Pollination ecology of *Lantana camara* and its relationship with honey bees (*Apis mellifera scutellata*) in South Africa

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by

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Declaration

I, Eileen Engelbrecht, declare that this thesis, which I hereby submit for the degree of MSc Entomology at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Eileen Engelbrecht

Signature Eileen

Date 2023-07-19

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Abstract

Invasive species are considered a major biological threat to the planet, causing losses on economic and environmental fronts. *Lantana camara* is one of the worst invasive plant species, globally and in South Africa, with the cost of control of lantana amounting to millions of rands annually. The success of invasive plants, including lantana, is often dictated by the presence of potential, often native, pollinators in newly invaded environments. Shared pollinators are also an important way in which invasive plants interact with sympatric, co-flowering native plant species. Pollinator preferences can influence the success of natives and invasives in a shared environment. This study aims to investigate the pollination ecology of *Lantana camara* in South Africa, specifically focusing on African honey bees (*Apis mellifera scutellata*), and how lantana interacts with three native flowering plant species, *Aloe greatheadii, Aloe marlothii,* and *Kalanchoe rotundifolia*, via their shared native pollinators. The diversity of flower-visitors of lantana in an urban nature reserve was explored in four sampling seasons (spring, summer, autumn, and winter, from Nov 2021 to Jun 2022). We show the pollinator population utilising lantana is diverse (31 families from 5 orders) and the main pollinators of lantana in our study sites are thrips. Honey bees were the second most abundant pollinators. The variation in the number of honey bees observed on lantana was only explained by observation season. We were further interested to see how different pollinators influence the pollination success of lantana. This was accomplished with exclusion experiments, designed to exclude pollinators based on size. We found no significant differences in the numbers of viable fruits produced by lantana between the different exclusion treatments. To investigate the potential impact of lantana on indigenous flowering plants two experiments were conducted to investigate the preferences of native pollinators at a species level and a community level. For the species-level experiments, preference experiments were conducted on African honey bees. Honey bee colonies were presented with a choice between lantana and two indigenous bee plants, *Aloe greatheadii* and *Aloe marlothii*. These data show that honey bees chose aloes significantly more often than lantana, and took less time to choose aloes, indicating a preference for the indigenous plants. At a community level, pollinator communities on the indigenous *Kalanchoe rotundifolia* in the inflorescences were compared between plots invaded with *Lantana camara* and those where the lantana had been removed. No differences in the pollinating communities of invaded plots versus cleared plots were found. Post hoc analysis revealed that the power of the statistical analysis is not high enough to confidently reject the alternative hypothesis that lantana influences the number of pollinators per observation bout between the cleared and invaded sites. Improvements to the study design are discussed. This study provides insights into the interaction of native South African pollinators with *Lantana camara* and to what extent lantana relies on these interactions for successful fruit production. Many native pollinators utilize the resources provided by lantana, which likely contributes to its success in South Africa. Honey bees, however, are not at the forefront of this success, showing a clear preference for indigenous aloes.

Keywords

Lantana camara, pollination ecology, African honey bees, *Apis mellifera*, invasive plants, pollinators, exclusion experiments, preference tests

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List of abbreviations

AB	Abundance of *Lantana camara* inflorescences
AD	Average distance of honey bee colonies
AIC	Akaike Information Criterion
AIS	Alien and Invasive Species Regulations
FGNR	Faerie Glen Nature Reserve
GLMM	Generalised linear mixed model
H’	Shannon diversity index
ICNCP	International Code of Nomenclature for Cultivated Plants
IUCN	International Union for the Conservation of Nature
LS	Lesotho
NEMBA	National Environmental Management: Biodiversity Act
S	Season
s.l.	*Sensu lato*
SD	Standard deviation
SE	Standard error
SW	eSwatini
T	Temperature

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Chapter 1

General introduction

Biological invasions

A major biological threat to the planet is alien species invasions (Vitousek *et al.* 1997, Pejchar and Mooney 2009). It is among the most pressing problems experienced by natural ecosystems globally and one of the most detrimental effects of humans on the planet (Sharma *et al.* 2005). The invasion of alien plants specifically, constitutes a large proportion of biological invasions, causing monetary losses in several economic sectors, as well as losses on the environmental front, mainly in terms of the loss of biodiversity (Traveset and Richardson 2006, Sandham *et al.* 2010, Berry *et al.* 2011, Pereira *et al.* 2012). An overview of the costs of control programmes for invasive species in six different countries, including South Africa, estimates the global costs of such programmes at US$314 billion per year (Pimentel *et al.* 2001). Based on average global inflation alone (World Economic Outlook Data 2022), such costs would have reached over US$ 700 billion per year in 2021.

Although biological invasion is an ancient and natural process, the current enhanced rate of invasion of alien species is human-induced (Sharma *et al.* 2005). Human activities have played a critical role in facilitating the introduction of invasive plants. The intentional and large-scale distribution of plants over long distances began with the establishment of European colonies (Le Maitre *et al.* 2004). Not all non-native species, however, are considered invasive. The term invasive species is used in the context of taxa that can aggressively spread in regions where they are not native (Hobbs 2000, Rejmánek 2000). This is opposed to naturalised species which form sustainable populations without human intervention but do not necessarily spread. In addition to this, not all naturalised or invasive species are even considered to be weeds (Rejmánek 2000).

The focus species of the present thesis, *Lantana camara* Linnaeus (*sensu lato*) (hereafter *L. camara* or lantana) is, in most introduced areas, considered an invasive weed. Although, it exists solely as a naturalised plant in some areas as well. The spread of lantana is limited in areas with undisturbed rainforests and intact canopies, such as native forests in India, Papua New Guinea, and Australia (Duggin and Gentle 1998, Day *et al.* 2003, Sharma *et al.* 2005). Lantana is especially susceptible to shading from neighbouring plants (Duggin and Gentle 1998). In the drier central parts of South Africa, lantana is restricted to gardens and river sides (Vardien *et al.* 2012).

Global distribution of *Lantana camara*

The intentional introduction of plants can fall into two categories, the introduction of a species for utilitarian reasons such as food sources, fuel, medicines, or aesthetic reasons (Mack and Lonsdale 2001). Since the motivation for most of the initial distribution of lantana was for ornamental purposes, most introductions of *L.* fall in the latter (Morton 1994, Urban *et al.* 2011, Vardien *et al.* 2012).

Lantana is widely accepted to be a native of the West Indies, Mexico, and Central and South America (Cilliers 1983, Broughton 2000, Day *et al.* 2003, Urban *et al.* 2011). The first recorded introduction of *Lantana spp.* occurred in the 1640s when Dutch explorers brought the plant from Brazil to the Netherlands as ornamentals (Stirton 1977, Morton 1994, Vardien *et al.* 2012). In Europe, lantana was a popular garden and glasshouse plant, where it was bred and hybridised repeatedly, and was then exported, as ornamentals, to other countries worldwide, often between lantana clubs (Day *et al.* 2003, Vardien *et al.* 2012). Lantana was considered a prized ornamental because of its brightly coloured inflorescences, easy propagation, and hardy nature of the plant (Urban *et al.* 2011).

The most notable and problematic introductions of lantana occurred between the early 19th century and 20th century (Figure 1.1) (Thakur *et al.* 1992, Goulson and Derwent 2004, Urban *et al.* 2011, Carrión-Tacuri *et al.* 2014, August-Schmidt *et al.* 2015). Today, around 650 hybrid varieties of *L. camara* are naturalised in over 60 countries worldwide and is an especially problematic invader in tropical, subtropical, and temperate regions of invaded countries (Day *et al.* 2003, Zalucki *et al.* 2007).

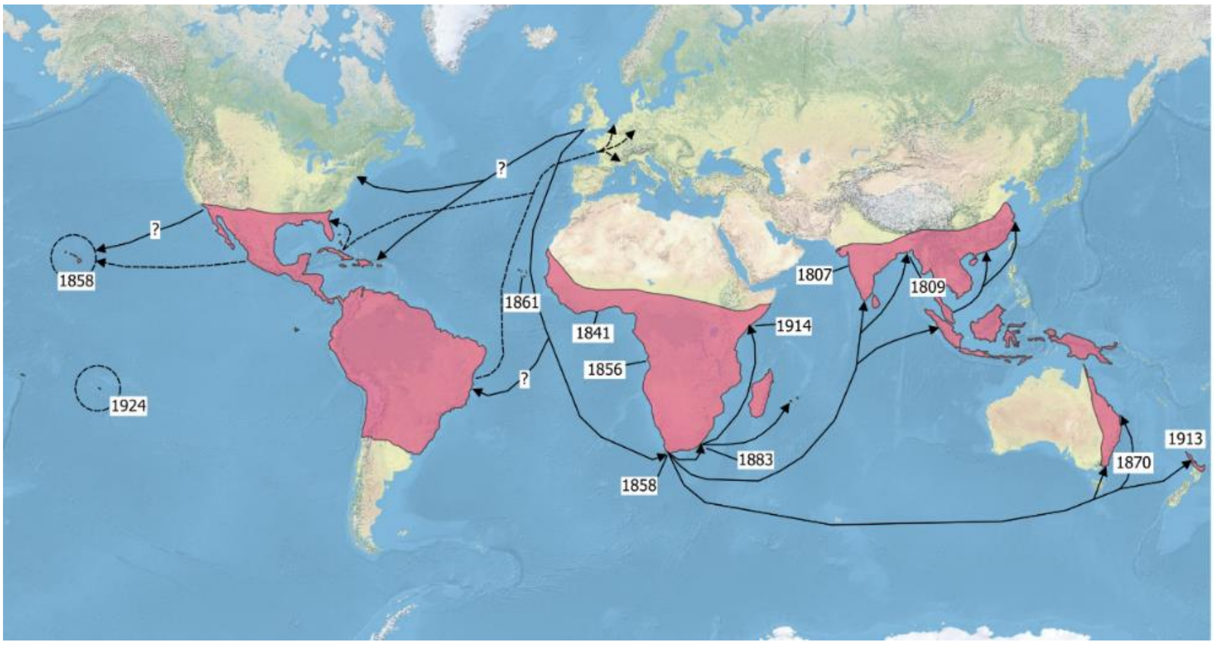

Figure 1.1 Documented intentional introductions of *Lantana camara* L. (*sensu lato*) throughout the world. Dashed lines indicate the movement of *Lantana camara* from its native range, while solid lines show the movement of hybridised lantana plants for ornamental purposes. (Figure redrawn from Stirton (1978)).

Lantana camara in South Africa

South Africa undoubtedly faced many unrecorded introductions of lantana, and there are some conflicting reports on the exact timing of the recorded introductions. Cilliers and Neser (1991) state that the first introductions to South Africa occurred in Kwa-Zulu Natal from Mauritius, and in Cape Town from Europe, in 1883 and 1885 respectively. Urban *et al.* (2011) report that there was an earlier introduction to Cape Town in 1858, but that lantana was unable to spread very far at the time on account of the Mediterranean climate in the region. It is, however, in the subtropical climate of the Kwa-Zulu Natal that lantana eventually got a foothold, from where it was able to flourish and spread in South Africa (Stirton 1977, Cilliers and Neser 1991, Day *et al.* 2003). By 1909 lantana had become thoroughly established in the coastal areas of KwaZulu-Natal, and by 1946 the region accounted for 80% of the lantana in the country (Cilliers 1983, Urban *et al.* 2011). In 1954, after the discovery that the leaves of lantana are poisonous to cattle, it was finally declared a noxious weed throughout South Africa (Cilliers and Neser 1991). Despite this, its spread continued to the surrounding subtropical and temperate regions, and by 2000 lantana had invaded more than 2 million ha, with a condensed invaded area of over 69 000 ha (Le Maitre *et al.* 2000).

