants pollinated by vertebrates (Paton 1993). In southern Australia insect pollinated plants have largely evolved with solitary bee species, but
many ecosystems today are strongly disturbed by the presence of the non-native, eusocial *A. mellifera* (Paton 1996).

In this study, using a combination of surveys and experiments, we investigate whether highly attractive Australian plant species, that we predict have the potential to act as a pollinator magnet, can influence the rate or pattern of visitation to surrounding co-flowering native plants. Here we aim to answer the following questions using a comparative approach before and after magnet removal (1) Do putative magnets attract a greater number and more diverse suites of pollinators? (2) Does seed set and seed weight vary with distance from magnet plants? And using a manipulative experiment (3) Does the quantity, diversity and fidelity of pollinators vary with distance from magnet plants?

### 4.3 Methods

*Selection of species:*

All study sites supported a flowering putative magnet population (*Boronia ledifolia*, *Grevillea sericea* or Heathland (*Banksia ericifolia* and *Darwinia fascicularis*)), surrounded by one of three native co-flowering species (*Dillwynia elegans*, *Dillwynia bruniioides* or *Acacia suaveolens*) (see Table 4.1 for details of magnet and co-flowering combinations). All magnet populations at each site beside those within the heathland experiment were characterised by having a discrete population of magnet plants surrounded by co-flowering plants at various distances from the magnet population. At all sites there were no other conspicuous co-flowering plant species besides those within
our experiment. The study was conducted throughout flowering seasons in 2013 and 2014 within natural areas in eastern New South Wales, Australia (Table 4.1).

The putative magnet and co-flowering species were chosen after preliminary observation had revealed that all were receiving flower visitation. We also carried out pollinator exclusion experiments to test the hypotheses that two of the co-flowering species (*D. elegans* and *D. brunioides*) were unable to set seed through spontaneous autogamy (i.e. seed set without the need for a pollen vector). This characteristic was essential as we planned to monitor seed set in these species to test the hypothesis that seed set varies with distance from magnet plants (which is expected if magnet plants are influencing pollinator visitation to co-flowering species). We did not monitor seed set in *Acacia suaveolens* as a pollination study by Morrison and Myerscough (1989) found that this species undergoes self-fertilization and that it can set seed through spontaneous autogamy.

For *D. elegans* and *D. brunioides* we tested for spontaneous autogamy by excluding pollinators using a fine mesh cloth (organza) to bag one branchlet with virgin flowers on each of five plants at all study sites (Table 4.1). Following the bagging of ~250 and ~300 virgin flowers on *D. brunioides* and *D. elegans* respectively, neither species displayed any seed set.

**Experimental design**

For each of the three putative magnet species we replicated the study within each of three or four sites with a total of 100 study plants. Within each site n= 22 to 40 co-flowering study plants were selected extending from within the magnet population to a maximum distance of 90 m. Plants were chosen that displayed open flowers and that
were separated from conspecifics by at least one meter. Study sites were separated by at least three kilometres to limit the effect of neighbouring magnet patches. The details of experimental sites, array structure and replications are presented in Table 4.1. To test the effect of the presence of the putative magnet species on the adjacent co-flowering species surveys were replicated after preventing pollinator access to the magnet using a bagging experiment (detailed below). Although, removal of the magnet species is confounded with time, measures were taken to ensure consistency over the study days such as ensuring that all observations were carried out during the peak flowering and that weather conditions were restricted to fine weather days.
Table 4-1 Location and number of sites for each species pair, details of autogamy trials (including the number of plants and flowers utilized) and year of experimentation.

<table>
<thead>
<tr>
<th>Floral neighbourhood</th>
<th>Site Coordinates</th>
<th># of Co-flowering Plants</th>
<th>Site Location*</th>
<th>Test for Spontaneous Autogamy</th>
<th>Month &amp; Year</th>
<th>Flowering season</th>
<th># of flowering magnet plants</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. ledifolia</em> (m) &amp; <em>D. elegans</em> (c)</td>
<td>S1 - 33°39'18.75&quot;S 150° 22'04.4&quot;E</td>
<td>30</td>
<td></td>
<td>15 plants, 5 from each site, ~300 flowers</td>
<td>August 2013, 2014</td>
<td>Winter</td>
<td>223</td>
</tr>
<tr>
<td></td>
<td>S2 - 33° 39'06.8&quot;S 150° 22'55.7&quot;E</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>S3 - 33° 38'54.5&quot;S 150° 23'35.2&quot;E</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>165</td>
</tr>
<tr>
<td></td>
<td>S1 - 33° 41'04.2&quot;S 150° 20'3.9&quot;E</td>
<td>25</td>
<td>Blue Mountains NP</td>
<td></td>
<td></td>
<td></td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>S2 - 33° 41'04.2&quot;S 150° 20'53.9&quot;E</td>
<td>25</td>
<td></td>
<td>20 plants, 5 from each site, ~250 flowers</td>
<td>October 2013, 2014</td>
<td>Summer</td>
<td>21</td>
</tr>
<tr>
<td><em>G. sericea</em> (m) &amp; <em>D. brunioides</em> (c)</td>
<td>S3 - 33° 41'04.2&quot;S 150° 20'53.9&quot;E</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>S4 - 33° 41'04.2&quot;S 150° 20'53.9&quot;E</td>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>S1 - 34° 09'23.6&quot;S 150° 58'53.9&quot;E</td>
<td>30</td>
<td>Waterfall</td>
<td>NA</td>
<td>May, 2014</td>
<td>Winter</td>
<td>As above</td>
</tr>
<tr>
<td>Heathland community (m) &amp; <em>A. suaveolens</em> (c)</td>
<td>S2 - 34° 09'27.6&quot;S 150° 58'52.2&quot;E</td>
<td>24</td>
<td>Waterfall</td>
<td>NA</td>
<td></td>
<td></td>
<td>As above</td>
</tr>
<tr>
<td></td>
<td>S3 - 34° 09'30.8&quot;S 150° 58'50.7&quot;E</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>As above</td>
</tr>
<tr>
<td></td>
<td>S4 - 34° 15'33.2&quot;S 150° 52'36.9&quot;E</td>
<td>22</td>
<td>Dharawal NP</td>
<td></td>
<td></td>
<td></td>
<td>As above</td>
</tr>
</tbody>
</table>

(m) magnet, (c) co-flowering
* All sites are in New South Wales, Australia
Documenting flower visitors and behaviour

Flower visitor surveys were undertaken using a combination of direct observations and full HD Digital Video Recording (following the methodology of Gilpin et al. (2017)). Direct human or video observations were undertaken on each plant for 21 minutes each day (Fijen and Kleijn 2017). The initial minute of recording or observation was excluded to reduce the potential for observer presence to impact pollinator visitation. Throughout the peak of the flowering season, each study plant was observed for a total of four days, with the magnet species present, and a further four days following the magnet species exclusion through bagging (detailed below). The exception was the *G. sericea, D. bruniodes* 2014 study which only had 3 days of observations due to weather conditions. During the pollinator surveys, any flower visitor that accessed the reproductive parts of a flower was recorded, as was the number of flowers visited and the length of time spent foraging per flower. We estimated pollinator visitation rates expressed as the number of flower visitors per floral unit, i.e. the individual flower for which species and for *G. sericea, A. suaveolens, B ericifolia* and *D. fasicularis* the inflorescence. The number of flowers was recorded each time observations were undertaken. In order to compare relative flower visitation for magnet and co-flowering species, we compared flower visitation for 40 magnet and 40 co-flowering species in the middle of the day (10:00am to 1:00pm) for four days at each site.

To estimate the influence of magnet species on the behaviour and visitation rates of pollinators to co-flowering plants, a bagging experiment was undertaken. A fine synthetic mesh was used to completely envelope each magnet plant in the study area excluding all floral resources. After all individual magnet plants had been completely
covered, for 24 hours, we recommenced flower visitor surveys on co-flowering species. Observations were conducted upon the bagged magnets to ensure no pollinator visitation occurred at any site, although pollinator behaviour may still have been influenced. This treatment was not repeated in the heathland magnet experiment due to the large scale over which the magnet community was found.

Determining the species specificity of insect visitors

Following flower visitor surveys any foraging flower visitors on the study species were caught in a specimen jar and frozen for storage. Entire insects were examined to determine if they carried pollen either on their body or within their corbiculae. For honeybees, in addition to whole body examination, a smear of both corbiculae was made on a glass microscope slide. A subsample of each slide was analysed by examining ten randomly chosen fields of view with a 40x objective lens. The percentage of non-target species pollen was assessed by calculating the proportion of pollen grains from non-target species over the total number of pollen grains.

Seed set and weight

To determine whether distance from magnet plants affects seed set or seed weight, the seed of all of the observed co-flowering plants were allowed to mature in situ. We then collected all mature seeds and recorded the number of individual seed and seed pods per plant. During each flower visitor survey we also recorded the number of open flowers on each study plant to allow the number of seeds produced to be standardised against the plants’ flowering effort. We estimated seed set for each plant
using the average number of flowers (per plant) recorded over the study period and we weighed 30 seed per plant to produce an estimate of average seed weight.

Data analysis

To determine whether proposed magnet species received higher honeybee visitation than their respective co-flowering species, the number of honeybees observed during contemporaneous observations were analysed using a two factor Generalised Linear Model (GLM) with Poisson error distribution and log link function. The number of flower visitors were standardised by dividing the number of flower visitors by the number of open flowers on each plant at the time of observations and site and species were used as the two explanatory variables. The GLM was performed using SPSS statistics version 21.

In addition to comparing the number of species of flower visitors on putative magnet and co-flowering species we also compared the proportion of visits made by HBs using a heterogeneity chi-square test using SPSS v21.

To determine the effect of distance from magnet species on visitation by honeybees to the study species and whether the pattern of visitation is maintained after the exclusion of the magnet species, Generalised Linear Mixed Models (GLMMs) with Poisson distribution (Bolker et al. 2009) in Lme4 package (Bates et al. 2015). All GLMMs had fixed effects of distance of study plants from the magnet population, whilst the D. elegans and D. brunioides case studies also had a fixed effect of treatment (before or after magnet species exclusion). Plant ID and site were included in the model as a random effect to account for non-independence. We found that in all of the case studies, honeybee visitation was a positive function of flower number, therefore, we
divided the number of honeybee visits by the number of open, sexually mature flowers recorded at each observation time. The data was then transformed by multiplying by 1000 and rounding to whole numbers. To analyse seed set data, Generalised Linear Model (GLMs) were undertaken with quasipoisson distribution which accounted for the non-normality and overdispersion. To give a more accurate indication of seed set, the number of pods were standardised by the average number of flowers observed throughout the study period. Seed weight data were analysed using GLMMs with site included as a random effect after initially determining no significant interaction between distance and site. Open source R 3.0.3 statistical platform (R Core Development Team., 2014) were used to analysed seed set, seed weight and the effect of distance on visitation data.

4.4 Results

In all case studies we found that the hypothesised magnet plant species attracted a greater number, however, did not consistently attract a greater diversity of flower visitors than the co-flowering species. At all sites, the overwhelming majority (90 to 100%) of visits to the study plants and the magnet plants were made by *A. mellifera*. Most surprisingly, we found no evidence of indirect interactions (spill-over effects) between magnet plants and the pollination of co-flowering species: exhibited either as variation in pollinator visitation rates; diversity of pollinators or pollinator fidelity with distance from the magnet plants.

*Do putative magnets attract a greater number of pollinators?*
Across all sites and species, as expected the putative magnet plant species experienced significantly more pollinator visits than the co-flowering plant species measured as visits per flower or per inflorescence (Table 4.2). The ratio of mean numbers of visitors per flower across all sites on putative magnets versus co-flowering species ranged from 3.6:0.5 to 11.1:0.4 (Table 4.2) (GLM $p < 0.0001$). Moreover, patterns of visitation were highly consistent across sites for each species (GLM $p >0.05$).