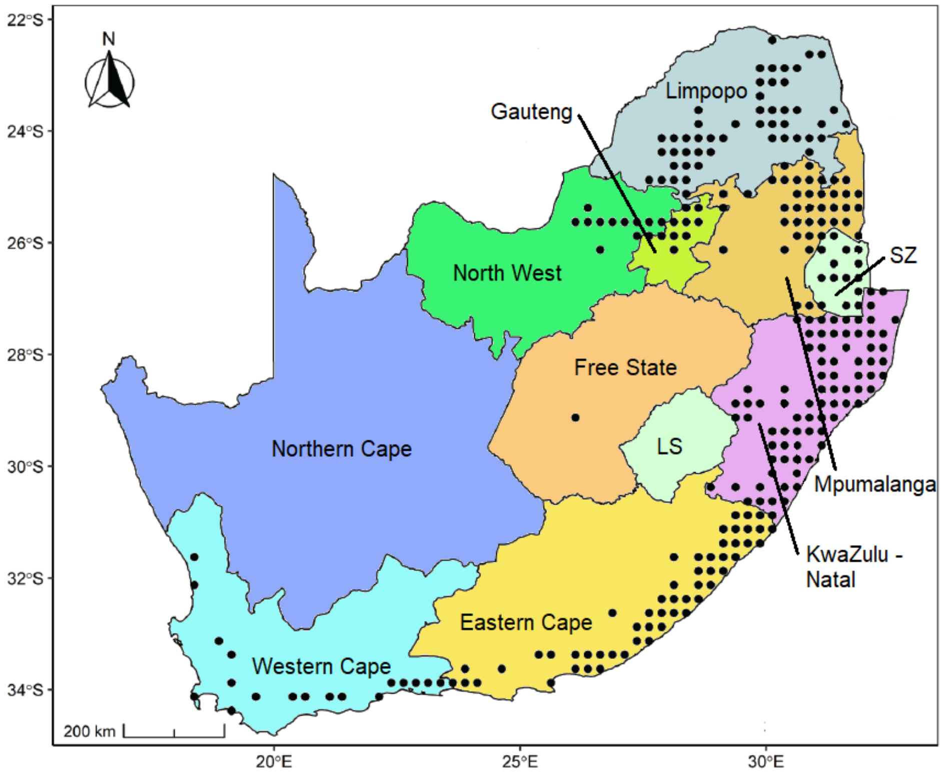

Figure 1.2 The distribution of *Lantana camara* L. (*sensu lato*) in South Africa. Provinces of South Africa are indicated in multicolour. The nations of Lesotho (LS) and eSwatini (SZ) are shown. Original map drawn by Guy Sutton. Data source: SAPIA database, Agricultural Research Council-Plant Protection Research Institute, Pretoria. **(Figure redrawn from Simelane *et al.* (2021))**

Simelane *et al.* (2021) present the most recent distribution of lantana in South Africa. Lantana is found in eight of the nine provinces of South Africa, distributed along the southern and eastern coast, and inland in the northern and north-western regions of the country (Figure 1.2). The distribution range and density of lantana are continuously increasing in all areas where it occurs (Urban *et al.* 2011). The National Environmental Management: Biodiversity Act (NEMBA) of South Africa categorises invasive plant species based on the threat level of the species and management requirements. Currently, lantana is declared a category 1b invasive species, indicating that it is regarded to be highly invasive and requires compulsory control as part of an invasive control programme in respect of the Alien and Invasive Species Regulations (AIS), NEMBA (Act No 10 of 2004).

Control of *Lantana camara*

The control and management of lantana is a very popular topic of study but to little avail. The shallow root system of the plant allows for the use of lantana levers or tree poppers for the removal of the plant (Cilliers 1983, Graaff 1986). Extracted plants need to be stacked, allowed to dry, and burnt. It is further important for reclaimed land to be cultivated to minimise the re-establishment of lantana seedlings (Cilliers 1983). Lantana has been shown to have a persistent seed bank (Vivian-Smith and Panetta 2009). Activities that increase the light intensity or soil temperature, for example, the removal of large lantana plants with wide and shallow root systems, will stimulate the germination of deposited lantana seeds (Duggin and Gentle 1998, Sharma *et al.* 2005). Combined, these factors make the sustained management of lantana via mechanical removal, very inefficient.

Herbicides are often preferred over the expensive and labour-intensive process of mechanical control. However, herbicidal control of lantana has been variable and mostly unsuccessful (Ferrell *et al.* 2012). This has been attributed to a large number of hybrid varieties of lantana that exists (Graaff 1986, Ferrell *et al.* 2012). Regardless of whether mechanical or chemical control is used, lantana is known for its prolific regrowth and re-establishment after treatment, and treatments need to be repeated many times to achieve complete eradication. At high levels of infestation, and in inaccessible areas, the eradication of lantana using chemical methods is extremely costly and inefficient (Goncalves *et al.* 2014).

A third alternative treatment for lantana is biological control. Biological control directed at weeds was initiated in 1902 when 23 insect species were sent to Hawaii from Mexico for the control of lantana (Perkins and Swezey 1924, Day and Neser 2000). However, despite lantana being at the forefront of the development of biological control and extensive efforts afterwards, attempts at its biological control have remained largely unsatisfactory worldwide (Broughton 2000, Day and Neser 2000, Zalucki *et al.* 2007). In South Africa, the first attempts at the control of lantana using insects were in the 1960s, when five herbivorous insect species were imported to South Africa from Hawaii, where they were successful in the control of the lantana invasion (Cilliers and Neser 1991). Since then, a further 16 biocontrol agents have been established, but their control over lantana infestations remains ineffective (Simelane *et al.* 2021). This, again, is attributed to many hybrid varieties of lantana. Several established biocontrol agents in South Africa have displayed varietal preferences, limiting the agents to a subset of the varieties available to them. The effect of varietal preferences of biocontrol agents is exacerbated by climatic mismatches of the agent's native range and that of the target host in the area of introduction (Broughton 2000).

The cost of the control and management of lantana is immense in every invaded country. In India, over 13 million ha of pasture lands alone has been invaded, racking up a cost of control and management of over UD$70 million per ha (Negi *et al.* 2019). In 2004 the annual estimated expenditure on the control of lantana in Australia exceeded Aus$10 million, while decreased stocking densities and cattle deaths due to lantana poisoning cost the livestock industry a further Aus$7.7 million every year (Goulson and Derwent 2004). Marais *et al.* (2004) reported the estimated costs associated with invasive alien plants in South Africa during the 2002/03 financial year. A condensed area of 5 407 ha of lantana was treated in that year, with an initial cleaning cost of R8.97 million, and the cost of follow-up cleaning of R7.02 million. These costs exclude the costs of herbicides used. It also does not account for the economic losses associated with lantana infestations, which are certain to be substantial.

Brief taxonomic overview of *Lantana camara* L. (*sensu lato*)

The considerable number of lantana taxa that exist as invasives worldwide are different from lantana in their native range in nearly every way possible. It is the former that technically holds the name *Lantana camara* Linnaeus (Urban *et al.* 2011). Despite this, there is a convention in the scientific literature to apply this name to weedy lantana, which is taxonomically incorrect (Sanders 2006). Weedy lantana can produce intermediate hybrids, which is what makes it so difficult to apply appropriate taxonomic nomenclature to lantana (Munir 1996). The taxonomic nightmare which is the species complex *L. camara* L. has been explored at length (Day *et al.* 2003, Sanders 2006, Urban *et al.* 2011). The use of the name *L. camara* L. (*sensu lato*) has been widely applied to refer to weedy lantana (Munir 1996, Baars and Neser 1999, Day *et al.* 2003), and this is correct under the International Code of Botanical Nomenclature and the International Code of Nomenclature for Cultivated Plants (ICNCP) (Urban *et al.* 2011). In the present study, we will follow the naming convention described by Day *et al.* (2003) and applied by Urban *et al.* (2011), using the common name 'lantana' to refer to the weedy taxa of *Lantana* L. section *Camara* in the broad sense (*sensu lato*) abbreviated (*s.l.*).

Biology of *Lantana camara*

L. camara L. (*s.l.*) (also commonly known as largeleaf lantana or wild sage) is a perennial woody shrub, belonging to the family Verbenaceae. It is a hardy and adaptable plant, able to utilise a variety of habitats and soil types (Sharma *et al.* 2005). Lantana grows well in open, unshaded areas, especially disturbed areas such as pastures, the edges of forests, roadsides, railway tracts, and canals (Munir 1996, Sharma *et al.* 2005). While lantana tolerates low-light environments, it relies on sufficient light to flourish (Duggin and Gentle 1998). In Australian rainforests, established infestations of lantana only became severe once the closed canopy was damaged (Fensham *et al.* 1994, Duggin and Gentle 1998). Lantana is susceptible to frost and is rarely found in areas with temperatures frequently below 5°C (Cilliers 1983, Sharma *et al.* 2005).

Lantana produces large numbers of small florets, grouped into paired inflorescences in leaf axils, and each plant is capable of producing high numbers of inflorescences (Sharma *et al.* 2005, Carrión-Tacuri *et al.* 2014). Inflorescences are often multicoloured (Figure 1.3). Newly opened florets are yellow, and changes to orange, red, purple, pink, or white, depending on the colour variant. In some variants, only a yellow ring is present around the opening of the corolla (Barrows 1976, Sharma *et al.* 2005). Only the yellow, newly opened florets contain pollen and nectar (Barrows 1976). These colour changes act as a visual cue to pollinators, with the pollination of the floret inducing the colour change (Mohan Ram and Mathur 1984). There are large variations in floret colour, size, and shape.

Figure 1.3 Colour variants of *Lantana camara* L. (*s. l.*) found in the Faerie Glen Nature Reserve, Pretoria, South Africa. The colour changes of the florets act as a visual cue to pollinators. Yellow florets are newly opened, containing pollen and nectar rewards. Florets that have undergone colour changes contain negligible amounts of pollen and no nectar. The colour change is induced by pollination. © Eileen Engelbrecht.

Lantana flowers are attractive to several charismatic insect pollinators, and birds seeking to forage on the fruit it produces (Urban *et al.* 2011, Lonare *et al.* 2012). The florets have long, narrow corolla tubes, accumulation of nectar, and grouped inflorescences (Carrión-Tacuri *et al.* 2014), flower characteristics that are often associated with pollination by butterflies (Faegri and Pijl 1979, Proctor *et al.* 1996). Despite this, a study conducted in Queensland, Australia, claims that honey bees, *Apis mellifera* (an introduced species in Australia) are the main pollinators of lantana (Goulson and Derwent 2004). Pollinators were observed on 24 789 lantana inflorescences at 63 sites throughout coastal Queensland. Despite the large numbers of inflorescences observed only 272 insects were recorded, of which 171 were honey bees (Goulson and Derwent 2004). They also found that the fruit set of lantana was significantly correlated with honey bees and butterflies in their study sites. Studies conducted on the Galapagos Islands found that butterflies are the main pollinators associated with invasive lantana (Carrión-Tacuri *et al.* 2014). In India, both thrips and butterflies are pollinators of lantana (Mathur and Mohan Ram 1978, Mukherjee *et al.* 2015). There are no analogue studies of this nature in South Africa.

Due to the wide variety of pollinators frequenting lantana flowers, the plant is considered a pollinator-generalist plant species (Carrión-Tacuri *et al.* 2014). There are conflicting reports as to whether lantana relies on insect pollination for reproduction, or whether it can effectively produce fruits by autonomous self-pollination (Kritasampan *et al.* 2016). Initial studies of Barrows (1976) concluded that the lantana inflorescences did not self-pollinate, while Mohan Ram and Mathur (1984) determined that lantana was self-compatible but needed insects for effective pollination. Carrión-Tacuri *et al.* (2014) found that lantana is capable of autonomous self-pollination. In their experiments, pollinators were excluded from inflorescences, using fine-mesh nylon bags. The bagged inflorescences were still able to produce seeds, albeit in lower numbers than the control inflorescences. With similar experiments, Goulson and Derwent (2004) concluded that the fruit set of lantana is significantly enhanced when inflorescences were pollinated by insects compared to inflorescences from which pollinators were excluded.