*Does pollinator abundance vary with distance and is this altered by bagging?*

*Apis mellifera* visitation did not vary significantly with distance before or after magnet exclusion ($P >0.10$) (Table 4.6) (Fig 4.2 Fig 4.3 and Fig 4.4). We found no consistent effect of magnet removal on the number of visitors to co-flowering species with three of the case studies showing a decrease in *A. mellifera* visitation following magnet removal (*D. elegans* 2014, $n = 184$ and $n = 96$ ($t_{(399)} = 3.7$ $p = <0.001$) and *D. bruniodides* 2013, $n = 128$ and $n = 85$ ($t_{(399)} = 0.075$ $p = 0.94$) and *D. bruniodies* 2014, $n = 96$ and $n = 75$ ($t_{(299)} = 1.73$ $p = 0.083$) *A. mellifera* in the presence of the magnet and following magnet exclusion respectively) and one case study which showed an increase in visitation (*D. elegans* 2013, $n = 248$ and $n = 334$ respectively ($t_{(399)} = -8.12$ $p = <0.0001$) (Table 4.3). In all cases where there was a decline in visitation following magnet exclusion the change in visitation was immediate (Table 4.4).
Table 4-2 Mean ± SE contemporaneous counts of *A. mellifera* standardised by flower number on the magnet and co-flowering species at each of three or four sites. At each site counts were made on 40 plants per day for four days. The significance of variation among sites, species and site*species interactions was assessed using GLM.

<table>
<thead>
<tr>
<th>Study system</th>
<th>Year</th>
<th>Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
<th>Site 4</th>
<th>GLM test statistic and P values</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. ledifolia</em> (magnet)</td>
<td>2013</td>
<td>0.68 ±0.09</td>
<td>0.73 ±0.08</td>
<td>0.76 ±0.07</td>
<td>na</td>
<td>Site $\chi^2 (2) = 1.37$ p = 0.50</td>
</tr>
<tr>
<td><em>D. elegans</em> (co-flowering)</td>
<td></td>
<td>0.13 ±0.03</td>
<td>0.1 ±0.02</td>
<td>0.09 ±0.02</td>
<td>na</td>
<td>Species $\chi^2 (1) = 210.4$ p = &lt;.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Site*Species $\chi^2 (2) = 2.0$ p = 0.36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>1.52 ±0.25</td>
<td>1.16 ±0.2</td>
<td>1.04 ±0.25</td>
<td>na</td>
<td>Site $\chi^2 (2) = 1.35$ p = 0.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.16 ±0.09</td>
<td>0.08 ±0.06</td>
<td>0.12 ±0.06</td>
<td>na</td>
<td>Species $\chi^2 (1) = 42.4$ p = &lt;.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Site*Species $\chi^2 (3) = 0.32$ p = 0.86</td>
<td></td>
</tr>
<tr>
<td><em>G. sericea</em> (magnet)</td>
<td>2013</td>
<td>0.88 ±0.14</td>
<td>1.24 ±0.40</td>
<td>0.76 ±0.17</td>
<td>1 ±0.13</td>
<td>Site $\chi^2 (3) = 0.33$ p = 0.96</td>
</tr>
<tr>
<td><em>D. bruniodes</em> (co-flowering)</td>
<td></td>
<td>0.1 ±0.04</td>
<td>0.08 ±0.06</td>
<td>0.08 ±0.05</td>
<td>0.09 ±0.04</td>
<td>Species $\chi^2 (1) = 57$ p = &lt;.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Site*Species $\chi^2 (3) = 0.13$ p = 0.99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>1.35 ±0.13</td>
<td>1.75 ±0.2</td>
<td>1.65 ±0.21</td>
<td>1.55 ±0.18</td>
<td>Site $\chi^2 (3) = 0.43$ p = 0.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.15 ±0.05</td>
<td>0.1 ±0.06</td>
<td>0.1 ±0.07</td>
<td>0.1 ±0.07</td>
<td>Species $\chi^2 (1) = 56.1$ p = &lt;.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Site*Species $\chi^2 (3) = 0.83$ p = 0.84</td>
<td></td>
</tr>
<tr>
<td><em>B. ericifolia</em> (magnet community)</td>
<td>2014</td>
<td>2.08 ±0.42</td>
<td>1.68 ±0.29</td>
<td>3.44 ±0.85</td>
<td>1.68 ±0.28</td>
<td>Site $\chi^2 (3) = 2.89$ p = 0.41</td>
</tr>
<tr>
<td><em>D. fasicularis</em> (magnet community)</td>
<td></td>
<td>1.16 ±0.19</td>
<td>0.44 ±0.13</td>
<td>1.16 ±0.23</td>
<td>0.56 ±0.16</td>
<td>Species $\chi^2 (1) = 49.8$ p = &lt;.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Site*Species $\chi^2 (2) = 0.75$ p = 0.69</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Site $\chi^2 (2) = 1.2$ p = 0.56</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Species $\chi^2 (1) = 51$ p = &lt;.0001</td>
<td></td>
</tr>
<tr>
<td><em>A. suaveolens</em> (co-flowering)</td>
<td></td>
<td>0</td>
<td>0.08 ±0.06</td>
<td>0.12 ±0.08</td>
<td>0.12 ±0.06</td>
<td>Site*Species $\chi^2 (2) = 0.49$ p = 0.72</td>
</tr>
</tbody>
</table>
Do putative magnets attract a greater diversity of pollinating species?

In contrast to expectations for magnet plants our putative magnets did not attract consistently more diverse suites of flower visitors than their co-flowering neighbours. The majority of visits to all magnet and co-flowering plant species were made by *A. mellifera* which accounted for 95 to 100% and 90 to 100% of visits respectively (Fig 4.1). In most cases the magnet and co-flowering species were also visited by the same suite of native flower visitors and we never detected more than three species at a site (Fig 4.1). Indeed, in 3 out of 6 cases the magnet and co-flowering species were also visited by exactly the same suite of flower visitors with the remaining 3 case studies differing by a combination of two species of native bees and hoverflies that were found in comparatively very low numbers (Fig 4.1). Moreover, the proportional contribution of honeybees to the total number of flower visitors did not vary significantly between magnet and co-flowering species for any of the case studies (p >0.05) (Fig 4.1).

Does pollinator diversity vary with distance and is it altered by bagging?

As described above, *A. mellifera* was the dominant flower visitor to all co-flowering species and indeed this was true across all sites, species and years (Table 4.3) with the remainder of visits made by native flower visitors. Co-flowering species across all treatments received a limited suite of native flower visitors with only *Trichocolletes* sp, *Exoneura* sp and hoverflies, *Trichophalma* sp, observed during the study period. Chi square test revealed no significant difference between the proportion of visits made by honeybees to the magnet and respective co-flowering species for any of the case studies (P > 0.05). Following magnet removal, the suite of flower visitors did not differ dramatically to those observed when the magnet was present with the same suite of
flower visitors recorded before and after magnet removal in all but one case (*D. elegans* 2013) (Table 4.3). Unsurprisingly, there were no cases where flower visitor diversity appeared to vary with distance from the magnet plant.
Figure 4-1 Percentage of visits per species observed on the magnet and co-flowering species. Chi square test revealed no significant difference between the proportion of visits made by honeybees to the magnet and respective co-flowering species for any of the case studies (P > 0.05). Numbers above each column represent the number of flower visitor individuals observed over the study period.
Table 4-3 Number of flower visitors observed before and after bagging of magnet plants the number of foraging movements made by honeybees and the average time spent foraging per flower. For each case study the total observation time before and after magnet removal was 133hrs.

<table>
<thead>
<tr>
<th>Study system</th>
<th>Before (B) or after (A) bagging of magnets</th>
<th>Number of A. mellifera</th>
<th>Number of foraging movements by A. mellifera</th>
<th>Proportion of interplant movements by A. mellifera</th>
<th>Av time A. mellifera spent foraging</th>
<th>Trichocolletes sp.</th>
<th>Exoneura sp.</th>
<th>Hoverflies</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. elegans</td>
<td>B</td>
<td>248</td>
<td>741</td>
<td>17%</td>
<td>3.7 ± 0.09</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>334</td>
<td>1094</td>
<td>14%</td>
<td>2.7 ± 0.05</td>
<td>11</td>
<td>17</td>
<td>0</td>
</tr>
<tr>
<td>D. elegans</td>
<td>B</td>
<td>184</td>
<td>615</td>
<td>0.5%</td>
<td>4.4 ± 0.19</td>
<td>45</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>96</td>
<td>400</td>
<td>1%</td>
<td>3.5 ± 0.15</td>
<td>18</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>D. brunioides</td>
<td>B</td>
<td>128</td>
<td>428</td>
<td>17%</td>
<td>3.0 ± 0.1</td>
<td>28</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>85</td>
<td>150</td>
<td>13%</td>
<td>2.4 ± 0.07</td>
<td>12</td>
<td>36</td>
<td>0</td>
</tr>
<tr>
<td>D. brunioides</td>
<td>B</td>
<td>96</td>
<td>549</td>
<td>0%</td>
<td>2.2 ± 0.05</td>
<td>16</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>75</td>
<td>226</td>
<td>4%</td>
<td>2.3 ± 0.1</td>
<td>15</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>119</td>
<td>588</td>
<td>0%</td>
<td>3.0 ± 0.05</td>
<td>0</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>A. suaveolens</td>
<td>A</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
Table 4-4 Average ± standard error of *A. mellifera* on each day before and after magnet exclusion, all sites combined.

<table>
<thead>
<tr>
<th>Study System</th>
<th>Day 1 Before</th>
<th>Day 2 Before</th>
<th>Day 3 Before</th>
<th>Day 4 Before</th>
<th>Day 1 After</th>
<th>Day 2 After</th>
<th>Day 3 After</th>
<th>Day 4 After</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. ledifolia/D. elegans</em> 2013</td>
<td>0.27 ±0.6</td>
<td>0.27 ±0.05</td>
<td>0.26 ±0.05</td>
<td>0.33 ±0.06</td>
<td>1.0 ±0.13</td>
<td>1.1 ±0.14</td>
<td>0.64 ±0.13</td>
<td>0.61 ±0.11</td>
</tr>
<tr>
<td><em>B. ledifolia/D. elegans</em> 2014</td>
<td>0.28 ±0.05</td>
<td>0.59 ±0.10</td>
<td>0.38 ±0.07</td>
<td>0.29 ±0.07</td>
<td>0.36 ±0.06</td>
<td>0.31 ±0.06</td>
<td>0.2 ±0.06</td>
<td>0.3 ±0.06</td>
</tr>
<tr>
<td><em>G. sericea/D. brunioides</em> 2013</td>
<td>0.23 ±0.07</td>
<td>0.19 ±0.04</td>
<td>0.3 ±0.06</td>
<td>0.24 ±0.05</td>
<td>0.14 ±0.03</td>
<td>0.15 ±0.04</td>
<td>0.2 ±0.04</td>
<td>0.35 ±0.05</td>
</tr>
<tr>
<td><em>G. sericea/D. brunioides</em> 2014</td>
<td>0.20 ±0.05</td>
<td>0.41 ±0.10</td>
<td>0.35 ±0.06</td>
<td>na</td>
<td>0.24 ±0.05</td>
<td>0.28 ±0.05</td>
<td>0.23 ±0.05</td>
<td>na</td>
</tr>
</tbody>
</table>
Figure 4-2 The total number of *A. mellifera* visiting *D. elegans* during 20 minute observations over four days before and after bagging of the magnet plants plotted against distance from the magnet *B. ledifolia* at each site in 2013. (A) In the presence of the magnet at site 1. (B) After magnet removal at site 1. (C) In the presence of the magnet at site 2. (D) After magnet removal at site 2. (E) In the presence of the magnet at site 3. (F) After magnet removal at site 3
Figure 4-3. The number of *A. mellifera* visiting *D. brunioides* over four days before and after bagging of the magnet plants plotted against distance from magnet *G. sericea* in 2013. (A) In the presence of the magnet at site 1. (B) After magnet removal at site 1. (C) In the presence of the magnet at site 2. (D) After magnet removal at site 2. (E) In the presence of the magnet at site 3. (F) After magnet removal at site 3. (G) In the presence of the magnet at site 4. (H) After magnet removal at site 4.
Figure 4-4. The number of *A. mellifera* visiting *D. brunioides* over four days before and after bagging of the magnet plants plotted against distance from magnet *G. sericea* in 2014. (A) In the presence of the magnet at site 1. (B) After magnet removal at site 1. (C) In the presence of the magnet at site 2. (D) After magnet removal at site 2. (E) In the presence of the magnet at site 3. (F) After magnet removal at site 3. (G) In the presence of the magnet at site 4. (H) After magnet removal at site 4.
**Table 4-5** GLMM analysis testing for variation in honeybee visitation with distance when observations were undertaken with or without access to the magnet plant species (treatment).

<table>
<thead>
<tr>
<th>Study system</th>
<th>Distance * Treatment</th>
<th>Test and significance</th>
<th>Distance</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. elegans</em> 2013</td>
<td>( \chi^2_{(1)} = 98.7, p = &lt;0.0001 )</td>
<td>( \chi^2_{(1)} = 305.5, p = &lt;0.0001 )</td>
<td>( \chi^2_{(1)} = 3.7, p = 0.054 )</td>
</tr>
<tr>
<td><em>D. elegans</em> 2014</td>
<td>( \chi^2_{(1)} = 0.049, p = 0.82 )</td>
<td>( \chi^2_{(1)} = 13.6, p = 0.0002 )</td>
<td>( \chi^2_{(1)} = 0.09, p = 0.75 )</td>
</tr>
<tr>
<td><em>D. brunioides</em> 2013</td>
<td>( \chi^2_{(1)} = 5.66, p = 0.017 )</td>
<td>( \chi^2_{(1)} = 11.45, p = 0.0007 )</td>
<td>( \chi^2_{(1)} = 3.0, p = 0.083 )</td>
</tr>
<tr>
<td><em>D. brunioides</em> 2014</td>
<td>( \chi^2_{(1)} = 6.7, p = 0.0094 )</td>
<td>( \chi^2_{(1)} = 89.0, p = &lt;0.0001 )</td>
<td>( \chi^2_{(1)} = 1.69, p = 0.19 )</td>
</tr>
<tr>
<td><em>A. suaveolens</em> 2014</td>
<td>na</td>
<td>na</td>
<td>( \chi^2_{(1)} = 3.1, p = 0.08 )</td>
</tr>
</tbody>
</table>

**Table 4-6** GLMM analysis testing for variation in honeybee visitation with distance when observations were undertaken before or after magnet exclusion.

<table>
<thead>
<tr>
<th>Study system</th>
<th>Before (B) or After (A) magnet exclusion</th>
<th>Test and significance</th>
<th>Distance</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. elegans</em> 2013</td>
<td>B</td>
<td>( \chi^2_{(1)} = 0.15 p = 0.69 )</td>
<td></td>
</tr>
<tr>
<td><em>D. elegans</em> 2013</td>
<td>A</td>
<td>( \chi^2_{(1)} = 0.14 p = 0.35 )</td>
<td></td>
</tr>
<tr>
<td><em>D. elegans</em> 2014</td>
<td>B</td>
<td>( \chi^2_{(1)} = 1.60 p = 0.20 )</td>
<td></td>
</tr>
<tr>
<td><em>D. elegans</em> 2014</td>
<td>A</td>
<td>( \chi^2_{(1)} = 0.87 p = 0.35 )</td>
<td></td>
</tr>
<tr>
<td><em>D. brunioides</em> 2013</td>
<td>B</td>
<td>( \chi^2_{(1)} = 0.91 p = 0.34 )</td>
<td></td>
</tr>
<tr>
<td><em>D. brunioides</em> 2013</td>
<td>A</td>
<td>( \chi^2_{(1)} = 9.86 p = 0.10 )</td>
<td></td>
</tr>
<tr>
<td><em>D. brunioides</em> 2014</td>
<td>B</td>
<td>( \chi^2_{(1)} = 4.40 p = 0.10 )</td>
<td></td>
</tr>
<tr>
<td><em>D. brunioides</em> 2014</td>
<td>A</td>
<td>( \chi^2_{(1)} = 4.92 p = 0.20 )</td>
<td></td>
</tr>
</tbody>
</table>
**Honeybee plant fidelity**

The overwhelming majority of pollen found both on the body and within the corbiculae of honeybees was from the plant species on which it was found foraging regardless of whether these were putative magnets or co-flowering species (Table 4.7). Indeed 383 of the 388 inspected honeybees captured upon co-flowering species carried only the same species’ pollen in their corbiculae, as what they were caught foraging upon and all 388 carried only same that species’ pollen on their bodies. The only striking exception to this high level of species fidelity was one honeybee detected on *D. brunioides* that carried 45% unidentified pollen within its corbiculae. Therefore, despite our initial prediction that *A. mellifera* might transfer magnet species pollen to co-flowering species we found no evidence that of this was these for any of the three magnet x native species comparisons.

With one exception, all of the n = 1365 *A. mellifera* across all case studies were seen to be collecting pollen. We found honeybees made relatively few inter plant movements (0-17% of total movements observed) compared to intra plant movements flower visits (83-100%) with the proportion remaining relatively constant before and after magnet exclusion at each site (Table 4.3).
<table>
<thead>
<tr>
<th>System</th>
<th>Specimens caught on</th>
<th>Number of honeybees analysed</th>
<th>Number of honeybees with heterospecific pollen on body</th>
<th>Average percentage of foreign pollen</th>
<th>Type of foreign pollen</th>
<th>Number of honeybees with heterospecific pollen in corbiculae</th>
<th>Average percentage of foreign pollen</th>
<th>Type of foreign pollen</th>
<th>Other species with pollen on their body</th>
<th>Heterospecific pollen</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. elegans 2013</td>
<td>D. elegans</td>
<td>108</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>3</td>
<td>2.85% ±3</td>
<td>Unknown</td>
<td>Hoverflies (n = 10)</td>
<td>0</td>
</tr>
<tr>
<td>D. elegans 2014</td>
<td>B. ledifolia</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>D. brunioides 2013</td>
<td>D. elegans</td>
<td>60</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>D. brunioides 2013</td>
<td>B. ledifolia</td>
<td>45</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>D. brunioides 2013</td>
<td>D. brunioides</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>D. brunioides 2014</td>
<td>G. sericea</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>D. brunioides 2014</td>
<td>D. brunioides</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>1</td>
<td>44.50%</td>
<td>Unknown</td>
<td>Exoneura sp (n = 3) and Tricholletes sp (n = 3)</td>
<td>Exonuera (n = 1) 9.1% unknown</td>
</tr>
<tr>
<td>A. suaveolens 2014</td>
<td>G. sericea</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>1</td>
<td>1.20%</td>
<td>Unknown</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. suaveolens 2014</td>
<td>A. suaveolens</td>
<td>22</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>B. ericifolia 2014</td>
<td>D. fasicularis</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
</tbody>
</table>
Does seed set and seed weight vary with distance from magnet plants?

We found no clear relationship between seed set and distance from the magnet plants as might be expected, given that we detected no significant effect of distance from the magnet plants on pollinator diversity, visitation or fidelity (Fig 4.5 and Table 4.8). There was a significant effect of distance on seed weight in one case study (*B. ledifolia/D. elegans*) ($\chi^2_{(1)} = 4.02$, $p = 0.04$) with seed weight being negatively correlated with distance from magnet plants (Fig 6 a-c) (see supplementary table).
Figure 4-5 The number of pods (fruit set) as a proportion of the average number of flowers observed over the study period against distance (m) from the magnet population. (A) *D. elegans* site 1, 2013 (B) *D. elegans* site 2, 2013. (C) *D. elegans* site 3, 2013. (D) *D. brunioides* site 1, 2013. (E) *D. brunioides* site 2, 2013 (F) *D. brunioides* site 3, 2013 (G) *D. brunioides* site 4, 2013 (H) *D. brunioides* site 1, 2014 (I) *D. brunioides* site 2, 2014 (J) *D. brunioides* site 3, 2014 (K) *D. brunioides* site 4, 2014
Figure 4-6. The average (±se) weight (mg) of 30 seeds for each case study against distance (m) from the magnet population. (A) *D. elegans* site 1, 2013 (B) *D. elegans* site 2, 2013. (C) *D. elegans* site 3, 2013. (D) *D. elegans* site 1, 2014. (E) *D. elegans* site 2, 2014 (F) *D. elegans* site 3, 2014 (G) *D. brunioides* site 1, 2013. (H) *D. brunioides* site 2, 2013 (I) *D. brunioides* site 3, 2013 (J) *D. brunioides* site 4, 2013 (K) *D. brunioides* site 1, 2014 (L) *D. brunioides* site 2, 2014 (M) *D. brunioides* site 3, 2014 (N) *D. brunioides* site 4, 2014.
Table 4-8. GLM analysis testing for variation in fruit set against distance and site within each case study.

<table>
<thead>
<tr>
<th>Study system</th>
<th>Test and significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Distance*Site</td>
</tr>
<tr>
<td>D. elegans 2013</td>
<td>$\chi^2(2) = 16975, p = &lt;0.0001$</td>
</tr>
<tr>
<td>D. elegans 2014</td>
<td>$\chi^2(2) = 36368, p = 0.06$</td>
</tr>
<tr>
<td>D. bruniodes 2013</td>
<td>$\chi^2(3) = 7729, p = 0.022$</td>
</tr>
<tr>
<td>D. bruniodes 2014</td>
<td>$\chi^2(3) = 29435, p = 0.015$</td>
</tr>
</tbody>
</table>
Table 4-9. GLMM analysis testing for variation in seed weight against distance and site within each case study.

<table>
<thead>
<tr>
<th>Study system</th>
<th>Distance*Site</th>
<th>Distance</th>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\chi^2_{(1)} = 1.47$, $p = 0.20$</td>
<td>$\chi^2_{(1)} = 4.02$, $p = 0.04$</td>
<td>$\chi^2_{(1)} = 0.028$, $p = 0.85$</td>
</tr>
<tr>
<td><em>D. elegans</em> 2013 (n = 32)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>D. elegans</em> 2014 (n = 43)</td>
<td>$\chi^2_{(1)} = 3.31$, $p = 0.047$</td>
<td>$\chi^2_{(2)} = 0.0008$, $p = 0.92$</td>
<td>$\chi^2_{(1)} = 2.03$, $p = 0.012$</td>
</tr>
<tr>
<td><em>D. bruniodes</em> 2013 (n = 43)</td>
<td>$\chi^2_{(1)} = 0.39$, $p = 0.24$</td>
<td>$\chi^2_{(1)} = 1.54$, $p = 0.21$</td>
<td>$\chi^2_{(1)} = 10.83$, $p &lt;0.0001$</td>
</tr>
<tr>
<td><em>D. bruniodes</em> 2014 (n = 49)</td>
<td>$\chi^2_{(1)} = 0.02$, $p = 0.69$</td>
<td>$\chi^2_{(1)} = 0.03$, $p = 0.87$</td>
<td>$\chi^2_{(1)} = 2.43$, $p &lt;0.0001$</td>
</tr>
</tbody>
</table>
4.5 Discussion

The results of our surveys of flower visitation to three potential Australian native pollinator magnets and co-flowering species, together with pollinator exclusion trials only partly supported our expectations based on northern hemisphere studies.

Do putative magnets attract more pollinators and greater diversity?

As predicted each of our putative magnets attracted significantly more flower visitors than their co-flowering neighbours (Diekötter et al. 2010; Holzschuh et al. 2011) but we mixed evidence that ‘magnets’ attracted more diverse suites of visitors or indeed obviously influenced the quantity or quality of pollen transfer within groups of co-flowering neighbours (Ghazoul 2006) We acknowledge that in defining a magnet plant it is difficult to know whether it is the pulling power of the individual plant, the density of flowers per plant or mass of plants within a population that is enabling the species to act as a magnet plant. Likewise, it may be that the co-flowering species is comparatively less abundant then the magnet species or has fewer flowers and under certain circumstances can itself be a magnet species. However, in both the D. elegans and D. brunioides case studies, the number of flowers per plant were similar for both the magnet and co-flowering species as was the number of individual plants within the population, however, visitation rates were drastically higher upon magnet plants. In the A. suaveolens, case study the number of magnet plants (B. ericifolia and D. fasicularis) greatly outnumbered A. suaveolens as in this case we were aiming to determine whether the effects of magnets varied if an entire magnet community was used. Differing flowering effort may have impacted upon scenarios where we examined species which displayed highly obvious and resource rich inflorescences. However, these factors were
only present in species determined to be magnets, and we attribute such floral morphology in assisting the plant in drawing pollinators from the floral community.