Given sufficient moisture and light conditions, it is not uncommon for lantana to flower prolifically all year round (Graaff 1986, Sharma *et al.* 2005). Lantana produces aggregate fruits (called infructescences), composed of several round, fleshy drupes, each approximately 0.5 mm in diameter (Sharma *et al.* 2005). Each infructescence contains multiple drupes, and each drupe contains one to two seeds. As with the flowers, lantana seeds are also able to germinate year-round, given adequate soil moisture, light conditions, and temperature. Seeds are readily spread by animal agents, especially birds (Graaff 1986, Swarbrick *et al.* 1995). Frugivorous birds feed on the fruits, and in turn spread the seeds of the plant, facilitating the invasion, and spread of the plant into natural ecosystems (Loyn and French 1991, Lonare *et al.* 2012).

After establishment, lantana is capable of high rates of vegetative growth (Sharma *et al.* 2005). They can spread vegetatively via prostrate stems. Prostrate stems are branches of a plant that grow just above or on the ground. In lantana, these stems can form roots at the nodes when covered by moist soil, or other natural debris. This vegetative growth facilitates the formation of dense thickets in infested environments.

The effects of *Lantana camara* on natural and agricultural environments

Lantana is considered by the International Union of the Conservation of Nature (IUCN) to be one of the 100 worst invasive alien plants in the world (Lowe *et al.* 2000). In their global review on woody invasive plants, Richardson and Rejmánek (2011) state that lantana may be one of the ten worst invasive plant species in the world. Le Maitre *et al.* (2000) named lantana number nine on their list of the 10 worst invaders by condensed invaded area in South Africa.

Lantana is described as an aggressive grower, able to tolerate a wide range of environmental conditions (Baars and Neser 1999). It produces allelochemicals that adversely influence the growth and competitiveness of plants growing alongside it (Ferrell *et al.* 2012). Lantana is resistant to herbivory (Ghisalberti 2000) and has a high tolerance for defoliation (Broughton 2000). These factors are the main contributors to the status of lantana as a major agricultural and environmental weed. Lantana mainly occurs in clumps with <1 m diameters in its native range. In invaded areas, however, lantana can form highly persistent, monospecific thickets, reaching up to four meters in height (Sharma *et al.* 2005).

On agricultural lands, lantana rapidly encroaches on plantations, orchards, and pastures (Gentle and Duggin 1997b). It forms dense thickets that are impenetrable to both humans and animals, obstructing access to water sources, and leading to a reduction in the productivity of stock farming where lantana has invaded (Thakur *et al.* 1992, Swarbrick *et al.* 1995, Gentle and Duggin 1997a). The shoots, leaves, and fruits of lantana are also extremely toxic to livestock when ingested, and cause a wide array of organ damage, usually leading to rapid death or chronic poisoning in horses, sheep, and cattle (See Morton 1994, Day *et al.* 2003, and references therein). Afflicted animals rarely recover to resume productive gains (Ferrell *et al.* 2012). Due to the difficulty of eradicating lantana, and the adverse effects thereof, lantana infestations on agricultural lands frequently result in the loss of value of that land (Urban *et al.* 2011). Lantana eagerly invades timber plantations in South Africa, forming thickets beneath the trees and in fire breaks. These infestations impede thinning and felling activities increase fire hazard, and competes with young trees for resources, reducing productivity (Graaff 1986).

The encroaching nature of lantana also has negative effects on natural environments. The composition of natural vegetation may be severely altered due to the reduction of the density, biomass, species diversity, richness, and evenness of the native species sharing a habitat with lantana (Bhatt *et al.* 1994, Fensham *et al.* 1994, Singh *et al.* 2014). These alterations can at least partly be attributed to the ability of lantana to restrict the regeneration and growth of surrounding plants via allelopathic suppression (Mishra 2014, Singh *et al.* 2014). The presence of lantana has been found to change the soil condition and composition, which in turn affects the composition and productivity of native plants (Raizada *et al.* 2008, Ruwanza and Shackleton 2016). Changes in the fire regime of dry rainforests in the north of Australia have been linked to the presence of lantana. Lantana alters the distribution of fuel, shifting the available fuel closer to the ground, and creating a continuous fuel layer leading into the forest from the edge (Berry *et al.* 2011). Taylor *et al.* (2019) studied small frugivorous, insectivorous, and nectarivorous birds at the Ferncliff nature reserve in KwaZulu-Natal, South Africa, which is infested with lantana. They found birds with hard, black deposit clogging their feet, which they suggest originated from stem secretions of lantana shrubs, on which these birds often forage. The deposit encloses their toes, making their feet unusable, ultimately resulting in the demise of the birds (Taylor *et al.* 2019). There is an alarming overlap in the distribution of lantana (Day *et al.* 2003) and the Myers biodiversity hotspots (Myers *et al.* 2000), indicating a severe threat to ecosystems in these overlaps.

The facilitative interactions between native and alien invasive species

In many instances, the interactions between invasive species, and the fauna and flora of invaded areas may prove to be a double-edged sword. In general, invasive species compete with native species for resources such as space, light, water, and nutrients (Brown *et al.* 2002, Ghazoul 2002) and often invasive species are found to suppress or even eliminate native species (Richardson *et al.* 2000). Introduced plant species rely on newly established mutualisms in their “new habitats” to first become naturalised and thereafter invasive (Richardson *et al.* 2000, Nel *et al.* 2017). These mutualisms established by invaders often serve to facilitate the propagation of invasive species (Richardson *et al.* 2000). In Australia pollination services of bees to invasive plants have been recognised as a key process threatening native biodiversity (Gross *et al.* 2010).

Situations do exist, however, in which the presence of an alien plant may have positive effects on native fauna and flora (Feldman *et al.* 2004, Rodriguez 2006). Invasive plants can act to facilitate the pollination of natives by acting as an attractant to pollinators and subsequently leading to an increase in the number of pollinator visits received by the native cohabitant species (Rathcke 1988, Laverty 1992). Albrecht *et al.* (2016) discuss three mechanisms by which co-flowering species may facilitate pollinator visitation, pollination, and reproductive success, which can be applied to co-flowering native and invasive plant species as well. The mechanisms are as follows: (a) per capita visitation to relatively unattractive plant species may be enhanced in the presence of a more attractive (often invasive) species, (b) pollination attraction and visitation may be increased due to the sheer volume of floral displays of co-flowering species, or because of higher floral resource diversity, and (c) increased numerical response of pollinations in the presence of a co-flowering species across years.

This facilitative effect is lost, however, if the pollinators in question show a preference towards the invasive plant species rather than the native species. In situations like this, the visitation of, and by extension, the pollination of native plant species may be negatively affected (Larson *et al.* 2006). Brown *et al.* (2002) found that the presence of an invasive herb, *Lythrum salicaria* negatively affected the reproduction of the native *Lythrum alatum*. Pollinators showed a preference for invasive species, which resulted in reducing the pollination services to the native plant. In addition to this, visitor facilitation does not always translate to increases in reproductive success. The quality of the visit plays a role. Pollinators with low flower constancy may increase the transfer of heterospecific pollen transfer, which may lead to decreased pollination success (Morales and Traveset 2008).

Besides the effects of invasive plants on native flora, native fauna may also be affected. For example, fleshy-fruited invasive plants provide food that supports indigenous frugivore populations (Buckley *et al.* 2006). The presence of invasive species may have a positive influence on native pollinating species, by providing limiting resources to higher trophic levels (Rodriguez 2006). In Kolkata, India, it was found that lantana is a host to 25 butterfly species in urban and rural areas, with the abundance of butterflies found to be proportional to the density of lantana flowers across rural and urban sites (Mukherjee *et al.* 2015). They conclude that the presence of lantana could facilitate the conservation efforts of butterflies. It is important to take the facilitative interactions between native and invasive species into consideration since they may present management dilemmas. Pollinators face many challenges in urban environments (Baldock 2020). There is an increasing need to ensure access to adequate resources for declining pollinator communities, which some have suggested might include invasive species (Salisbury *et al.* 2015, Bartomeus *et al.* 2016, Wood *et al.* 2018). However, a recent paper highlights how native gardens can be designed intentionally to support increased numbers of insects (Breed *et al.* 2022).

Project rationale

In many other parts of the world where lantana presents challenges as an invasive, some studies have been conducted to determine the pollinator associations of the plant in that region, however, similar studies for lantana is South Africa are lacking.While lantana is well researched in South Africa, much of this research has been focussed on the biological control of lantana (eg. Cilliers 1983, Cilliers and Neser 1991, Baars and Neser 1999, Urban *et al.* 2011, Simelane *et al.* 2021). In contrast, extremely little research has been dedicated to the pollinating insects associated with lantana and its interaction with our native pollinators in South Africa. A search of the Web of Science database with the search terms “*Lantana camara”* and “pollinators” resulted in only three hits when the results are filtered to South Africa (Accessed 20/04/2023). The three hits were Nel *et al.* (2017), Bitani and Downs (2022), and Santana *et al.* (2022), and none of these three hits are similar to the present study.

Which of our pollinators exploit lantana as a food resource, and in turn, how reliant is lantana on the pollination services it receives from our pollinators? What are the possible effects that lantana has on native co-flowering plants? These are some of the main themes of the present thesis.

Contrasting goals might arise between the management of lantana and the conservation of native insect species. Improving our understanding of the factors that influence the propagation of lantana could be of great value in understanding the ecological role fulfilled by lantana in our natural environments, enabling the design of appropriate and effective control programmes for this weed. This study aims to fill this gap in South African research and contribute to the pool of knowledge on lantana in South Africa by investigating aspects of the pollination biology of *L. camara*.

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Chapter 2

Interactions between *Lantana camara* and pollinating insects in an urban nature reserve in South Africa.

Note: The chapter is written to be submitted to Weed Research, hence the structure and format.

Abstract

Invasive species are considered a major biological threat. The presence of potential pollinators in an invaded environment often dictates the success of an invasive plant species. *Lantana camara* is one of South Africa's worst invasive plants. This study explores the diversity of flower visitors on *Lantana camara* in an urban nature reserve in South Africa (the Faerie Glen Nature Reserve in Pretoria), with a specific focus on honey bee (*Apis mellifera scutellata*) visitors. Insect observations were conducted in four seasons (summer, autumn, spring, and winter, from Nov 2021 to Jun 2022). In total 3409 arthropods were recorded on *L. camara,* 2967 of which were pollinating insects. We show that lantana has a diverse pollinator population spanning 30 families from five insect orders, making lantana a pollinator-generalist plant. The most abundant pollinating insects on lantana were thrips (63% of all observed arthropods). After thrips, honey bees were the second most abundant pollinators in all seasons, apart from spring (making up 4% of all observed arthropods). The number of honey bees observed on lantana varied between seasons, with significantly higher numbers observed in summer and autumn. Season was the only significant explainer of variation in honey bee numbers on lantana inflorescences. We were further interested to see how important pollinators are to the pollination success of lantana. Using exclusion experiments designed to exclude pollinators based on size, we investigated the importance of different pollinators on the viable fruit set of lantana. These experiments resulted in no significant differences between the different pollinator exclusion treatments. This study provides insights into the interaction of native pollinators with *Lantana camara* and to what extent lantana relies on these interactions for successful fruit production. Many native pollinators utilize the resources provided by lantana, which likely contributes to its success in South Africa, however, honey bees are not at the forefront of this success.

Keywords

Invasive plants, honey bees, *Apis mellifera scutellata*, pollinators, *Lantana camara*, exclusion experiments

Introduction

Most plant species, including many alien plant species, are dependent on animals for pollination (Ollerton *et al.* 2011). The success of the invasion of alien invasive plants is often reliant on the presence of potential pollinators in the invaded environment, whether the pollinators are native or introduced (Parker and Haubensak 2002), and the ability of the alien plant to form novel mutualistic relationships with these pollinators in its newly invaded environment. Few invasive plants have been as successful as *L. camara* L. (*sensu lato*) (hereafter *L. camara* or lantana). This shrub is one of the world's most invasive weeds, having invaded more than 60 countries worldwide (Day *et al.* 2003, Zalucki *et al.* 2007). It is considered one of the 100 worst invasive plants in the world (Lowe *et al.* 2000), and the ninth worst invader in South Africa by condensed invaded area (Le Maitre *et al.* 2000).