*Do magnets alter the pollination of co-flowering species and do effects vary with distance from magnet plants?*

We found some evidence that when the flowers of the magnet species were excluded from the study area, visitation subsequently declined to the co-flowering plant by 23-51% although removal of the magnet is confounded with time of observation. In all cases where there was a decline in visitation following magnet exclusion the change in visitation was immediate and hence not a gradual effect of changing conditions. This suggests that there is a link between the magnet and the co-flowering plant in relation to honeybee visits. Such findings are supported by Laverty (1992), whose study revealed similar links between a magnet plant and their co-flowering neighbours. Laverty (1992) investigated the pollinator visitation rates and subsequent seed set of the co-flowering mayapple (sp) in relation to their proximity to the magnet, lousewart (sp). In the presence of the magnet, visitation rates to the co-flowering mayapple were approximately 4 times greater than rates without magnet presence, while interestingly, unlike our study, they revealed a positive increase in seed set in the presence of a magnet. A key finding was revealed through their removal of magnet flowers, which led to a decline in pollinator visitation rates, as was found in our study, although removal may be more definitive than exclusion by bagging.

Similar interactions were investigated through a manipulative experiment by Lopezariza-Mikel et al. (2007), in Great Britain, using paired plots (invaded and not invaded) examining the effects of the magnet invasive plant Himalayan balsam.
Impatiens glandulifera) on co-flowering natives. Importantly, while revealing that invaded plots had significantly higher pollinator species richness, visitor abundance and flower visitation rates, seed set declined in the presence of a magnet, in contrast to Laverty (1992). Lopezariza-Mikel et al. (2007) also found that foreign pollen was frequently present on insect visitors. Increased visitation may therefore not result in increased pollination due to the deposition of heterospecific pollen on co-flowering stigmas. Similarly, a study of insect visitation and pollen deposition on native plants within and away from an invasive plant species (Euphorbia esula) in North Dakota found significantly less conspecific native plant pollen on stigmas of plants located within infested plots (Larson et al. 2006). Although our study revealed a high degree of pollinator faithfulness, our findings in regard to seed set in the presence of a magnet plant reveal a complex mix of potential outcomes for co-flowering plants.

Several studies have found that if two plants flower simultaneously and share a pollinator they are likely to compete for pollination, such as Waser (1978) who looked at competition for pollination by hummingbirds and Mitchell et al. (2009) who recently review the field. However, there is a growing body of literature that suggests that under certain circumstances there may be facilitative interactions among plants via the sharing of pollinators (e.g., Moeller 2004). Liao et al. (2011) investigated interactions between the magnet Pedicularis monbeigiana and the co-flowering Vicia dichroantha (both alpine perennial herbs), which share the same suite of bumblebee pollinators. They demonstrated facilitative effects in the presence of the magnet, leading to an increase in the reproductive success of the co-flowering V. dichroantha in the bumblebee dominated system. Similarly, Ghazoul (2006) conducted manipulative field experiments involving a magnet plant community, and the co-flowering Raphanus raphanistrum....
(wild radish), which were serviced by a suite of pollinators including wasps, butterflies and solitary bees. His work supports the concept of facilitative interactions led by magnet species, as his findings revealed increased visitation rates and reproductive output in the co-flowering *R. raphanistrum*, depending upon the specific floral makeup of the magnet plant community.

The spatial scale of magnet influence investigated in our study is supported by a range of similar manipulative experiments which utilised a co-flowering species array within 150 meters of a magnet patch. Samnegård et al. (2011) who found that in an agricultural area in Sweden, pollinator visitation was higher in a native outcrossing plant (*Campanula persicifolia*), closer (<15 m) to gardens (500m²) than further away (>140 m) although, they also found that seed set was higher. Despite studies by Samnegård et al. 2011 finding decreased visitation over distances of <15m - 140m we found very little evidence of a decrease in honeybee visitation to co-flowering species out to a distance of 90 meters. This may be due to the fact that previous studies have primarily found bumblebees to be the most frequent visitors (Liao et al. 2011; Samnegård et al. 2011).

**Does seed set and seed weight vary with distance from magnet plants?**

We found honeybees made relatively few inter plant movements compared to intra plant movements, with the proportion remaining relatively constant before and after magnet exclusion at each site. Honeybees have also been shown to make fewer inter plant movements than native Australian flower visitors (Whelan et al. 2009; Gilpin et al. 2016). Although the striking difference in flower visitor behaviour demonstrated
in Gilpin et al. (2016) did not correspond to a difference in early life-history fitness, there may be an effect depending on the mating system of the plant. Grabas and Laverty (1999) demonstrated the impacts of the foraging behaviour of honeybees upon a co-flowering plant community in the presence of the magnet, *Lythrum salicaria* (purple loosestrife). The study documented a honeybee dominated pollination system, with over 90% of documented visits made by honeybees. Unlike our study, Grabas and Laverty (1999) found a decrease in seed set and visitation rates to co-flowering species in the presence of the magnet species. This demonstrates that the effects of honeybee foraging behaviour upon plant reproductive outcomes in the presence of a magnet may be dependent upon the characteristics (including mating systems) of the plants involved.

Variation among plant mating systems goes some way toward explaining the significant interaction we found between distance and site on fruit set in four of five of our case studies. In each case this was largely driven by differences between sites, indicating the inherit variances in reproductive output between plant individuals and populations. We also found very little evidence that seed weight was influenced by distance from magnet species, although, as expected, there were differences between sites.

It is important to acknowledge that our study did not investigate seed set or seed weight from samples collected throughout the entire flowering season, although we expect that this is unlikely to affect the results obtained as there was no clear pattern of seed set or weight with distance from magnet plants across sites and years in any case study.
Conclusions

Future research should consider building upon this experiment to determine the
distance over which honeybee spill-over is found within an Australian context and to
determine if honeybee visitation differs to that of native flower visitors, both in the
foraging behaviour and whether this corresponds to an effect on the number of seed
produced or seed fitness. Consideration to plant population size, density and the amount
of floral reward on offer is likely to drive pollinator visitation. In conclusion, we found
evidence that the flowering community combined with the foraging behaviour of
honeybees has the potential to influence visitation to co-flowering species within
distances of at least 100 m.
References


Hermansen TD, Britton DR, Ayre DJ, Minchinton TE (2014) Identifying the real pollinators? Exotic honeybees are the dominant flower visitors and only effective pollinators of *Avicennia marina* in Australian temperate mangroves. Estuar Coasts 37: 621-635.


5- Are exotic magnet plants in Australian agricultural areas influencing the pollination of surrounding co-flowering native plants?

This chapter has been formatted for submission to Agriculture, Ecosystems and Environment.
5.1 Abstract

Agricultural landscapes in Australia are dominated by northern hemisphere crop species and exotic weeds, which may disrupt plant-pollinator interactions for adjacent native plants. In northern hemisphere systems, crop plants have been shown to act as ‘magnets’ drawing pollinators from co-flowering plants, while in other cases there is spill-over of pollination services that declines with distance from the crop. However, the impact of highly attractive (magnet) plant species (either introduced crops or endemics) have not been investigated in Australia where plants have evolved with a different suite of pollinators, although pollination is now often dominated by the European honeybee (*Apis mellifera*). We tested how distance from potential magnet species (lavender, nectarines and the pasture weed *Echium plantagineum*) affected the pollination biology of Australian native and European plant species, with and without the addition of honeybee hives. We found that honeybees were the dominant visitor of all crop species. Pollinator diversity was unexpectedly higher on co-flowering species compared to crop species. In contrast to expectations, we detected no clear positive or negative effects of proximity to any crop species. Flower visitor abundance varied significantly with distance from crop species in two case studies although there was no significant effect of proximity on flower visitor diversity. Pollinator abundance was relatively low on all co-flowering species, but the pollen loads on flower visitors show little contamination with crop species pollen (0.78%). We found evidence that populations of resource-rich European crop or weed species are highly attractive to the available pollinators. Although we found some evidence that on the spatial scale investigated, proximity to these species altered the pollination of less attractive experimental populations of co-flowering species. We argue that the basis for this unexpected contrast with northern hemisphere studies likely reflects the low diversity of pollinators observed and the
highly targeted foraging behaviour of *A. mellifera*, the dominant pollinator of both exotic and native species.

Key words: Agroecosystems, Pollinator spill-over, *Apis mellifera*, honeybee abundance, Australian native plants, southern hemisphere.

### 5.2 Introduction

Large-scale agriculture has produced vast monocultures of florally resource-rich crop species that often act as “magnet” populations, drawing pollinators from existing plant communities (Lopezaraiza-Mikel et al., 2007; Diekötter et al., 2009; Holzschuh et al., 2011; Gibson et al., 2013; Montero-Castaño et al., 2016). To date, research has focussed on the effect of magnet plants within northern hemisphere plant communities, with two broad outcomes reported. Firstly, magnets have been shown to enhance the pollination success of neighbouring co-flowering plants, due to the magnet plant species supporting either a greater abundance or diversity of pollinators. This can result in spill-over effects from the magnet onto co-flowering neighbours (Westphal et al., 2003; Hagen and Kraemer 2010; Hanley et al., 2011). Alternatively, co-flowering neighbours may be overlooked as existing pollinators are drawn to magnet species, which have higher floral density and reward (Holzschuh et al., 2011).

Several detrimental impacts of magnet crop species on co-flowering species have been documented (Holzscuh et al., 2011). For neighbouring vegetation, magnet influenced pollinator behaviour may cause pollen of lower quality to be deposited, producing stigma clogging and sub-optimal matings (Lopezaraiza-Mikel et al., 2007) and decreased visitation and altered foraging behaviour may reduce rates of pollen
deposition and removal (Grabas and Laverty 1999; Chittka and Schürkens 2001; Brown et al., 2002). Nevertheless, these effects are also dependent on the degree of specialisation of the pollinators involved (Lázaro et al., 2009; Diekötter et al., 2010).

Of the few published studies that investigate the effect of mass flowering crop plants on co-flowering species, most have used oilseed rape as the focal crop species (Westphal et al., 2003; Cussans et al., 2010; Diekötter et al., 2010; Holzschuh et al., 2011; Kovács-Hostyanszki et al., 2013). Studies are split in terms of the impact of the crop on co-flowering species with three studies finding that the interaction was dependent on the stage of flowering (Cussans et al., 2010; Kovács-Hostyanszki et al., 2013; Grab et al., 2017). In the majority of these studies the main pollinator were bumblebee spp (Westphal et al., 2003; Cussans et al., 2010; Diekötter et al., 2010; Hanley et al., 2011; Holzschuh et al., 2011; Kovács-Hostyanszki et al., 2013). Within Australia bumblebees are not found on the mainland thus the interaction between crop plants, co-flowering plants and pollinators may differ.

In comparison to the well-studied northern hemisphere plant-pollinator systems, little is known about the interaction of northern hemisphere crops with Australian native vegetation that has evolved with a different suite of pollinators. Furthermore, crops in Australia and many native Australian plants are now visited by the highly successful invasive generalist, the European honeybee (*Apis mellifera*). Honeybees have successfully invaded a variety of ecosystems globally (Paton 1993; Oldroyd et al., 1997) because they are able to forage on an extremely wide range of plant species. However, as predicted by optimal foraging theory (Schoener 1971; Waddington and Holden 1979; Marden and Waddington 1981) they are more likely to exploit plant
species that provide the greatest reward for least effort, such as those potentially found within flowering agricultural crop settings (Thom et al., 2017). Presumably then, if honeybee abundance is high and floral resources are limited, honeybees will potentially forage on other co-flowering floral resources. Therefore, we decided to test whether experimental addition of honeybee hives effects flower visitation to both the crop and co-flowering test species.

This study aims to determine whether, within Australia, northern hemisphere mass-flowering agricultural species are both highly attractive to native and exotic pollinators and whether they influence the pollination biology of co-flowering native Australian and exotic plant species occurring in close proximity. We compare the effects on pollinator diversity, abundance and species fidelity across multiple sites and years in three potential magnet crop populations surrounded by experimental arrays of co-flowering native or exotic species. Specifically we ask:

(1) Do the potential magnets attract a greater abundance and diversity of pollinators than co-flowering species? (2) Within the co-flowering arrays does pollinator abundance, diversity and fidelity vary with distance from the magnet population? (3) Within the co-flowering arrays are these aspects of pollination biology altered by the experimental addition of managed honeybee hives?

5.3 Materials and methods
The study was conducted throughout 2013-2014 within agricultural areas in eastern New South Wales, Australia. Study sites consisted of a flowering magnet population of non-native plants (crop or weed species), surrounded by grassland that
was bordered by remnant bushland. At all sites, conspicuous co-flowering plants other than those within our experimental arrays were removed.