The typical floral characteristics of lantana, such as grouped inflorescences, the deep and narrow corollas of the florets, and nectar accumulation, are often associated with pollination by lepidopteran species, especially butterflies (Faegri and van der Pijl 1979, Proctor *et al.* 1996). In its native range of central and south America (Stirton 1977, Day *et al.* 2003), the main pollinating insects of lantana are Lepidoptera (Barrows 1976, Mathur and Mohan Ram 1986). Several studies have investigated the relationships between invasive lantana and its pollinators in the invaded regions but results from these studies have varying accounts of which insects act as major pollinators for the weed (Kritasampan *et al.* 2016).

Goulson and Derwent (2004) suggest that in Australia, the most abundant pollinator on lantana are feral honey bees (*Apis mellifera*), accounting for 62.9% (only 171 out of a total of 272 total insects observed on 24789 lantana inflorescences) of all recorded visits. Lepidopteran (30%) and Dipteran pollinators were also identified on lantana. They found that fruit set closely correlated with honey bee and butterfly abundances, with significantly higher fruit set at sites where these insects are abundant. Carrión-Tacuri *et al.* (2014) found 12 insects species from three orders, Lepidoptera, Hymenoptera, and Diptera, pollinating lantana in the Galapagos Islands, but showed that the main pollinators were Lepidoptera. The endemic butterfly, *Urbanus galapagensis*, accounted for more than 80% of the visitor frequency on lantana in their study. They recorded two Hymenoptera species visiting lantana inflorescences, however, only one (*Xylocopa darwinii*, family: Apidae) behaved as a pollinator. Mukherjee *et al.* (2015) investigated the association between lantana and butterfly species in rural and urban sites in Kolkata, India, and found that 25 different butterfly species preferred *L. camara* as host plants in their study areas.

Pollinators on lantana in canopy gaps in the Mabira forest reserve in Uganda were observed by Kato *et al.* (1999), who found that the most frequent visitors to lantana belonged to the orders Lepidoptera, Hymenoptera, and Diptera. Kritasampan *et al.* (2016) conducted field observations and inflorescence collections on lantana to investigate the total arthropod community in lantana inflorescences in Thailand. From field observations, they found that bees and butterflies had the highest frequency of visits to lantana, with 32% and 28% of the visits respectively. From the inflorescence collections, thrips (Thysanoptera) and mites (Trombidiformes) were the most frequently sampled arthropods. They conclude that bees, butterflies, and thrips all play roles in the pollination of lantana. Mathur and Mohan Ram (1986) found that both butterflies and thrips visited lantana inflorescences in Delhi, India, but that thrips were more regular and efficient pollinators to lantana, resulting in a high fruit set. It is clear, even with the pollination syndrome of lantana pointing to butterflies as pollinators, many other insects can and do utilise lantana inflorescences as a foraging resource in its invaded ranges. Lantana can be considered a generalist flower, ecologically and functionally (Ollerton *et al.* 2007).

The role that mutualisms play in the facilitation of alien plant invasions is generally understudied (Stokes *et al.* 2006, Stout and Tiedeken 2017), and in South Africa specifically, very little research has been aimed at understanding the relationships of *L. camara* with native pollinating species. The present study is aimed at investigating pollinating insect visitors of lantana in a heavily infested urban nature reserve in South Africa. The relationship between lantana and African honey bees was specifically observed. Honey bees have a reputation for facilitating the spread of alien invasive plants through pollination (Barthell *et al.* 2001, Goulson and Derwent 2004). Their frequent presence on invasive plants has been attributed to their generalist morphology and behaviour (Stokes *et al.* 2006, Aslan *et al.* 2016). In South Africa, only about half of the 1000 plant species that African honey bees visit, are indigenous, and many alien plant species are important forage plants for honey bees, including eucalyptus, acacia, and *L. camara* (Illgner 2002). Honey bees have been shown to prefer abundant plants (Hung *et al.* 2019). In invaded areas, lantana plants often form large, monospecific thickets with very high numbers of inflorescences. Therefore, in the flower-scarce winter months, lantana might be an important and preferred food resource for African honey bees. If this is the case, I postulate that there would be an increase in the number of honey bees visiting lantana in winter months when other resources are relatively scarce.

In addition to the pollinator observations, the importance of different pollinators was further assessed by performing pollinator exclusion experiments. The fruit set and viable fruit set of inflorescences subjected to different exclusion treatments were investigated. Lantana is often stated to be capable of autonomous self-pollination within bagged inflorescences (Carrión-Tacuri *et al.* 2014), however, cross-pollination has been found to increase the seed set of lantana (Barrows 1976). Insects have also been found to be important to the reproduction and spread of lantana, by significantly increasing the fruit set of lantana compared to hand-pollinated inflorescences (Goulson and Derwent 2004).

The lack of knowledge about the pollinators utilising lantana in South Africa, and their importance to its pollination success, is surprising considering the influence these interactions have, not only on the fitness and success of the invasive but also on the native flower visitors foraging on lantana. This study aims to investigate the pollination ecology of *L. camara* in an urban nature reserve in South Africa.

Materials and methods

Study site

The Faerie Glen Nature Reserve (FGNR) is an urban reserve in the southeast of Pretoria, a major urban settlement in Gauteng, South Africa (Figure 2.1). The reserve forms a part of the larger Bronberg Nature Conservancy, which in turn is part of the Bronberg, a prominent ridge in the residential suburbs of eastern Pretoria. The reserve itself encompasses about 120 hectares. Lantana has been established in the FGNR reserve for over a decade (V. van de Wiel, personal communication, November 2020). The ongoing management of lantana in the reserve is spearheaded by volunteer groups facilitated by the Friends of Faerie Glen. The main control method used is mechanical removal using tree poppers, supplemented by chemical control in the cases of very large lantana plants of which root systems have become too extensive to remove.

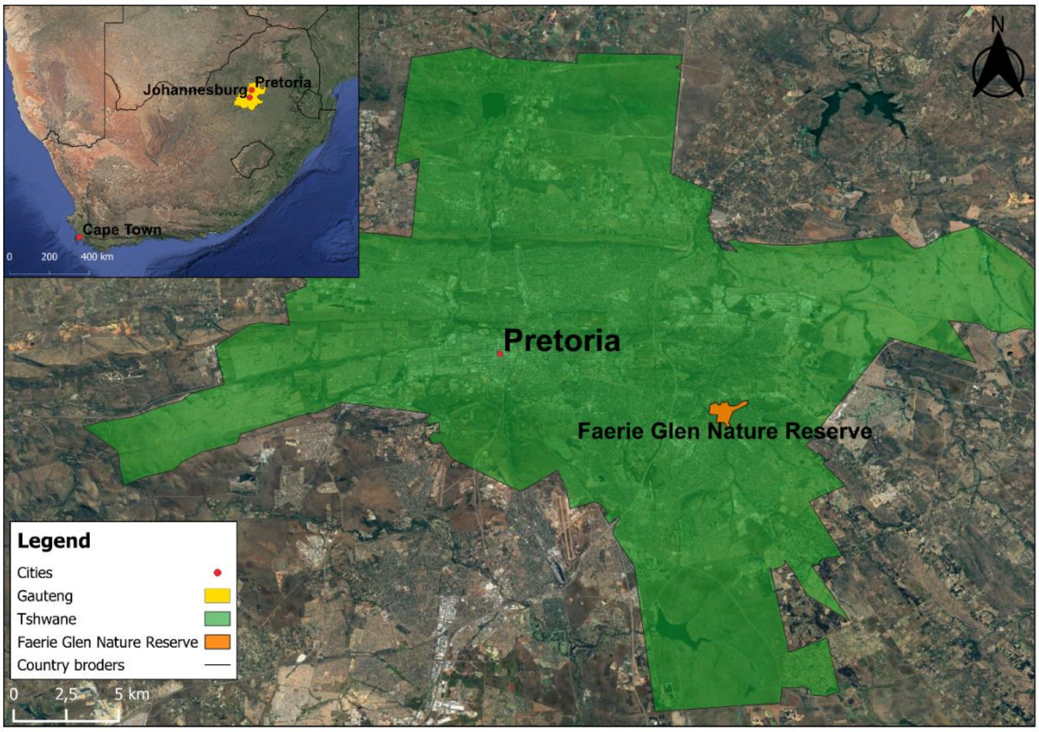

Figure 2.1 Map of South Africa and the city of Pretoria, where the reserve is situated. The province of Gauteng is highlighted in yellow in the map inlay. The green polygon depicts the Tshwane metropolitan area, with the reserve in orange.

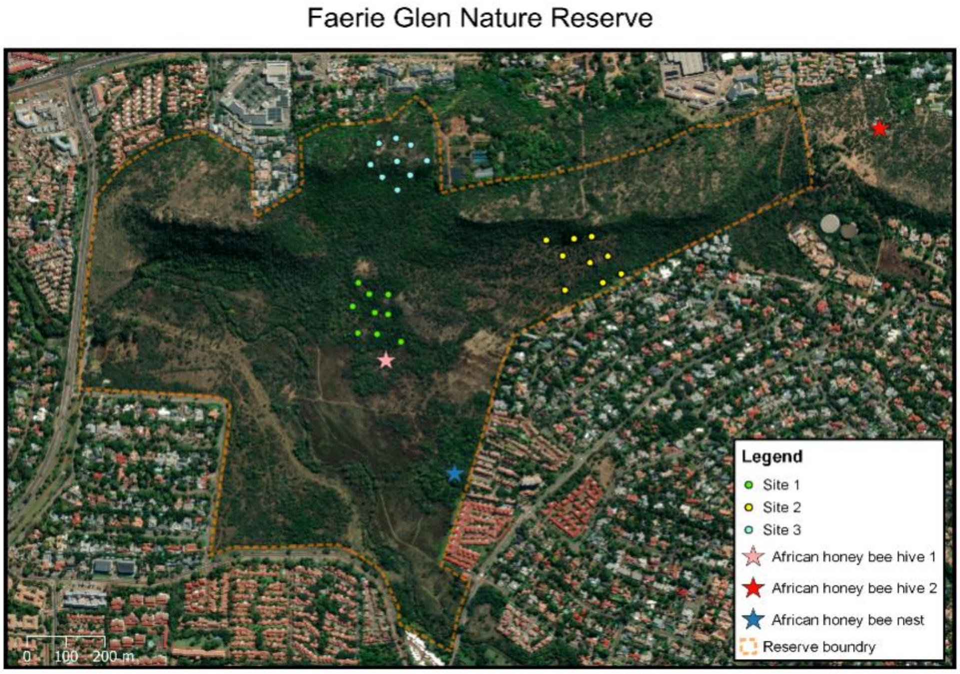

Figure 2.2 Map of the Faerie Glen Nature Reserve, Pretoria, South Africa. The locations of three observational sites, each with 9 replicate plots (point markers), are shown. Pollinator observations and exclusion experiments were conducted at these sites. The stars indicate the positions of the African honey bee (*Apis mellifera scutellata*) colonies, which were used to record honey bee activity. The different colours of the stars distinguish the different colonies, the pink star represents hive one, the red star represents hive 2, both of which were Langstroth hives. The blue star represents the honey bee nest, which was located underground beneath a large rock.

Observations of pollinating insects of lantana

Three independent observation blocks (approximately 100 m x 100 m) were established in the reserve (Figure 2.2) in areas invaded by *L. camara*. The distances between the observation blocks were roughly 350m. In each of the three blocks, nine observational plots (1 m x 1 m) were set up in a grid formation (represented by the coloured point markers, Figure 2.2). Observations on the pollinating insects visiting lantana inflorescences in the 27 observation plots, were conducted in four seasons: spring (November 2021), summer (January 2022), autumn (March 2021), and winter (June 2022).