To assess the generality of the magnet plant effects in Australian ecosystems, we compared visitation to co-flowering Australian and European plant species arranged in experimental arrays along cardinal directions around each of three potential magnet species. We chose two native Australian species (Melaleuca thymifolia and Backhousia myrtifolia) and two northern hemisphere plant species (Lavendula stoechas and Thymus citriodorus). These species were chosen as they flower profusely and for an extensive period, are visited by a range of insect pollinators and easily maintained in pots (personal observations). The details of experimental sites, array structure and replication is presented in Table 5.1. In each case potted co-flowering plants were simultaneously placed at each test distance from the magnet patch up to a distance of 250 m. The placement of plants was deliberately staggered to reduce the potential of trap-line foraging.
Table 5-1 Site specifications for each year detailing the study species used, relative size of each magnet area, number of magnet plants and experimental distances used for each of four transects at each site.

<table>
<thead>
<tr>
<th>Site</th>
<th>Coordinates</th>
<th>Year</th>
<th>Relative size of magnet area</th>
<th># of magnet plants</th>
<th>Test species</th>
<th># of potted plants</th>
<th>Experimental distances from magnet (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1 Thirlmere</td>
<td>34°13’41.3”S 150°33’41.6”E</td>
<td>2013-2014</td>
<td>Summer</td>
<td>100 x 200m (2ha)</td>
<td>350 M. thymifolia</td>
<td>28</td>
<td>-10 (within the magnet patch), 10, 20, 30, 40, 80 and 100.</td>
</tr>
<tr>
<td>S2 Cobargo</td>
<td>36°24’08.6”S 149°58’05.3”E</td>
<td>2013-2014</td>
<td>Summer</td>
<td>200 x 200m (4ha)</td>
<td>800 M. thymifolia</td>
<td>28</td>
<td>-10 (within the magnet patch), 10, 20, 30, 40, 80 and 100.</td>
</tr>
<tr>
<td>S1 Thirlmere</td>
<td>34°13’41.3”S 150°33’41.6”E</td>
<td>2014-2015</td>
<td>Summer</td>
<td>100 x 200m (2ha)</td>
<td>350 M. thymifolia, B. myrtifolia, T. cāridorus</td>
<td>44</td>
<td>-10 (within the magnet patch), 0 (on the edge of the magnet patch) 10, 20, 30, 40, 80, 100, 150, 200, 250.</td>
</tr>
<tr>
<td>S2 Cobargo</td>
<td>36°24’08.6”S 149°58’05.3”E</td>
<td>2014-2015</td>
<td>Summer</td>
<td>200 x 200m (4ha)</td>
<td>800 M. thymifolia, B. myrtifolia, T. cāridorus</td>
<td>44</td>
<td>-10 (within the magnet patch), 0 (on the edge of the magnet patch) 10, 20, 30, 40, 80, 100, 150, 200, 250.</td>
</tr>
<tr>
<td>S3 Orange</td>
<td>33°18’47”S 149°08’56.5”E</td>
<td>2013</td>
<td>Summer</td>
<td>Several ha</td>
<td>M. thymifolia</td>
<td>28</td>
<td>-10 (within the magnet patch), 10, 20, 30, 40, 80 and 100.</td>
</tr>
<tr>
<td>S4 Orange</td>
<td>33°18’47”S 149°08’56.5”E</td>
<td>2013</td>
<td>Summer</td>
<td>Several ha</td>
<td>M. thymifolia</td>
<td>28</td>
<td>-10 (within the magnet patch), 10, 20, 30, 40, 80 and 100.</td>
</tr>
<tr>
<td>Site</td>
<td>Coordinates</td>
<td>Year</td>
<td>Relative size of magnet area</td>
<td>Test species</td>
<td># of potted plants</td>
<td>Experimental distances from magnet (m)</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>---------------------------</td>
<td>-----------</td>
<td>------------------------------</td>
<td>-------------------------------</td>
<td>--------------------</td>
<td>---------------------------------------</td>
<td></td>
</tr>
<tr>
<td>S3 Orange</td>
<td>33°18'47&quot;S 149°08'56.5&quot;E</td>
<td>2014 Summer</td>
<td>300 x 200 (6ha)</td>
<td>M. thymifolia, L. stoechas</td>
<td>44</td>
<td>-10 (within the magnet patch), 0 (on the edge of the magnet patch) 10, 20, 30, 40, 80, 100, 150, 200, 250.</td>
<td></td>
</tr>
<tr>
<td>S4 Orange</td>
<td>33°18'47&quot;S 149°08'56.5&quot;E</td>
<td>2014 Summer</td>
<td>200 x 200 (4ha)</td>
<td>M. thymifolia, L. stoechas</td>
<td>44</td>
<td>-10 (within the magnet patch), 0 (on the edge of the magnet patch) 10, 20, 30, 40, 80, 100, 150, 200, 250.</td>
<td></td>
</tr>
</tbody>
</table>

**Nectarine**

<table>
<thead>
<tr>
<th>Site</th>
<th>Coordinates</th>
<th>Year</th>
<th>Relative size of magnet area</th>
<th>Test species</th>
<th># of potted plants</th>
<th>Experimental distances from magnet (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S 5 Tahmoor</td>
<td>34°10'49.9&quot;S 150°34'02.9&quot;E</td>
<td>2014 Spring</td>
<td>100 x 200 (2ha)</td>
<td>L. stoechas</td>
<td>60</td>
<td>-10 (within the magnet patch), 0 (on the edge of the magnet patch) and then randomly out to 130m.</td>
</tr>
<tr>
<td>S6 Bilpin</td>
<td>33°29'47.6&quot;S 150°30'52.3&quot;E</td>
<td>2014 Spring</td>
<td>300 x 400m (12ha)</td>
<td>L. stoechas</td>
<td>60</td>
<td>-10 (within the magnet patch), 0 (on the edge of the magnet patch) and then randomly out to 130m.</td>
</tr>
</tbody>
</table>
**Flower visitor surveys**

Using a combination of human observation techniques and Digital Video Recording using a GoPro Hero 3 Silver Edition™ (2013, gopro.com) (Gilpin et al., 2017) each test plant was monitored for four days, for 21 minutes per day (the initial minute of recording or observation was excluded from the analyses and was used to minimise disturbance to the test species) for both the lavender and *E. plantagineum* case studies (Fijen and Kleijn 2017). Cameras were set to record in full HD (920 x 1080 pixels) whilst ensuring the test plant was captured entirely within the field of view. Analysis of recording was undertaken using Windows Media Player™. These approaches were varied for the nectarine study system since it was not possible to make comparable video recordings of visits to the more structurally complex nectarine plants (Gilpin et al., 2017). Here both Nectarines and *L. stoechas* plants were monitored by human observers for three minutes per day for four days.

During each survey, any flower visitors that were seen foraging on a flower were identified (to genus) as well as the number of open sexually mature flowers it visited was recorded and the number of open flowers that were present on the plant. Direct comparisons of the relative attractiveness of the potential magnets and co-flowering species are complicated by differences in the structural complexity of the plant species including variation in flower structure and density. In order to accurately determine the relative abundance of honeybees to crop and co-flowering species, a 30 cm x 30 cm quadrat was placed on the flowering plant and pollinator visitation to flowers within the quadrat within a one minute period was recorded. Within each quadrat, the number of
open flowers or inflorescences was recorded and used as a measure of pollinator visitation to flowering effort.

To determine the number of flower visitors on the magnet *E. plantagineum* and Lavender a 10 meter transect was randomly selected through the magnet patch. Any flower visitors within one meter either side were counted. This was repeated (16-22) times throughout the study period before and after the addition of hives.

*Comparing the pollen loads of insect visitors*

Honeybees and other foraging insects that visited the test species were caught and frozen individually. Insect specimens were later examined to determine if they carried pollen either on their body (by a whole body inspection under a microscope) or (in the case of honeybees) in their corbiculae. A smear of pollen from both corbiculae was made on a glass microscope slide. A subsample of each slide was analysed by examining ten randomly chosen fields of view with a 40x objective lens. The percentage of other species of pollen was then assessed by calculating the proportion of pollen grains that were from non-target species over the total number of pollen grains.

*Effects of experimental addition of honeybees*

To test whether an increase in honeybee abundance affects visitation to *M. thymifolia* (2013 and 2014) or *L. stoechas* (2014 only) and the magnet *E. plantagineum*, honeybee hives were introduced into the *E. plantagineum* agro-ecosystem study areas. In 2013 and 2014 we introduced three and five honeybee hives respectively to each study site which was predicted to increase the local abundance of honeybees by
~150,000 and ~250,000 workers respectively. Two days were allowed to pass to allow normal honeybee foraging to commence before undertaking flower visitor surveys. However, it should be noted that honeybee addition was confounded with time.

Data Analysis

To determine whether proposed magnet species received higher visitation than their respective co-flowering species, the number of flower visitors observed during contemporaneous observations were analysed using two factor Generalised Linear Models (GLMs) with Poisson error distribution and log link function. The number of flower visitors were standardised by the number of open flowers on each plant at the time of observations and site and species (crop or co-flowering) were used as the two explanatory variables. The GLM was performed using SPSS statistics version 21.

To determine if the species diversity of flower visitors differed between crop and co-flowering species, Chi square tests were used. Species diversity of flower visitors was represented as a proportion of *A. mellifera* visitation compared to all other visitors. Species diversity was analysed using SPSS v21.

To determine the effect of distance from crop species on visitation by *A. mellifera* to the study species and whether the pattern of visitation was maintained after the addition of hives for the *E. plantagineum* case studies we used Generalised Linear Mixed Models (GLMMs) with Poisson distribution and the models were fitted with the Laplace function (Bolker et al., 2009) in Lme4 (Bates et al., 2015) and car (Fox and Weisberg 2011) packages. All GLMMs had fixed effects of distance of study plants from the magnet crop population and site and random effects of plant identification
number to account for non-independence. In the case of the *E. plantagineum* case study, an additional fixed effect of treatment was used to assess whether there was a difference in honeybee visitation before and after the introduction of honeybee hives. However, the analysis could only be run on data obtained after the introduction of *M. thymifolia* in 2013. Due to low honeybee visitation, GLMMs could not be undertaken on all other *E. plantagineum* case studies, but was analysed using a paired *t*-test using R software. Assumptions of normality were tested by examining plots and a Shapiro-Wilks test. To verify homogeneity of variances a Levene’s test were performed. The mean total number of honeybees were normally distributed after a square root transformation. For the nectarine case study, we transformed the standardised honeybee visits by multiplying them by 1000 and rounding to whole numbers. We used ANOVA using type III sums-of-squares for all GLMMs. Open source R 3.0.3 statistical platform (R Core Development Team., 2014) were used to analyse the effect of distance and the effect of hive addition on visitation data.

### 5.4 Results

As expected, each of the three northern hemisphere crop plant species were highly attractive to European honeybees but contrary to expectation, they did not attract a greater diversity of pollinators than co-flowering plants and examination of pollen loads revealed little interspecific pollen transfer between crops and co-flowering plants. Even more surprisingly, we found that for each of the two co-flowering native and northern hemisphere species tested, pollinator visitation, diversity and species fidelity was not significantly affected by proximity to the crop plants.
The co-flowering plant species received far fewer visits than magnet plants across all sites and species than the adjacent crop plants (Table 5-2). We detected a significant interaction between distance and site from magnet plants on honeybee visitation in only one case-study, co-flowering *L. stoechas* within the nectarine case study 2014, (GLMM p = 0.0001), although this was driven by variation in honeybee visitation between sites. There was also no clear effect in regard to the origin of the co-flowering species (either Australian or European), although the highest honeybee visitation upon a co-flowering species was recorded on a European species (*L. stoechas*) within the nectarine agro-ecosystem. The addition of honeybees did not alter the visitation rates to co-flowering species (Table 5-4).
Table 5-2  Average number of honeybees visiting the study species within a 30cm x 30cm quadrat during 1 minute (n = 10) or per branch in the case of the nectarine study. The number of honeybees was then standardised by the number of flowers or inflorescences within the quadrat.

<table>
<thead>
<tr>
<th>Study site/ species</th>
<th>Average ± SE number of honeybees/ 900cm²</th>
<th>Number of honeybees per flower or inflorescence</th>
<th>Average number of flowers</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lavender 2013</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lavender s1</td>
<td>4.2 ±0.53</td>
<td>0.096</td>
<td>43.7 ±1.94</td>
</tr>
<tr>
<td>M. thymifolia s1</td>
<td>0.1 ±0.33</td>
<td>0.004</td>
<td>24.2 ±1.33</td>
</tr>
<tr>
<td>Lavender s2</td>
<td>3 ±0.54</td>
<td>0.073</td>
<td>41.1 ±2.05</td>
</tr>
<tr>
<td>M. thymifolia s2</td>
<td>0 ±0</td>
<td>0</td>
<td>19.1 ±1.40</td>
</tr>
<tr>
<td><strong>Lavender 2014</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lavender s1</td>
<td>8 ±1.25</td>
<td>0.20</td>
<td>40.6 ±2.86</td>
</tr>
<tr>
<td>M. thymifolia s1</td>
<td>0 ±0</td>
<td>0</td>
<td>22.2 ±1.51</td>
</tr>
<tr>
<td>Lavender s2</td>
<td>1.5 ±0.03</td>
<td>0.038</td>
<td>39.5 ±2.44</td>
</tr>
<tr>
<td>M. thymifolia s2</td>
<td>0 ±0</td>
<td>0</td>
<td>11.2 ±1.04</td>
</tr>
<tr>
<td>Lavender s1</td>
<td>9.1 ±0.97</td>
<td>0.18</td>
<td>51.7 ±3.45</td>
</tr>
<tr>
<td>B. myrtifolia s1</td>
<td>0 ±0</td>
<td>0</td>
<td>21.6 ±1.29</td>
</tr>
<tr>
<td>Lavender s2</td>
<td>3.5 ±0.5</td>
<td>0.076</td>
<td>45.8 ±2.58</td>
</tr>
<tr>
<td>B. myrtifolia s2</td>
<td>0 ±0</td>
<td>0</td>
<td>13.1 ±1.28</td>
</tr>
<tr>
<td>Lavender s1</td>
<td>9.5 ±0.96</td>
<td>0.27</td>
<td>34.8 ±4.11</td>
</tr>
<tr>
<td>T. citrodorus s1</td>
<td>0 ±0</td>
<td>0</td>
<td>40.1 ±3.21</td>
</tr>
<tr>
<td>Lavender s2</td>
<td>5.3 ±0.82</td>
<td>0.14</td>
<td>36.9 ±3.12</td>
</tr>
<tr>
<td>T. citrodorus s2</td>
<td>0 ±0</td>
<td>0</td>
<td>37.3 ±3.45</td>
</tr>
<tr>
<td><strong>E. plantagineum 2013</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. plantagineum s3</td>
<td>1.1 ±0.37</td>
<td>0.076</td>
<td>14.4 ±1.15</td>
</tr>
<tr>
<td>M. thymifolia s3</td>
<td>0 ±0</td>
<td>0</td>
<td>12.0 ±2.17</td>
</tr>
<tr>
<td>E. plantagineum s4</td>
<td>1.6 ±0.27</td>
<td>0.13</td>
<td>12.4 ±0.88</td>
</tr>
<tr>
<td>M. thymifolia s4</td>
<td>0.2 ±0.92</td>
<td>0.015</td>
<td>13.5 ±0.92</td>
</tr>
<tr>
<td>E. plantagineum s3</td>
<td>1.3 ±0.3</td>
<td>0.087</td>
<td>14.9 ±1.69</td>
</tr>
<tr>
<td>B. myrtifolia s3</td>
<td>0 ±0</td>
<td>0</td>
<td>24.2 ±0.98</td>
</tr>
<tr>
<td>E. plantagineum s4</td>
<td>1 ±0.25</td>
<td>0.059</td>
<td>16.7 ±2.56</td>
</tr>
<tr>
<td>B. myrtifolia s4</td>
<td>0 ±0</td>
<td>0</td>
<td>23.8 ±2.79</td>
</tr>
</tbody>
</table>
### E. plantagineum

#### 2014

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>P</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. plantagineum s3</td>
<td>0.4 ±0.22</td>
<td>0.032</td>
<td>12.4 ±2.21</td>
</tr>
<tr>
<td>L. stoechas s3</td>
<td>0 ±0</td>
<td>0</td>
<td>25.6 ±1.39</td>
</tr>
<tr>
<td>E. plantagineum s4</td>
<td>0.5 ±0.22</td>
<td>0.047</td>
<td>10.6 ±1.10</td>
</tr>
<tr>
<td>L. stoechas s4</td>
<td>0 ±0</td>
<td>0</td>
<td>23.3 ±2.10</td>
</tr>
<tr>
<td>E. plantagineum s3</td>
<td>0.4 ±0.22</td>
<td>0.03</td>
<td>13.1 ±1.99</td>
</tr>
<tr>
<td>M. thymifolia s3</td>
<td>0 ±0</td>
<td>0</td>
<td>16.6 ±0.95</td>
</tr>
<tr>
<td>E. plantagineum s4</td>
<td>0.8 ±0.24</td>
<td>0.057</td>
<td>14.0 ±1.27</td>
</tr>
<tr>
<td>M. thymifolia s4</td>
<td>0</td>
<td>0</td>
<td>21.2 ±2.34</td>
</tr>
</tbody>
</table>

### Nectarines 2014

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>P</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nectarine s5</td>
<td>2±0.2</td>
<td>0.045</td>
<td>44±2 /branch</td>
</tr>
<tr>
<td>Nectarine s6</td>
<td>3±0.4</td>
<td>0.045</td>
<td>67±9 /branch</td>
</tr>
</tbody>
</table>
Table 5-3 GLMM analysis testing for variation the number of honeybees foraging on the co-flowering and magnet species before and after the introduction of hives at each site. Results from GLMM with fixed effects of distance from the magnet population, site and treatment (before and after hive addition) in relation to the number of *A. mellifera* observed foraging on the test species. IDR = Insufficient Data Recorded.

<table>
<thead>
<tr>
<th>Study system and test species</th>
<th>Site 1</th>
<th>Site 2</th>
<th>GLMM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Echium plantagineum</strong> - 2013</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| *M. thymifolia* | $(t_{11}) = 0.13, p = 0.89$ | $(t_{11}) = 3, p = 0.01$ | Distance * Site * Treatment $\chi^2 = 0.00, p = 0.99$
| On E. plantagineum during M. thymifolia experiment | $(t_{15}) = -1.2, p = 0.20$ | $(t_{15}) = 0.69, p = 0.49$ | Distance * Treatment $\chi^2 = 0.00, p = 1$
| *B. myrtifolia* | IDR | $(t_{10}) = 2.8, p = 0.02$ | IDR |
| On E. plantagineum during B. myrtifolia experiment | $(t_{17}) = 0.7, p = 0.47$ | $(t_{17}) = 0.1, p = 0.91$ |      |
| **Echium plantagineum** - 2014 |        |        |      |
| *M. thymifolia* | $(t_{19}) = 0.10, p = 0.91$ | $(t_{19}) = 0.61, p = 0.54)$ | IDR |
| On E. plantagineum during M. thymifolia experiment | $(t_{19}) = -0.1, p = 0.92$ | $(t_{19}) = 0.39, p = 0.69$ | IDR |
| *L. stoechas* | IDR | IDR | IDR |
| On E. plantagineum during L. stoechas experiment | $(t_{21}) = 0.57, p = 0.58$ | $(t_{21}) = 0.66, p = 0.51$ |      |
Pollinator visitation and pollinator diversity on magnet plants

All three agricultural species received flower visitors (Table 5.2), although in all cases these were almost exclusively *A. mellifera* (Fig 5.1). The exceptions were small numbers of Calyptrate (Diptera) and *Zizina labradus* (Lepidoptera) foraging on lavender and *E. plantagineum* respectively (Fig 5.1). In all cases (n = 100) *A. mellifera* were carrying the magnet crop species pollen in their corbiculae and were observed in the field to be foraging for pollen (Table 5.4) and in addition only one *A. mellifera* was found to carry a small amount (1.5%) of heterospecific pollen within its corbiculae and no heterospecific pollen was found on their bodies.

Direct comparisons of the relative attractiveness of the crop plants and co-flowering species are potentially complicated by differences in the structural complexity of the plant species including variation in flower structure and density. However, the crop plants proved more attractive whether comparisons were made per unit of canopy, per flower or per plant (Table 5.2). 10m

In contrast to expectations, the resource rich potential magnet species attracted fewer pollinating species than the co-flowering native and exotic species, with *A. mellifera* the only flower visitor recorded visiting any of the crop species. For the co-flowering species, *A. mellifera* was just one of the two to six flower visiting species (Fig 5.1).
**Figure 5-1** Percentage of visits per species observed on the magnet and co-flowering species. (*) indicates a significant p value following a chi square test between the number of *A. mellifera* and other flower visiting species combined for both the magnet and respective co-flowering plant species. (A) lavender agroecosystem 2013, co-flowering species *M. thymifolia*. (B) lavender agroecossytem 2014, co-flowering species *M. thymifolia* ($\chi^2 = 98.5$ df = 1 $p = <0.0001$). (C) lavender agroecosystem 2014, co-flowering species *B. myrtifolia* ($\chi^2 = 130.6$ df = 1 $p = <0.0001$). (D) lavender agroecosystem 2014, co-flowering species *T. citrodorus* ($\chi^2 = 169.6$ df = 1 $p = <0.0001$). (E) *E. planatgineum* agroecosystem 2013, co-flowering species *M. Thymifolia* ($\chi^2 = 56.7$ df = 1 $p = <0.0001$). (F) *E. plantaginnum* agroecosystem 2013, co-flowering species *B. myrtifolia* ($\chi^2 = 196$ df = 1 $p = <0.0001$). (G) *E. plantatgineum* agroecosystem 2014, co-flowering species *M. thymifolia* ($\chi^2 = 200$ df = 1 $p = <0.0001$). (H) *E. plantaginuem* agroecosystem 2014, co-flowering species *L. stoechas* ($\chi^2 = 200$ df = 1 $p = <0.0001$). (I) *P. persica* agroecosystem 2014, co-flowering species *L. stoechas*. 
Table 5-4 Species specificity of collected foraging insects on both co-flowering and magnet species.

<table>
<thead>
<tr>
<th>System</th>
<th>Site</th>
<th>Specimens caught on</th>
<th>Number of honeybees analysed</th>
<th>Number of honeybees with heterospecific pollen on body</th>
<th>Average percentage of foreign pollen</th>
<th>Type of foreign pollen</th>
<th>Number of honeybees with heterospecific pollen in corbiculae</th>
<th>Average percentage of foreign pollen</th>
<th>Type of foreign pollen</th>
<th>Other species with pollen on their body</th>
<th>Heterospecific pollen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lavender 2013</td>
<td>1</td>
<td>M. thymifolia</td>
<td>16</td>
<td>1</td>
<td>0.99% ± 0</td>
<td>unknown</td>
<td>2</td>
<td>2.9% ± 3</td>
<td>Asteraceae</td>
<td>Hymenoptera = 3</td>
<td>Z. labradus = 4</td>
</tr>
<tr>
<td></td>
<td>1 &amp; 2</td>
<td>Lavender</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td>Hemiptera = 3 Z. labradus = 4 Lepidoptera = 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>M. thymifolia</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td>Hemiptera = 1 Z. labradus = 5 Hymenoptera = 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>M. thymifolia</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td>Hemiptera = 1 Z. labradus = 5 Hymenoptera = 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 &amp; 2</td>
<td>Lavender</td>
<td>20</td>
<td>0</td>
<td>1</td>
<td>1.5% ± 0</td>
<td>unknown</td>
<td>0</td>
<td></td>
<td>Hemiptera = 1 Z. labradus = 5 Hymenoptera = 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>B. myrtifolia</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td></td>
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<td>4</td>
<td>B. myrtifolia</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td>Hemiptera = 1 Z. labradus = 5 Hymenoptera = 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 &amp; 4</td>
<td>E. plantagineum</td>
<td>20</td>
<td>1</td>
<td>52% ± 0</td>
<td>E. plantagineum</td>
<td>1</td>
<td>2% ± 0</td>
<td>unknown</td>
<td>Hemiptera (3 foreign of 4 pollen grains)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>M. thymifolia</td>
<td>12</td>
<td>1</td>
<td>52% ± 0</td>
<td>E. plantagineum</td>
<td>1</td>
<td>100% ± 0</td>
<td>unknown</td>
<td>Hemiptera (3 foreign of 4 pollen grains)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>M. thymifolia</td>
<td>15</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td>Hemiptera = 1 Z. labradus = 5 Hymenoptera = 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 &amp; 4</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td>Hemiptera = 1 Z. labradus = 5 Hymenoptera = 3</td>
<td></td>
</tr>
<tr>
<td>Nectarines 2014</td>
<td>5</td>
<td>L. stoechas</td>
<td>20</td>
<td>1</td>
<td>72%</td>
<td>unknown</td>
<td>0</td>
<td>0</td>
<td></td>
<td>Hemiptera (3 foreign of 4 pollen grains)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>L. stoechas</td>
<td>65</td>
<td>3</td>
<td>62% ± 14</td>
<td>unknown</td>
<td>2</td>
<td>52.4% ± 17</td>
<td>unknown</td>
<td>Hemiptera (3 foreign of 4 pollen grains)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 &amp; 6</td>
<td>Nectarines</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td>Hemiptera = 1 Z. labradus = 5 Hymenoptera = 3</td>
<td></td>
</tr>
</tbody>
</table>
Effects of proximity to magnet plants on pollination biology of co-flowering species