Observations were conducted during times of peak insect activity (between 9h00 and 15h00) and were restricted to days on which the weather favoured insect activity. Each plot was observed in 15-minute bouts until an accumulated time of an hour was reached for each plot. The following was noted during the observations: 1. Pollinating insects visiting lantana flowers in the plot. An insect visitor was considered a pollinator if the insect could be observed inserting itself or its mouthparts into the corolla of the flower (Larson *et al.* 2006, Carrión-Tacuri *et al.* 2014), 2. A count of the number of African honey bees (*Apis mellifera scutellata*) visiting the observation plots, 3. An estimation of the number of inflorescences (on a five-point scale, from >100, <100, <75, <50, and <20) in the observation plot at the time of observation, and 4. Weather conditions at the time of observation as measured by a Kestrel 3000 weather meter. Photographs were taken to aid in the identification of the visiting insects (using a mobile phone, Samsung Galaxy A52s, R5CT11F6MXV). In addition to photographs, whenever it was necessary and possible, insects were caught, either with a net or an aspirator, and stored in 70% alcohol or pinned when appropriate, to be identified later.

Along with the observations made in the field, five inflorescences were collected during each observation bout. These inflorescences were checked for the presence of small insects such as thrips and small beetles, as it was often impossible to spot these insects during in-person observations, but they still play an important role in the pollination of lantana (Mathur and Mohan Ram 1978, Sharma *et al.* 2005). Additionally, throughout the entire study period, opportunistic observations (n=40) were made on the pollinators visiting lantana, whether or not the lantana was in the specified study plots. Insects were identified to their lowest classification possible (in some cases with the assistance of experts, see acknowledgements), when possible. When species-level identification was not possible, species were identified as morphospecies.

Honey bee colonies around the reserve

The honey bee colonies used as part of these observations were established in the reserve before the start of the observations and were the only colonies around the reserve at the time of the study. Hives one and two are both Langstroth hives, both of which were established by people not associated with this research project. The colony in hive one was not managed at the time of the observations, while hive two was part of an apiary managed independently of the reserve or the research project. The nest was located underground beneath a naturally occurring rock. The average distance of each observation plot to the honey bee colonies was determined using google maps to measure the distances from each plot to each of the three honey bee colonies, as indicated on the map of the reserve (Figure 2.2). Table 2.1 shows these distances for the centre plot for each of the three observation sites and the three locations for honey bee colonies. All the observation plots fell well within the foraging distance of honey bees (Winston 1987). Apart from the three colonies that were used, there were no other honey bee colonies around the reserve from which observations could be conducted.

Table 2.1 The distances (meters) between the centre plot for each observation site and the three honey bee colonies, as well as the average distances calculated from the three colonies.

Location	Bee hive 1	Bee nest	Bee hive 2	Average distance (SD)
Site 1	125.40	473.54	1387.67	662.2 (651.9)
Site 2	587.83	656.98	827.07	690.6 (123.1)
Site 3	524.28	838.75	1233.31	865.4 (355.3)

Seasonal variation in African honey bee visitations to lantana

To control for the expected seasonal variation in the activity of honey bees, the flight activity of honey bees was measured at three colonies (two hives and one nest) occurring within the foraging distance of the three observation blocks (Figure 2.2). Recordings of the honey bee activity were taken twice daily at 11h00 and 13h00 using a video camara (Canon Legria HF R76), set up on a tripod at the entrance of the colonies. Ten-minute videos were captured and used to count the number of foragers returning to the hive (or nest) and these numbers were used as a proxy for bee activity (Dukku *et al.* 2013). These data were used to control for the expected seasonal variation in honey bee visitations on lantana. Recordings of activity were alternated between colonies twice daily. For the observation periods in March 2021 and November 2021, honey bee activity videos were recorded from hive 1 and the nest in the nature reserve (Figure 2.2). For the next observation period in January 2022, the hive on the reserve had absconded, therefore activity was only measured at the nest. In the final observational season, June 2022, all honey bee activity videos were conducted in a private apiary (hive 2) adjacent to the reserve (Figure 2.2).

Pollinator exclusion experiments

In the same observation blocks as described above, lantana inflorescences were subjected to pollinator exclusion experiments in seven of the nine observation plots in November 2020. Exclusion of pollinators was achieved by erecting hand-made plastic mesh cages (cylindrical cage with height c. 20 cm, diameter c. 15 cm), with varying-sized holes, or fine-mesh nylon bags (20 cm x 18 cm), placed over a single or a pair of lantana inflorescences.

The experiment consisted of four exclusion treatments and a control. The treatments were: 1. Large-holed cages (hole dia. < 1.5 cm) to exclude large pollinating insects, including all Lepidoptera, large bee species, such as Carpenter bees (*Xylocopa sp.*), and large bee flies, such as *Bombomyia discoidea,* 2. Small-holed cages (hole dia. < 0.5 cm) to exclude all insects the size of honey bees and larger, including drone flies and syrphids, but allow through smaller pollinating insects such as small bees, beetles, and flies, 3. Fine mesh bags (hole dia. < 0.5 mm, dimensions 20 cm x 18 cm) which excluded all pollinators, and lastly 4. Single pollination, where the inflorescence was netted as in treatment 3, but a single visit by a pollinating insect was allowed. For the control of this experiment, the inflorescences were marked but otherwise left unaltered.

The cages and nets were secured over the inflorescences in the bud stage, and care was taken to ensure that the inflorescence was at least two centimetres away from the edge of the cage. The openings of the nets over the inflorescences were sealed using Plantex (Banded fruit weevil and ant barrier, Chempac (Pty) Ltd.). For the single pollination treatment, once most of the florets on the inflorescence had opened, the net was temporarily opened, and the inflorescence was exposed and observed until a single pollinator visited the inflorescence. The visiting pollinator was noted, along with the time it spent on the inflorescence. Hand pollination, using fibers from a paintbrush, was performed in instances where a pollinator did not visit the inflorescence after an hour. Where hand pollination was employed, plants were cross-pollinated, i.e., pollen from a different plant was used. Goulson and Derwent (2004) showed that cross-pollination of lantana resulted in a higher fruit set than self-pollination. After the single visitation (or hand pollination), the net was closed again.

Fruits were allowed to ripen on the plants and were collected when they were ripe. The proportion of florets that developed into fruits was recorded as the fruit set of the plant. Once collected, the fruits were dried and stored at room temperature. The viability of the collected seeds was tested with a tetrazolium seed test using 2,3,5-triphenyl tetrazolium chloride.

Tetrazolium test

The dried fruits were soaked in distilled water overnight to soften the fruit coating and allow for easy removal. The removal of the fruit flesh has been found to increase germination in lantana seeds (Sharma *et al.* 2005). After the fruit coat was removed the seeds were soaked again in distilled water for 16 hours at 25°C (Miller and Peter 2010). During all soaking times, the seeds were forced to stay below the solutions using cotton wool discs. After soaking, approximately two-fifths of the distal ends of the seeds were cut using a scalpel, just enough to expose the embryos inside the endocarp of the seed. The cut seeds were soaked in a 1.0% tetrazolium solution for 24 hours at 33°C (Miller and Peter 2010). The tetrazolium solution was prepared using a buffer solution to ensure that an ideal pH (between 6.5 and 7.5) was achieved (Peters 2000). After the tetrazolium treatment, the seeds were cut longitudinally to expose the embryos completely. The embryos were examined for signs of red staining. Staining in the embryos indicates that cell respiration taking place, and thus can be used as a proxy of living tissue, indicating viability. The proportions of florets that had developed into viable fruits were recorded as the viable fruit set of the plant.

Data analyses

Data gathered with regards to the pollinating insects visiting lantana inflorescences were used descriptively to determine which species are present on lantana in a South African environment. To visually present these data, pollinator webs were drawn using the plotweb function of the bipartite package (Dormann 2020) in RStudio (Version 2022.12.0.353). Differences in sampling effort between seasons were corrected by calculating the number of individuals observed per observation bout. Kruskal-Wallis *H*-tests with multiple comparisons were conducted to determine whether the mean number of insects per observation bout, and the number of African honey bee visitors per observation bout were significantly different between seasons. Spearman Rank Order Correlation was further used to correlate the number of honey bee sightings with the daily activity of honey bees. The activity data were normalised by mean activity per season. G*Power (Version 3.1.9.7) was used to perform a post hoc test to determine the statistical power of the correlation. Additionally, Shannon diversity indices (H') were calculated for the pollinator populations observed on lantana flowers in each of the four seasons.

A generalised linear mixed model (GLMM) selection procedure was performed in RStudio (Version 2022.12.0.353), where all combinations of explanatory variables (season, temperature, the average distance to honey bee colonies, and abundance of lantana inflorescences) were considered (using the glmer function of the lme4 package, Bates *et al.* 2015). The insect visitors observed on lantana were divided into the number of honey bee visitors, the number of all pollinator visitors, and the number of non-pollinating arthropods observed on lantana inflorescences as the three response variables. Residuals were not normally distributed; therefore, a Poisson distribution was fit for the honey bee visitors, and a Poisson-lognormal distribution was fit for the pollinators and the non-pollinating arthropods. Since observations were repeated for each plot, location (which includes site and plot combined, n=27) was added as a random effect. For the Poisson-lognormal distributions, an additional random effect of observation was included in the model. All models were tested for overdispersion using a convenience function overdisp_fun defined in glmm_funs.R (Bolker *et al.* 2011). The Akaike information criterion (AIC) was used to determine the most parsimonious model (the lowest AICc was used as the model selection criteria).

The differences in the fruit set and viable fruit set between the different exclusion treatments, as well as the effects of different pollinators during the single pollination treatments, were both analysed with Kruskal-Wallis *H*-tests with multiple comparisons. All Kruskal-Wallis *H*-tests were performed in Statistica Version 13 (TIBCO Software Inc.). All of the fruits collected during these experiments contained seeds, therefore fruit set, and seed set in this context can be used interchangeably.

Results

Pollinators on Lantana camara

A total of 3409 individual arthropods were recorded on lantana inflorescences in the entire study period. The highest number of arthropods were recorded in the summer and autumn observation periods, with 1320 and 1251 arthropods, for summer and autumn respectively (Figure 2.3, A). A total of 126 unique arthropod species from 45 unique families were identified.

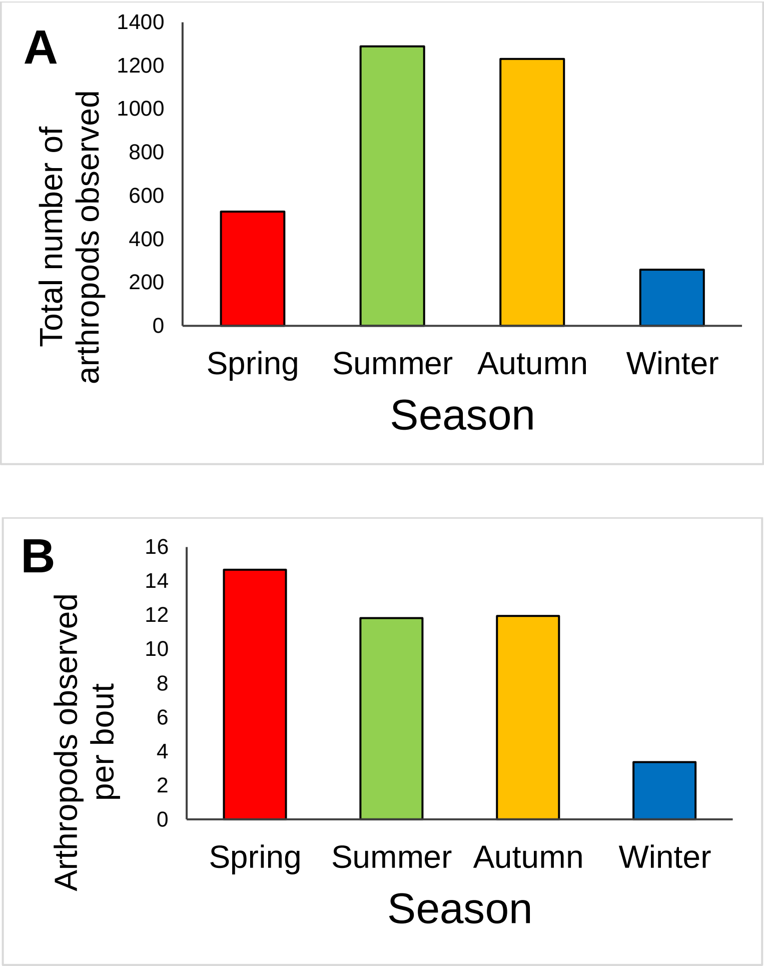

Figure 2.3 The total number of arthropods observed (A), and the total number of arthropods observed per observation bout (B) on *Lantana camara* inflorescences during four different sampling seasons. Seasonal observations were conducted for spring in November, summer in January, autumn in March, and winter in July.