Our surveys of flower visitation revealed an effect of proximity to crop plants on visitation rates in two cases, within the lavender and *E. plantagineum* case studies on *M. thymifolia* ($\chi^2_{(1)} = 7.08\ p = 0.008$) and ($\chi^2_{(1)} = 9.47,\ p = 0.002$) respectively (Table 5-6, Fig 5-2 and 5-3). Visitation declined with distance out to 100m in the lavender case study and out to a distance of 250m in the *E. plantagineum* case study. However, there was no significant effect of proximity to crop plants on the diversity of flower visitors to co-flowering plant species (Fig 5.1) regardless of their hemisphere of origin for four co-flowering species. In only one case study (nectarine 2014) was there a significant interaction between distance and site on *A. mellifera* visitation (GLMM $p = 0.0001$) (Table 5.5) although, this is likely accounted for through variation in visitation between sites (Fig 5.3). In both the lavender and *E. plantagineum* systems, extremely low numbers of honeybees were recorded at all sites, preventing the statistical analyses of data collected in 2014 on lavender and *E. plantagineum* for all co-flowering study species (Table 5.5). Similarly, in 2013, insufficient honeybee numbers prevented the statistical analyses of the *B. myrtifolia* study within the *E. plantagineum* agro-ecosystem.

Despite our finding that honey bees (the dominant flower visitor of all three crop species) made up 55.3% of flower visitors to the co-flowering species, we found that those honeybees that visited co-flowering species carried almost exclusively that species’ pollen (Table 5.4). Indeed, although we detected that 7 of the 128 honeybees that we caught foraging on co-flowering species (6.4%) carried heterospecific pollen,
pollen from the crop species was present on only one of these (Table 5.4). All *A. mellifera* on co-flowering species were observed in the field to be foraging for pollen.
Figure 5-2 The average number (± standard error) of *A. mellifera* observed foraging on the co-flowering species at each distance (m) from the magnet population over four days. (A) Visitation to *M. thymifolia* during the lavender study 2013, (B) Visitation to *M. thymifolia* during the lavender study 2014. (C) Visitation to *B. myrtifolia* during the lavender study 2014. (D) Visitation to *T. citrodorus* during the lavender study 2014. No bar is indicative of no *A. mellifera* observed at that distance.
Figure 5-3 The average number (± standard error) of *A. mellifera* observed foraging on the *L. stoechas* against distance (m) from the magnet nectarine population over four days. (A) - site 1. (B) - site 2
Table 5-5 GLMM analysis testing for variation in the number of *A. mellifera* observed against distance (m) from the magnet population at each site for each co-flowering study species. GLMM had fixed effects of distance from the magnet population and site in relation to the number of *A. mellifera* observed foraging on the test species. IDR= Insufficient data recorded.

<table>
<thead>
<tr>
<th>Study system and test species</th>
<th>Distance*Site</th>
<th>Distance</th>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lavender 2013</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. thymifolia</em></td>
<td>$\chi^2_{(1)} = 0.98, p = 0.32$</td>
<td>$\chi^2_{(1)} = 7.08, p = 0.008$</td>
<td>$\chi^2_{(1)} = 13.63, p = 0.0002$</td>
</tr>
<tr>
<td><strong>Lavender 2014</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. thymifolia</em></td>
<td>$\chi^2_{(1)} = 0.002, p = 0.98$</td>
<td>$\chi^2_{(1)} = 3.07, p = 0.080$</td>
<td>$\chi^2_{(1)} = 1.29, p = 0.26$</td>
</tr>
<tr>
<td><em>B. myrtifolia</em></td>
<td>IDR</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. citriodorus</em></td>
<td>IDR</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Echium plantagineum 2013</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. thymifolia</em></td>
<td>$\chi^2_{(1)} = 0.70, p = 0.40$</td>
<td>$\chi^2_{(1)} = 9.47, p = 0.002$</td>
<td>$\chi^2_{(1)} = 0.83, p = 0.36$</td>
</tr>
<tr>
<td><em>B. myrtifolia</em></td>
<td>IDR</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Echium plantagineum 2014</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>M. thymifolia</em></td>
<td>IDR</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. stoechas</em></td>
<td>IDR</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Nectarine 2014</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. stoechas</em></td>
<td>$\chi^2_{(1)} = 28, p = 0.0001$</td>
<td>$\chi^2_{(1)} = 1.00, p = 0.31$</td>
<td>$\chi^2_{(1)} = 4.65, p = 0.03$</td>
</tr>
</tbody>
</table>
Effects of increasing the number of honeybee hives on visitation rates

In 2013 there was no significant difference before or after the introduction of three hives to the number of *A. mellifera* foraging on the magnet *E. plantagineum* at any site during either the *M. thymifolia* or *B. myrtifolia* study. However, honeybee visitation to co-flowering plants during these experiments was mixed. In both the *M. thymifolia* and *B. myrtifolia* studies one out of two sites showed a significant increase in the number of honeybees after the introduction of hives ($t_{(11)} = 3, p = 0.01$) and ($t_{(10)} = 2.8, p = 0.02$) respectively (Table 5.3). Although at both sites where a significant increase was observed the number of honeybees recorded before and after hive introduction was low (Fig 5.4). Honeybees were also seen to visit study plants at distances not seen before the introduction of hives (Fig 5.4 b, d).

In 2014 honeybee visitation to the magnet *E. plantagineum* was not significantly different following the addition of five hives during the *M. thymifolia* experiment or *L. stoechas* experiment at any site (Table 5.3). In 2014 there no significant difference in the number of honeybees foraging on *M. thymifolia* before compared to after hive addition at both sites, and no honeybees observed before or after the addition of hives during the *L. stoechas* experiment (Table 5.3).
Figure 5-4 Average (± standard error) number of *A. mellifera* observed foraging on the co-flowering plant species against distance (m), during the *E. plantagineum* case study in 2013, before and after the addition of three hives. (A) Site 1, *M. thymifolia* (B) Site 2, *M. thymifolia*. (C) Site 1, *B. myrtifolia*. (D) Site 2, *B. myrtifolia*. 
5.5 Discussion

Examination of our surveys of patterns of pollinator visitation to northern hemisphere agricultural crop species and co-flowering species (both northern hemisphere and Australian origins) showed some evidence of competitive and facilitative interactions that have been reported in northern hemisphere studies (Westphal et al., 2003; Cussans et al., 2010; Diekötter et al., 2010; Hanley et al., 2011; Holzschuh et al., 2011; Kovács-Hostyanszki et al., 2013; Grab et al., 2017). We did find that, as expected, each of the crops were highly attractive to pollinators including the introduced honeybee *A. mellifera*, but contrary to expectation, they did not attract a greater diversity or number of native pollinators than co-flowering plants. Examination of pollen loads revealed little interspecific pollen transfer between crops and co-flowering species. Perhaps most importantly from both an ecological and conservation perspective, we found that for each of the two co-flowering native and northern hemisphere species tested, diversity and species fidelity was unaffected by proximity to the crop plants and that for only one native co-flowering species pollinator visitation declined with distance from the magnet species.

*Pollinator visitation and pollinator diversity on crop plants*

Although the numerical dominance of the honeybee, a flower visitor to each of the three crop species was expected, (Paton 1996; Cunningham et al., 2002) this finding highlights the degree to which Australian agro-ecosystems are influenced by this introduced pollinator. The ability of landholders to manage extremely large populations of honeybees makes them the pollinator of choice within an agricultural setting (Free 1993), while widespread feral populations increase the dominance of this generalist
pollinator across a diverse range of crop species (Cunningham et al., 2002). However, more work will be required to clarify the subsequent effects to co-flowering species reproductive success and additional impacts upon native pollinators.

It is also interesting that comparatively very few native flower visitors were observed visiting the crop species. In particular native bees were only observed during the lavender agro-ecosystem study and were only observed on co-flowering plant species. This may be due to the landscape that the lavender agro-ecosystem is part of having more native remnant vegetation surrounding the study site compared to other study sites.

*Effects of proximity to crop plants on pollination biology of co-flowering species*

The diverse interactions between crops and their co-flowering neighbours revealed in a range of northern hemisphere studies highlights the complexity of both competitive and facilitative interactions, that exist within plant-pollinator systems (Lopezaraiza-Mikel et al., 2007; Diekötter et al., 2009; Gibson et al., 2013; Montero-Castaño et al., 2016). These studies provide the framework for the interpretation of observed pollinator visitation rates as either competitive or facilitative. In the nectarine case study we found that flower visitation to co-flowering plants was not affected by a spatial scale of up to 250 meters. This is possibly due to a high abundance of honeybees in the study area. A high honeybee abundance in a floral resource limited landscape is likely to lead to spill-over of honeybees on to co-flowering plant species. In the other case studies we observed extremely high rates of visitation to all crops, however, in contrast to the nectarine case study, visitation to co-flowering plants in the lavender and *E. plantagineum* agro-ecosystems was exceedingly low to plants of all origins (both Australian and European). The extremely low pollinator visitation rates to our study co-
flowering plants leads us to infer a competitive interaction between the agricultural magnet and both European and Australian co-flowering test species within these agro-ecosystems. We also found in both the lavender and *E. plantagineum* case studies a significant decline in visitation to *M. thymifolia* with distance out to 100 and 250m respectively.

The deduction of a competitive interaction, based upon our observed lowered visitation rates to co-flowering plants in comparison to the crop plant, is supported by similar assumptions in the literature (Levin and Anderson 1970; Gross and Werner 1983; Callaway 1995; Palmer et al., 2003; Brown et al., 2002; Flanagan et al., 2009). Such studies highlight the degree to which competitive interactions occur between plants and their shared pollinators across a range of pollination systems with competitive effects strongest when plants share a generalist pollinator (Lázaro et al., 2009; Bartomeus et al., 2010; Diekötter et al., 2010; Samnegård et al., 2011; Holzschuh et al., 2012; Montero-Castaño et al., 2016) such as the European honeybee.

Honeybees were found to be overwhelmingly faithful to the test species, both crop and co-flowering. Therefore, we expect no negative interaction due to stigma clogging as a result of heterospecific pollen deposition as this is unlikely given the species specificity of honeybees (Grant 1950; Free 1963; Gilpin et al., 2014).

*Effects of increasing the number of honeybee hives on visitation rates*

Following the introduction of three and five honeybee hives there was no significant increase to observed honeybee abundance on any of the crops. Such results may indicate the capacity of magnet plant populations to provide for and support a huge
volume of insect pollinators, with the bountiful floral reward having the capacity to support more honeybees than were introduced in this study. A study by Sabbhahi et al., (2005) tested effects of no hives against 1.5 and three hives per hectare within a flowering canola crop and documented a prominent positive correlation between honeybee density and canola seed output, with three hives per hectare leading to 46% increase in seed yield. This highlights the capacity of such magnet flowering events to host significant numbers of honeybees, with the documented positive correlation in seed set inferring the capacity for magnet plants to host an even greater honeybee density. The foraging range of honeybees has been well studied, with a variety of results documented, including median ranges of 1.7 km (Vischer and Seeley 1982) and 6.1 km (Beekman and Ratnieks 2000). Even considering the shorter potential foraging ranges of honeybees, the floral resources available at such scales are extremely large within magnet communities, such as those found in the *E. plantagineum* study. Amongst the co-flowering plants, mixed results were found. In the 2013 study, honeybee abundance upon the co-flowering *B. myrtifolia* increased 4 times and on *M. thymifolia* honeybee abundance increased by 1.7 times after the addition of ~150,000 honeybees. The potential for these results to indicate possible competitive pressure upon the resources provided by the magnet is tempered by the non-significant results obtained in the 2014 study, whereby visitation rates to all species of co-flowering plants at all sites were not significantly altered by the addition of 5 hives (~250, 000 honeybees). To gain a clear insight into the effect of honeybee abundance towards the competitive interaction between crops and their co-flowering neighbours, even greater additions are required to ensure the full saturation of the crop’s floral resources.
Conclusions

Northern hemisphere magnet crop species may be negatively impacting adjacent co-flowering species. However, in contrast to effects reported for diverse suites of pollinators within northern hemisphere systems, detection of such impacts in Australia may require a landscape-level approach. Here, although the pollination systems of all species were dominated by the generalist pollinator *A. mellifera*, the combination of detectable and un-detectable spill-over effects in terms of pollinator visitation or pollinator fidelity even at scales of hundreds of metres may arguably reflect the normal foraging behaviour of honeybees as opposed to trapline foragers such as bumble bees (*Bombus* sp) and hoverflies reported as frequent visitors to crop plants in the northern hemisphere (Holzschuh et al., 2007; Diekötter et al., 2010). Perhaps most critically, we found very little cross-species pollen transfer although the effects of honeybees may still be either positive or negative depending on the foraging behaviour of honeybees and the mating system of the plant. Future research should determine whether the low number of native pollinators detected visiting the crop species is due to native pollinators inhabiting the area but displaying different foraging preferences or whether the native pollinator population is limited. Pollination rate of co-flowering species may vary depending on the floral neighbourhood particularly when crop plants are attracting and maintaining large numbers of honeybees at a regional level. When mass flowering magnet plants are not in flower, significant opportunities for pollination may arise for neighbouring plants with non-synchronous flowering seasons to that of the magnet, however, further research is required to fully document this process.
Acknowledgements
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References


6 General Discussion
Research summary

The reproductive success of any plant species is influenced by the breeding and pollination systems of the species of interest and by the surrounding assemblage of plant species. Furthermore, the relative density and spatial arrangement of plants, including both the species of interest and the extended flowering community (Feinsinger et al., 1991; Kunin 1997), is likely to affect the type of pollinator (Javorek et al., 2002; Klein et al., 2003), the behaviour of pollinators (Javorek et al., 2002; Seifen et al., 2014), the likelihood of pollination and the degree of outcrossing. In Australia, pollination systems have been greatly disrupted by western agriculture and urban development and by the successful invasion of most native and exotic ecosystems by the introduced European honeybee *Apis mellifera*. Prior to this invasion, many Australian native plants were pollinated by vertebrates such as birds and small mammals (Paton 1993; Whelan et al., 2009) with many of these plants now visited by *A. mellifera*. Studies have shown that some of these vertebrate co-evolved plant species are now serviced by *A. mellifera* (Goldingay et al., 1991; Vaughton 1992; Whelan et al., 2009). However, the subsequent effects of honeybee pollination on the reproductive output and early life-history stages of a typically vertebrate pollinated plant are poorly understood. I aimed to document the impacts of such combined effects as crucially, Australian pollination systems, already pressured by anthropogenic changes are vulnerable to a collapse of natural systems and processes.

Compounding the potential problem of an introduced highly prolific pollinator servicing native plants is the issue of *A. mellifera* spill-over and floral neighbourhood. Studies in the northern hemisphere have found both advantages (Laverty 1992; Ghazoul 2006; Samnegård et al., 2011) and disadvantages (Lopezaraiza-Mikel et al., 2007;
Diekötter *et al.*, 2010) for co-flowering plants in close proximity to “magnet” or highly attractive plant species. However, in Australia little is known about the distance over which spill-over from a highly attractive native magnet population onto native co-flowering species occurs when they are both serviced predominantly by the introduced *A. mellifera*.

Australia is also home to numerous introduced crop and weed species which often dominate agricultural landscapes. Floral visitation to these plant species is often dominated by *A. mellifera* which are often brought in to provide a pollination service in the form of managed hives or have formed feral colonies within the area. Remnant pockets of native vegetation often occur around these agricultural areas. In an effort to gain further understanding of such processes, I aimed to test the distance over which spill-over is observed for a range of Australian native plant species and European honeybee co-evolved plant species in order to determine *A. mellifera* foraging preferences.

I aimed to address key knowledge gaps by using a range of native Australian plant species within natural habitats and large-scale manipulative field experiments within agricultural areas primarily serviced by *A. mellifera*. First, I conducted a manipulative field-based experiment of the vertebrate pollinated *B. ericifolia*, which is now also serviced by *A. mellifera*, to determine the effect of honeybee versus vertebrate pollination on reproductive output and early life-history stages of *B. ericifolia* (Chapter 2). I then sought to improve the efficiency of time spent in the field collecting data by developing and utilising a novel pollination sampling technique (Chapter 3). Utilising this new sampling technique I sought to determine if, and over what distance, *A.
mellifera spill-over occurs within a native ecosystem and to determine whether magnet plants have a facilitative or competitive interaction with co-flowering plants in relation to pollinator visitation, seed set and seed weight (Chapter 4). Finally, I used a large-scale manipulative field experiment to test the distance over which spill-over occurs from agricultural areas and whether honeybee abundance influences the size and distance of spill-over effects (Chapter 5).

**Chapter 2 - Are introduced honeybees affecting the reproductive output and fitness of plants co-evolved for vertebrate pollination?**

Plants that are considered to be adapted to vertebrate pollination are now increasingly visited by the invasive pollinator *A. mellifera* (Paton and Turner 1985; Vaughton 1992; Hansen *et al.* 2002). Nevertheless, the consequences of this phenomenon are poorly understood (Traveset and Richardson 2006). My findings support those of other studies that have found that the foraging behaviour of birds and honeybees differ in regard to length of foraging bouts, exploitation of floral rewards (Hansen *et al.* 2002) and, importantly, the proportion of intra and inter-plant movements (Paton 1993). My data also supports earlier studies showing that honeybees make fewer inter-plant movements and more intra-plant movements as compared to avian pollinators (Richardson *et al.* 2000; Whelan *et al.* 2009). The difference in foraging behaviour of birds and honeybee pollinators might be expected to influence plant fitness by reducing seed set and altering the genotypic composition of seed produced. Most significantly, my study, which is the first to experimentally evaluate these predictions by using a vertebrate exclusion experiment, found no clear evidence that either seed set or seed quality were reduced when inflorescences were pollinated by honeybees.
Chapter 3 - The use of Digital Video Recorders in pollination biology

I designed, developed and implemented a range of parameters which determine the appropriate use of Digital Video Recording devices to gather pollination observation data efficiently at no cost to accuracy compared to human observations. The rugged portable and waterproof design alongside the affordable nature of modern action video cameras has led to their deployment across a range of scientific studies including marine, terrestrial and remote settings, however, their efficiency for use in pollination biology had not yet been studied.

I found that action video cameras are a very powerful tool for use by pollination biologists allowing multiple simultaneous recordings to be recorded remotely under a range of weather conditions by a single researcher. Importantly, the large storage capacity provided by micro SD cards allows a huge volume of data to be recorded in the field and observed later at the researcher’s discretion, with the ability to pause, rewind and observe in slow motion to document exact pollination visitation and behaviour to a time scale not possible by human observations.

Digital video recorders provide a superior means of conducting pollinator observations provided the target plant or subset of flowers can fit within the field of view of the camera. In such conditions these cameras surpass human observations given the significant advantage of multiple observations, reviewable footage and long battery and memory life.
Chapter 4 - The effect of natural magnet plants on pollinator visitation and seed set of less attractive native co-flowering species.

In light of recent and dramatic changes to Australian pollinator assemblages, brought about through the introduction of Apis mellifera, I sought to determine the combined effect of native magnet plant species and the role of A. mellifera, on the pollination biology and subsequent reproductive performance of co-flowering native plant species. Each native pollination system was, as expected, dominated by the introduced honeybee, although both magnet plants and co-flowering species had occasional native flower visitors. I found no evidence that highly attractive magnet species can increase honeybee visitation to co-flowering plants within 100 m of the magnet population. However, after magnet removal, we found that visitation to the co-flowering plant declined by 23-51%. This suggests that there is a link between the magnet and the co-flowering plant in relation to honeybee visits, although the spatial scale over which this relationship occurs is not known. Our study revealed a significant interaction between distance and site in relation to the number of fruit set in three out of four case studies, although in only one case was this driven by a significant decrease in fruit set with distance from the magnet. In all case studies we found a significant effect of distance from the magnet population in relation to seed weight, although the relationship varied between sites and species.

In conclusion, we found evidence that the flowering community combined with the foraging behaviour of honeybees has the potential to influence visitation to co-flowering species within distances of at least 100 m.
Chapter 5 - Are exotic magnet plants in Australian agricultural areas influencing the pollination of surrounding co-flowering native plants?

To further understand interactions between mass flowering plant species and co-flowering neighbours, I undertook a manipulative field experiment. Across all sites, distances and co-flowering species very few honeybee visits were observed in the presence of agricultural magnet species despite their highly attractive nature in other settings. Unexpectedly, most co-flowering species were visited by a greater diversity of native Australian flower visitors than their respective magnet species. Among co-flowering species, no difference was detected in visitation rates between European and Australian native species. Across a range of distances, we found in some case studies, evidence of spill-over from magnet populations to co-flowering species, regardless of co-flowering species origin. These effects were not influenced by an experimental increase of honeybee abundance, demonstrating the capacity of agricultural magnet plants to support a large population of pollinating insects.

Within all agro-ecosystems *A. mellifera* were the dominant flower visitor. I found that *A. mellifera* foraging on magnet plants were overwhelmingly faithful, with only 2.6% (n = 6) of honeybees analysed found to contain heterospecific pollen on their body and therefore potentially available for pollination.

My findings have significant implications for co-flowering species adjacent to agricultural magnets within Australia. I have shown that agricultural magnets are predominately serviced by honeybees. However, as my research solely focussed upon magnet plants during mass flowering events I cannot quantify the pollinator visitation to
co-flowering species when the agricultural magnets are not in flower. Albrecht et al., (2007) showed that remnant plant species host pollinators when agricultural magnets are not in flower, indicating a potential advantage for neighbouring plant species which do not overlap in their flowering season. However, when the predominant visitor is an introduced species, this may have a detrimental rather than beneficial effect on the neighbouring native plant species.

**Future research priorities**

Research that aims to understand the impact of introduced pollinators on plants needs to not only include details of pollinator visitation and behaviour but also needs to determine their effect on reproductive output, seed quality and effects on early and later life-history stages. The plants’ mating system should also be factored into any conclusions made. With more complete knowledge of introduced pollinators can come more targeted management of those pollinators or their impacts within natural areas.

Priority also needs to be given to research that focuses on understanding how entire flowering communities interact when they overlap in flowering time and share pollinators. Expanding upon the case studies within this thesis will enable insight into the interactions between plants with shared pollinators and may assist conservation efforts and management.

Future research should also pay particular attention to understanding the role that large scale mass flowering crops play on pollinator interactions between native co-flowering plants. Research should be conducted across all flowering seasons to gain a
complete understanding of these interactions. Research into these interactions has the capacity not only to aid pollination efforts of crop plants but also to maintain pollination systems within native remnants.

**Conclusion**

My thesis has clearly shown that the interactions and effects of the introduced honeybee in Australia are species-specific but also dependent upon the flowering neighbourhood and interactions between magnets and co-flowering species. My research suggests that the distance over which magnet plants and spill-over effects are found is dependent upon the species of plant, the spatial arrangement of plants and to a lesser extent (at least for the number of honeybees (~250,000) that I introduced) the abundance of honeybees. This body of knowledge forms a foundation upon which to build future work to quantify the combined effects of *A. mellifera* and magnet plants within an Australian context, in an effort to preserve existing native plant-pollinator interactions wherever possible.
References