It has been stated that lantana can flower throughout the year (Graaff 1986, Sharma *et al.* 2005). However, this was not the case for the lantana in this study area. For the duration of the study (November 2020 to June 2022), there was a rapid decline in the number of lantana inflorescences in the reserve from May onwards (personal observation). By the end of July, very few lantana plants in the reserve still carried inflorescences. The lantana plants started flowering again in November. As a result, fewer observation bouts were completed in the observation periods in winter (June) and spring (November). To account for this unequal sampling effort, differences in arthropod collections between seasons were considered in terms of the number of insects observed per sampling bout (Figure 2.3, B). Pairwise Kruskal-

Wallis tests revealed that the number of insects observed per observation bout did not differ significantly between spring, summer, and autumn. There were, however, significantly fewer insects observed per bout in the winter compared with all other seasons (Kruskal-Wallis test: $H_{3,\,89} = 38.8038$, $p < 0.0001$ for all season comparisons).

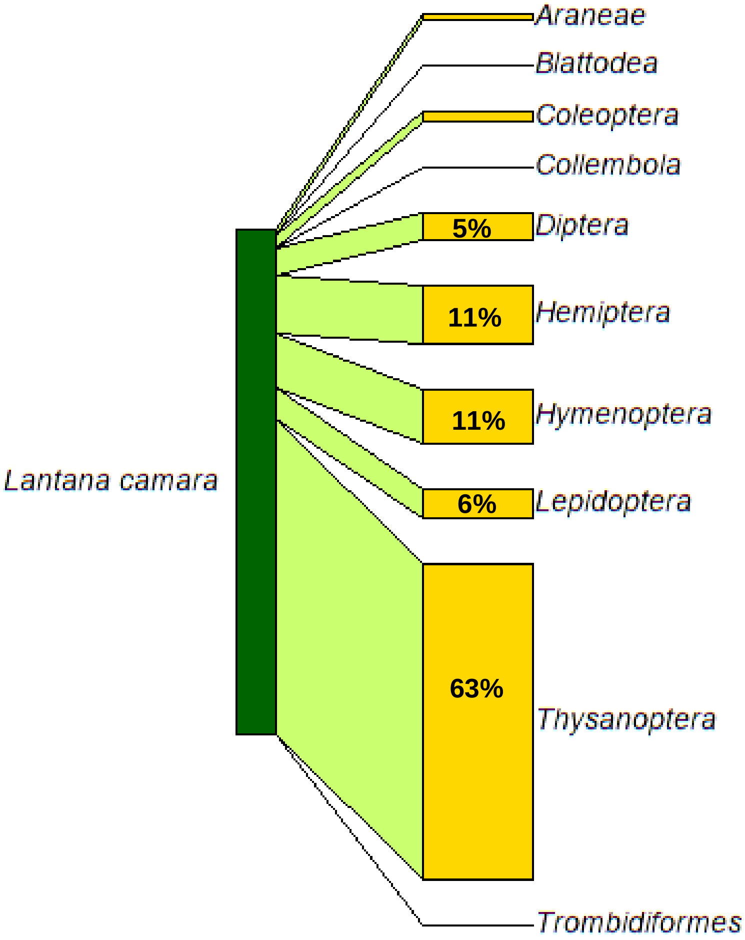

Figure 2.4 Orders of arthropods collected on *Lantana camara* inflorescences across all observation periods and collection methods combined. The thickness of the yellow bars represents the abundance of the order as a proportion of the total number of arthropods in the collection. Percentages are indicated for the five most abundant orders.

Arthropods collected from the three sampling methods and four sampling seasons spanned 10 orders (Figure 2.4). Thysanoptera was the most abundant order of arthropods sampled, accounting for 63% of recorded individuals. Hemiptera and Hymenoptera made up 11% of the sampled arthropods, and Lepidoptera and Diptera, 6% and 5%, respectively.

Of the 10 orders of invertebrates observed, only five, Thysanoptera, Hymenoptera, Lepidoptera, Diptera, and Coleoptera, were involved in the pollination of lantana. The proportions of each order of pollinators observed on lantana in each season are shown in Figure 2.5. In all seasons, Thysanoptera were the most abundant pollinators observed, with more than 72% of pollinators sampled on lantana inflorescences overall belonging to the order. Hymenoptera was the second most abundant across all seasons, accounting for more than 12% of all pollinators observed over all seasons and with honey bees making up 4% of all the pollinating species. Coleoptera had the lowest abundance of pollinators observed on lantana in all seasons except winter when Diptera was the least abundant order. Table 2.2 shows the numbers of families and morphospecies recorded for each of the five pollinator orders observed on lantana inflorescences.

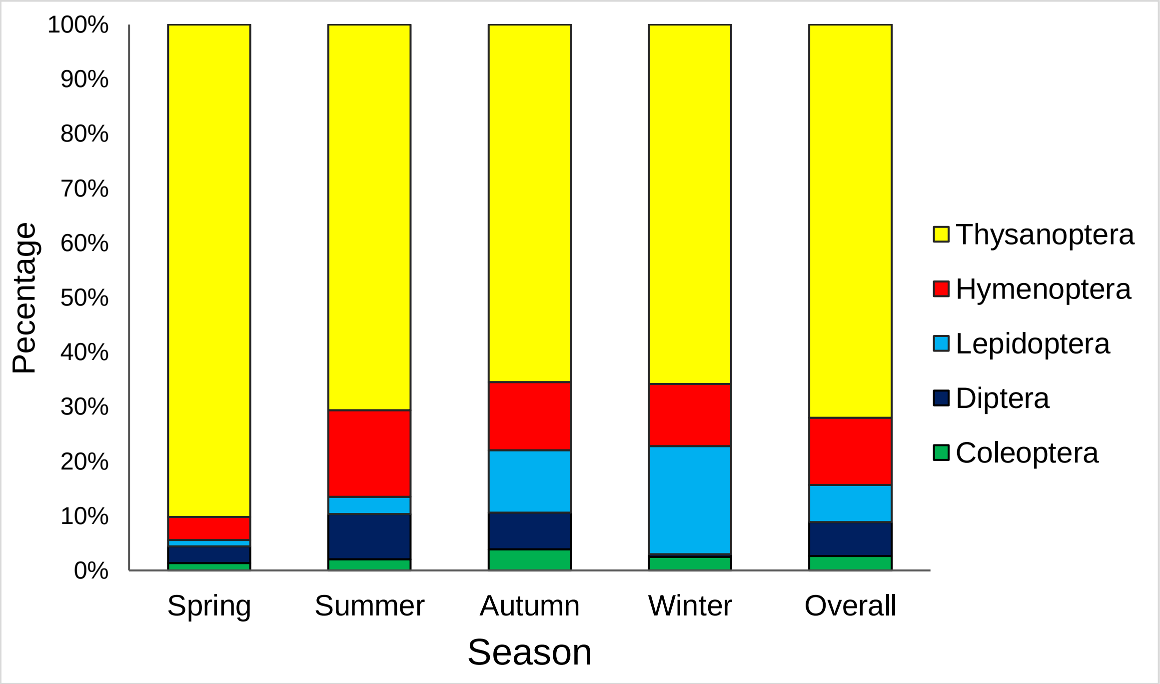

Figure 2.5 Proportions of orders of pollinating insects observed on *Lantana camara* inflorescences in four sampling seasons and overall. Seasonal observations were conducted for spring in November, summer in January, autumn in March, and winter in July. Different colours represent different orders; Thysanoptera (Yellow), Hymenoptera (Red), Lepidoptera (Blue), Diptera (Purple), and Coleoptera (Green).

Table 2.2 The numbers of unique families and unique species identified for each of the five pollinating orders of insects sampled on *Lantana camara* inflorescences in all sampling seasons.

Order	Unique families	Unique species
Diptera	10	27
Hymenoptera	7	27
Lepidoptera	6	23
Thysanoptera	2	5
Coleoptera	5	10
Total	30	93

There was a marked increase in the number of unique families and species observed on lantana in the summer observations compared to spring and winter (Table 2.3). Shannon diversity indices (H') show the highest diversity of arthropod visitors to lantana in the summer and autumn observation periods. Spring had the lowest diversity.

The proportions of pollinating species observed on lantana in autumn and winter are shown in Figure 2.6 and Figure 2.7. In all observation seasons, species from the order Thysanoptera were among the most abundant pollinators on lantana. The five morphospecies of thrips identified on lantana were consistently sampled on lantana in all the observation seasons (Figure 2.6 and Figure 2.7). *Frankliniella sp.* was the most abundant pollinator species recorded on lantana inflorescences in all seasons. Apart from the Thysanoptera, African honey bees (*Apis mellifera scutellata*) were the second most abundant species in all seasons except spring.

Table 2.3 Total number of observed pollinators on *Lantana camara* inflorescences, number of unique pollinator families, number of unique species, and the Shannon diversity index (H') based on the pollinating insect populations for each season. Samples for which the season was not recorded (n=14) are included in the overall row in brackets.

Observation season	The number of pollinators observed	Unique families	Unique species	Shannon Diversity Index (H')
Spring	521	15	32	1.614
Summer	1172	26	61	2.305
Autumn	1058	23	60	2.866
Winter	202	11	24	2.228
Overall	2953 (2967)	30	93	2.624

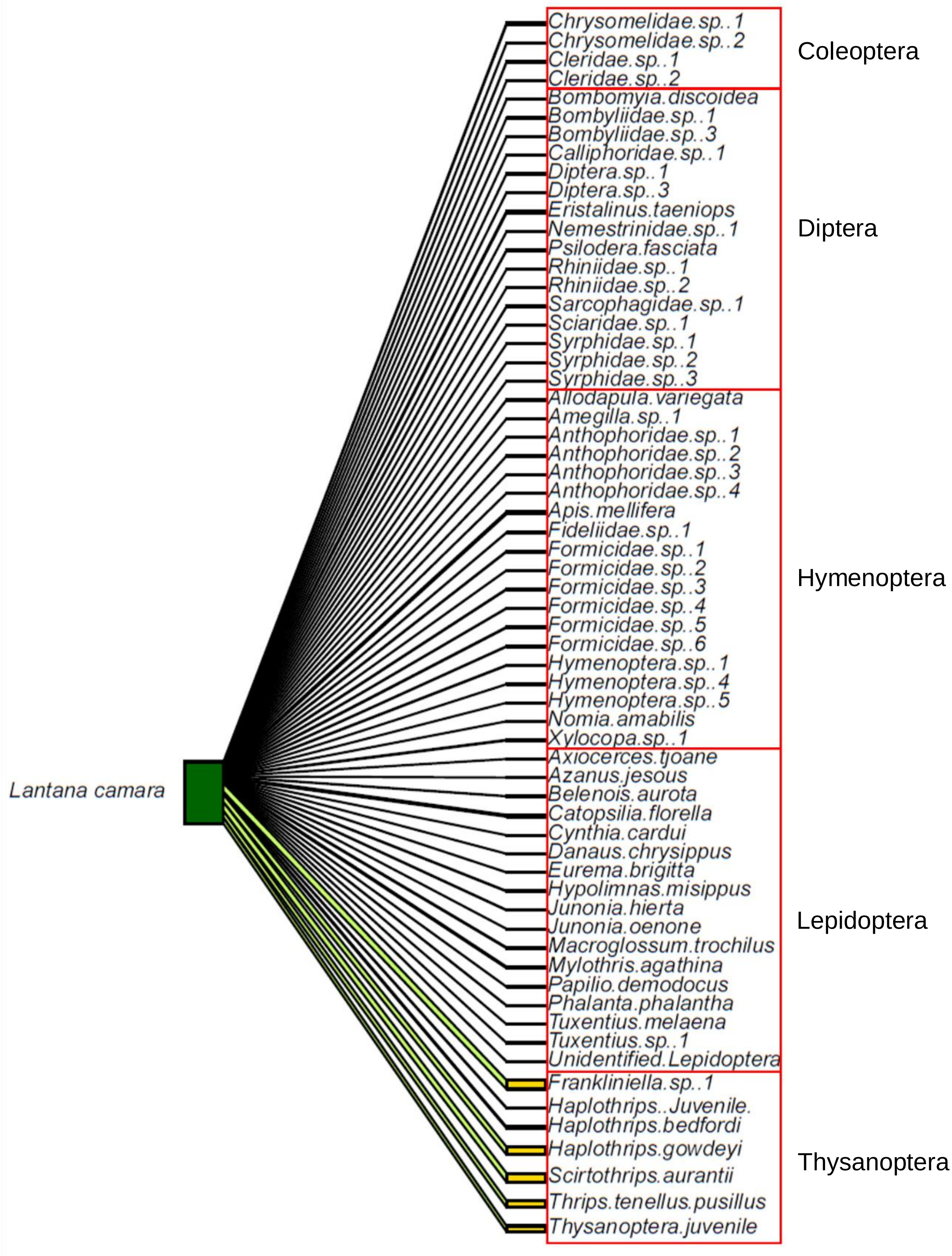

Figure 2.6 The pollinating species that were sampled on *Lantana camara* inflorescences in autumn (March) via three collection methods: in-person observations, opportunistic sampling, and inflorescence collections. The thickness of the bars represents the abundance of each species as a proportion of the total number of pollinators collected in the sampling season.

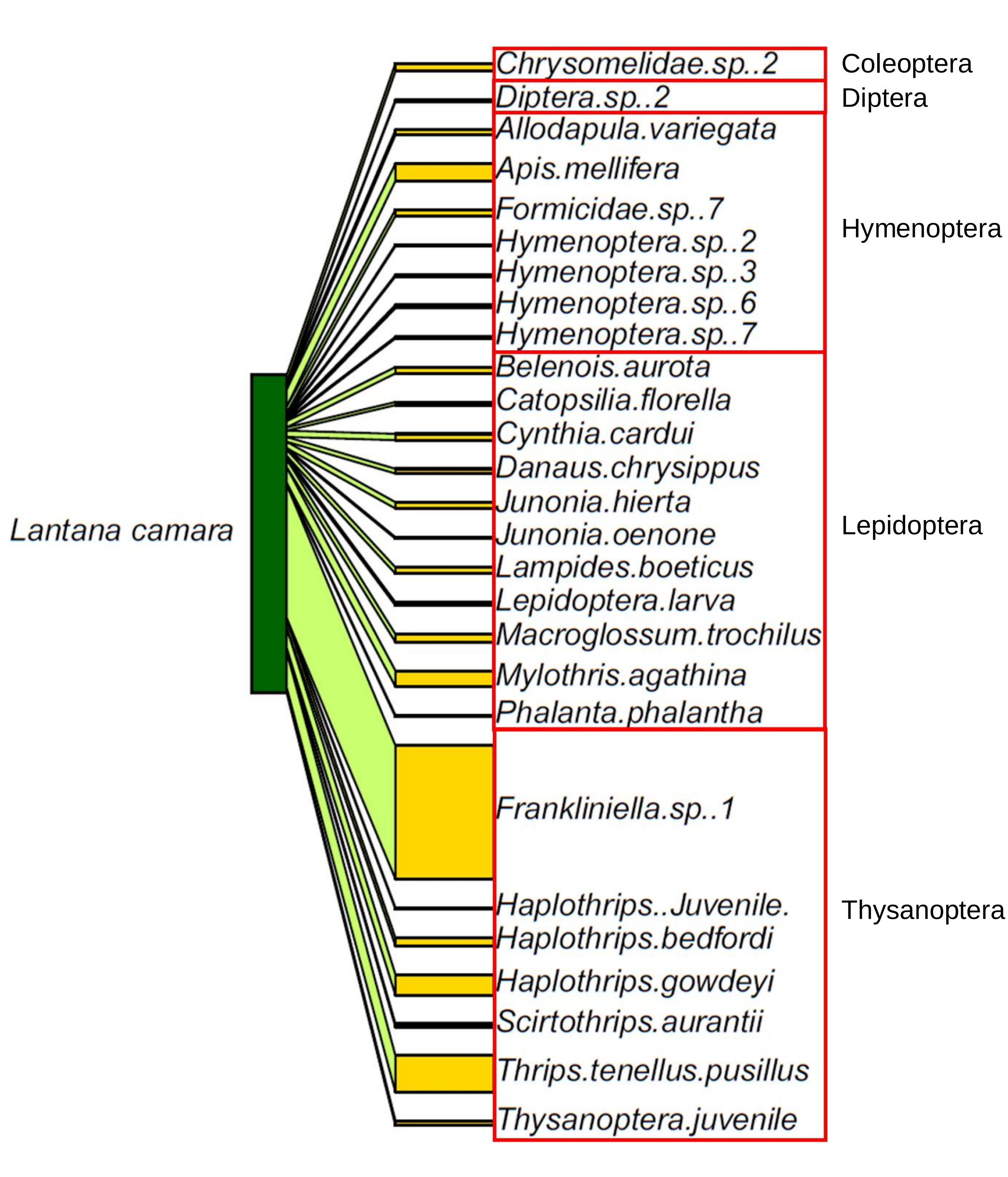

Figure 2.7 The pollinating species that were sampled on *Lantana camara* inflorescences in winter (June) via three collection methods, in-person observations, opportunistic sampling, and inflorescence collections. The thickness of the bars represents the abundance of each species as a proportion of the total number of pollinators collected in the sampling season.

Table 2.4 The effects of the explanatory variables, season, temperature, average distance to honey bee colonies, and abundance of lantana inflorescences, on the number of honey bees, the number of all pollinators, and the number of non-pollinating arthropods that were observed on lantana plots.

	Explanatory variables							
Response variables	S P-value	T P-value	AD P-value	AB P-value	AICc	ΔAICc	Overdispersion ratio	Overdispersion P-value
1. Honey bees								
Model 1 (Best model)	3.31E-15	-	-	-	465.146	2.043	0.855	0.969
Model 2	3.676E-15	-	n.s.	-	467.189		0.858	0.966
2. All pollinators (incl. honey bees)								
Model 1 (Best model)	2.20E-16	-	-	-	1910.923	0.838	0.509	1.000
Model 2	2.20E-16	-	n.s.	-	1911.761		0.513	1.000
3. Non-pollinating arthropods								
Model 1	8.71E-09	-	n.s.	-	985.322	0.664	0.786	0.998
Model 2 (Best model)	1.02E-08	-	-	-	985.987		0.779	0.998

Explanatory variables: Season (S); Temperature (T); Average distance to honey bee colonies (AD); Abundance of lantana inflorescences (AB). Only the two most parsimonious models for each of the three response variables considered (based on the lowest AICc) are listed. The p-value for each explanatory variable was obtained from a likelihood ratio test in which models with and without the variable in question were compared. n.s. represents p > 0.05. The symbol '-' represents the variables not included in the models that are shown in the table.

Temperature, the average distance to honey bee colonies, and the abundance of lantana inflorescences did not significantly explain the variation in either of the three response variables. The only effect that made a significant contribution to explaining the variance found in the number of honey bees, the overall number of pollinators, as well as the number of non-pollinating arthropods on lantana inflorescences in the observation plots was season (Table 2.4). Compared to the summer observation period, there was a slight decrease in the number of honey bees observed in autumn (Slope (SE) = -0.7005 (0.2062), $p = 0.0006$), and a more substantial decrease in spring (-3.1912 (1.005), $p = 0.0015$), and winter (-2.3367 (0.4608), $p < 0.0001$). There were no significant effects of autumn and spring on the overall numbers of pollinators on lantana, but compared to summer there was a significant decrease of pollinators in the winter observations (-1.6981 (0.1380), $p < 0.0001$). For the non-pollinating arthropods, even though model 1 had a lower AICc value, model 2 was chosen as the best model (Table

2.3), since the likelihood ratio test revealed that including the average distance to honey bee colonies as an explanatory variable did not significantly improve the model. Compared to summer, there was a significant increase in the number of arthropods observed on lantana inflorescences in autumn (0.4171 (0.1476), $p = 0.0047$). Spring had a significant decrease in the number of arthropods (-1.4582 (0.3583), $p < 0.0001$).

Seasonal variation of honey bee activity on Lantana camara

The proportions of African honey bees observed on lantana inflorescences in the four sampling seasons fluctuated between the seasons (Figure 2.8). There was a significant difference in the numbers of African honey bees observed per bout between the four seasons (Kruskal-Wallis test: $H_{3,89}$=37.2496, $p < 0.0001$). Multiple comparisons show that the numbers of honey bees observed per observation bout were significantly higher in the summer and autumn observation seasons, compared to spring and winter ($H_{3,89}$ = 37.2496, summer, $p < 0.0001$; autumn, $p < 0.05$).

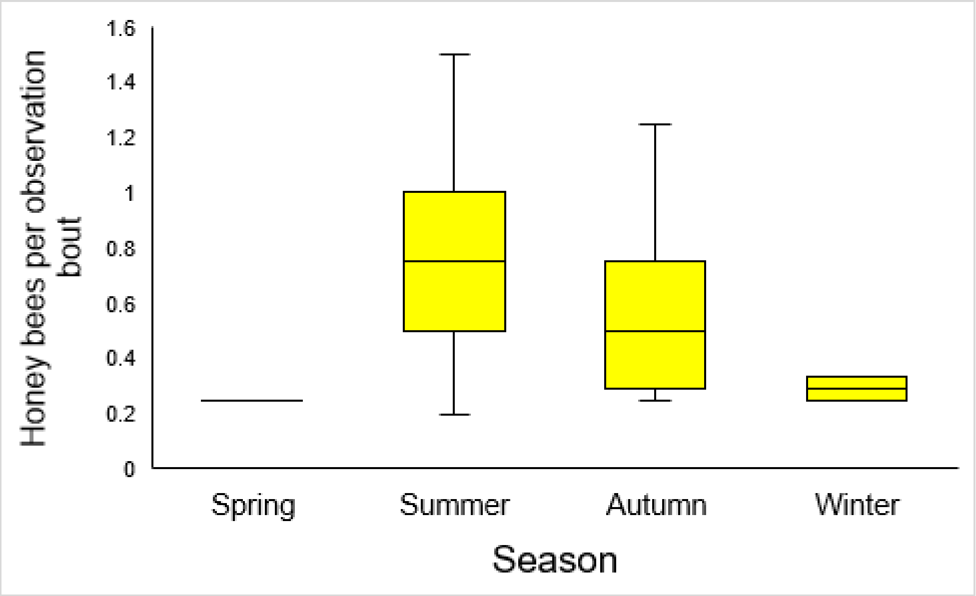

Figure 2.8 Box plots of the numbers of African honey bees (*Apis mellifera scutellata*) per observation bout on *Lantana camara* inflorescences during four sampling seasons. Seasonal observations were conducted in spring in November, summer in January, autumn in March, and winter in July. Boxes show the median, 25th and 75th percentiles; the whiskers show the minimum and maximum observations, excluding the outliers.

There was no correlation between the number of daily honey bee sightings on lantana inflorescences and the daily activity of honey bees (Spearman Rank Order Correlation, r (32) = 0.0384, p = 0.8522). A post hoc power analysis revealed that the statistical power of the correlation was not high enough to confidently make inferences about the relationship, or the lack thereof, between the honey bee sightings on lantana and the activity of the honey bees (1 – β error probability = 0.2946353).

Exclusion experiments

The number of inflorescences used in all treatments was never more than two, and the number of florets used in the treatments varied between 18 and 71. The average number of florets produced by lantana was 26 florets per inflorescence (SD, 4.8). Throughout all sites and across all treatments there was no significant difference in the number of florets used between the different treatments in the experiment (Kruskal-Wallis test: $H_{4,161}$ = 1.4807; p = 0.8301).

The control treatment had the highest proportion of florets that set fruit (mean ± SE; 21.5% ± 3.38), and the highest proportion of viable fruit set (13% ± 2.28). There was a significant difference in the fruit set between treatments (Kruskal-Wallis test: $H_{4,113}$ = 15.56543; p = 0.0037). Multiple comparisons show that the proportion of florets that set fruit on control inflorescences was significantly higher than for inflorescences with small cage and net treatments (Kruskal-Wallis test: $H_{4,113}$ = 15.56543, Small cage, p = 0.004; Net, p = 0.04), but not higher than the large cage and single pollinator treatments (Figure 2.9, purple bars).

Even though the nets in these experiments were meant to exclude all insects, it was noted during field observations that thrips were able to move freely through the nets.

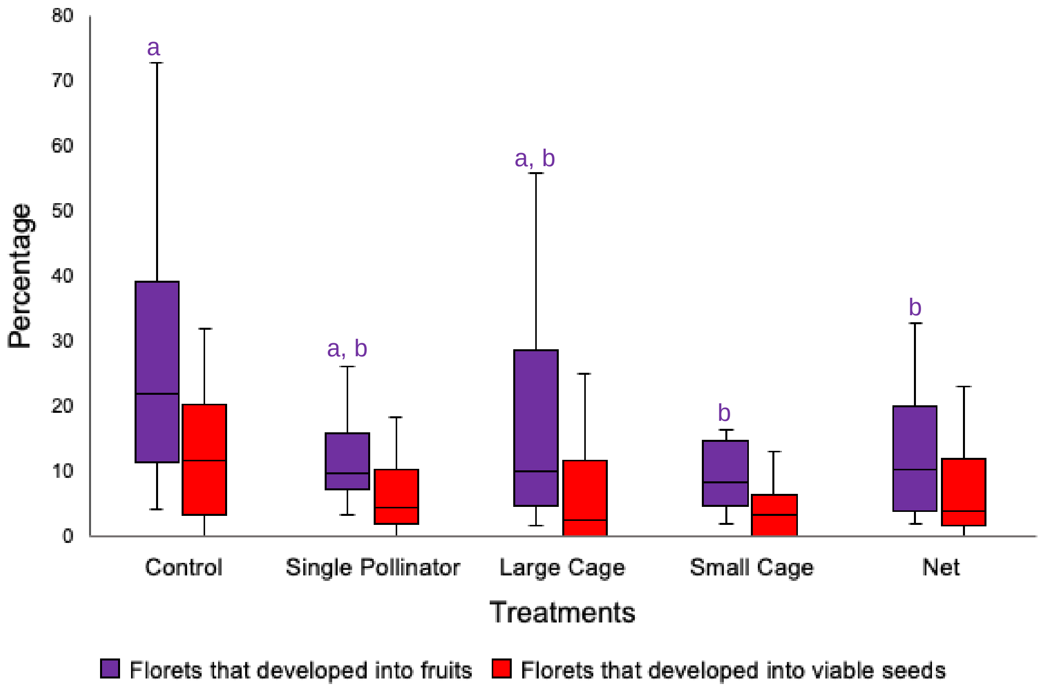

Figure 2.9 Box plot of the percentage fruit set (Purple) and the percentage viable fruit set (Red) from *Lantana camara* inflorescences subjected to five different pollinator exclusion treatments. Boxes show the median, 25th and 75th percentiles; the whiskers show the minimum and maximum observations, excluding the outliers. Significant differences between mean percentages of treatments are denoted by no letters in common.

The significant difference in the total fruit set between treatments did not translate to the viable fruit set (Figure 2.9, red bars). There was no significant difference in the percentage of viable seeds produced between any of the five treatments (Kruskal-Wallis test: $H_{4,113} = 6.5644$, p = 0.0676).

With regards to the single pollination treatment, there was no difference in the total fruit set or the viable fruit set depending on the pollen vector (Coleoptera, n = 4; Hymenoptera, n = 5; Thysanoptera, n = 2; and Hand pollination, n = 6) (Kruskal-Wallis test: Fruit set $H_{3,14} = 7.2588$, p = 0.0641, and viable fruit set $H_{3,14} = 3.6580$, p = 0.3008).

Discussion

In this study, insect visitors were recorded in four seasons, with a specific focus on the pollinating insects, and the importance of different pollinators on the fruit set and viable fruit set of *L. camara* in South Africa was investigated.

L. camara is shown to have an extremely diverse pollinator population. Overall, 93 species of pollinators from 30 families were found to visit lantana inflorescences. Ollerton *et al.* (2007) describe the different ways in which a plant can be considered a generalist: ecologically and functionally. Ecological generalisation refers to the number of pollinating species a plant interacts with, while functional generalisation involves the pollinators of a plant at a higher taxonomic level. These results show that lantana in South Africa can be considered a generalist plant in the ecological sense (having multiple species of pollinators) and in the functional sense (having pollinators from multiple families).

The diversity of the pollinators observed in this study was crudely compared to that of other studies. The Simpson diversity index (H') reveals higher overall diversity of pollinators observed on *L. camara* inflorescences in the present study (H' = 2.624) compared to that of Goulson and Derwent (2004) in Australia, H' = 1.656, and Carrión-Tacuri *et al.* (2014) the Galapagos Islands, H' = 0.809. The average diversity of butterflies on *L. camara* reported by Mukherjee *et al.* (2015), H' = 2.89 +- 0.04, was higher than that of the present study (H' = 2.576). The worldwide success of lantana as an invasive plant has been attributed to the presence of a wide range of pollinators foraging on lantana in its introduced ranges (Sharma *et al.* 2005).

The most abundant pollinators encountered on lantana inflorescences in our study sites were thrips (Thysanoptera). The effectiveness of a pollinator is determined by visitation frequency and the abundance of pollen transferred (Aslan *et al.* 2016). The sheer abundance of thrips sampled in *L. camara* inflorescences in this study indicates that they are likely the most effective pollinators. Five morphospecies of thrips were extracted from lantana inflorescences. The most abundant thrips sampled belonged to the genus *Frankliniella* sp. These results are following Mohan Ram and Mathur (1984) who found that thrips are regular pollinators on *L. camara* in Delhi, India. They reported the presence of two thrips, *Thrips hawaiiensis* (Morgan) and *Haplothrips tenuipennis* (Bagnall) in lantana inflorescences (Mathur and Mohan Ram 1978). Thrips from both these genera were also found on lantana in the present study. Thrips were also sampled in large proportions in the study of lantana arthropods conducted by Kritasampan *et al.* (2016), exhibiting behaviour, pointing to them being involved in the pollination of the weed. African honey bees were observed on lantana in the present study but to a much lesser extent than what was reported for lantana in Queensland, Australia. Goulson and Derwent (2004) reported that 62.9% of pollinators on lantana are honey bees. In the present study, only 12% of all pollinators on lantana overall were honey bees. Lantana has been reported as a major source of nectar for many species of butterflies (Sharma *et al.* 2005, Mukherjee *et al.* 2015). Mukherjee *et al.* (2015) identified 25 native butterfly species foraging on lantana inflorescences. This is comparable to results from the present study, where 24 different species of butterflies were associated with lantana inflorescences.

In this study, like most others, only diurnal pollinators were considered. Carrión-Tacuri *et al.* (2014) measured nectar consumption in lantana inflorescences and found that the highest consumption in their study sites occurred during the night, potentially pointing to nocturnal flower visitors foraging on lantana. Data collected in the present study does not provide any information on the possible nocturnal pollinators of lantana.

Only season was found to significantly explain the variations in the number of honey bees, the number of all pollinators, and the number of non-pollinating arthropods on *L. camara* inflorescences. The lack of an effect of the abundance of lantana inflorescences on the numbers of honey bees observed may indicate that honey bee visits to lantana are not driven by the abundance of resources provided by the plant. These results are in line with that of Urbanowics *et al.* (2020), who found that honey bees did not prefer either invasive or native flowers and that their preference did not change, regardless of high floral abundances, but contrary to those of Hung *et al.* (2019) who found that the numbers of honey bee visits increase with increases in flower abundance. The numbers of African honey bees observed on lantana varied between the four sampling seasons. The most honey bee visits were recorded in the summer and autumn observations, showing that African honey bees do not rely on lantana as a winter foraging resource. There was no correlation between the number of honey bees observed on the lantana inflorescences and the activity of honey bees, indicating that honey bee sightings on lantana might not reflect the number of honey bees that were active in the environment. However, the statistical power of the correlation is not high enough to make inferences from the data collected in the present study. Graaff (1986) states that lantana flowers prolifically throughout the year in South Africa. This was not the case for the lantana plants in the present study sites. Lantana is dependent on adequate amounts of water and is susceptible to frost (Sharma *et al.* 2005). Pretoria, South Africa, where the present study was conducted, is situated in a savannah biome, characterised by dry winters, with little to no rainfall between the months of May and September, and up to 16 mean frost days (Rutherford *et al.* 2006). This would explain the decline in the number of inflorescences on the lantana in the observation plots in the winter and spring observation periods. This also accounts for the significantly lower observations made on lantana in the winter observation period.

It has been reported that lantana is capable of autonomous self-pollination (Carrión-Tacuri *et al.* 2014), but our results show that fruit set is increased by cross-pollination and the presence of pollinators, confirming earlier findings (Barrows 1976, Goulson and Derwent 2004). The highest fruit set was recorded in the control inflorescences, where no pollinators were excluded. The fruit set of control inflorescences was significantly higher than that of inflorescences subjected to a small cage and net treatment, but not different than inflorescences where large pollinators were excluded (large cage treatments), or where pollination was limited to one pollinator (single pollination treatment). Netted and open inflorescences (control) in these experiments (mean ± SE, 9 ± 2.27 and 21.5 ± 3.38, respectively) produced fewer fruits than those of Carrión-Tacuri et al. (2014) (44.0 ± 4.5 and 17.8 ± 2.2, respectively) who performed similar experiments. It should be noted that even though the net was meant to exclude all pollinators, the nets used in these experiments were not able to exclude thrips. Thrips were seen freely moving in and out of the nets. Previous studies found that thrips are important flower visitors on lantana (Mathur and Mohan Ram 1978, Mohan Ram and Mathur 1984, Kritasampan *et al.* 2016). The fruit set from inflorescences with net treatments can thus only be attributed to the pollination services received by thrips, or autonomous self-pollination, which *L. camara* is capable of (Carrión-Tacuri *et al.* 2014), but from these experiments, it is impossible to separate these two factors. Despite the differences in the fruit set between the different treatments, there was no difference found in the proportions of viable fruits produced between the treatments. This shows that while the type of pollinators may influence how many fruits are produced, it did not influence the overall number of viable fruit that lantana produced. This also shows the necessity of viability testing in conjunction with the fruit set in determining the importance of pollinators.

Control of *L. camara* is proving to be a great challenge worldwide. The diversity of pollinators that forage on lantana inflorescences has been credited for the success of lantana as an invader (Sharma *et al.* 2005). Better understanding the interactions between non-native plants and native insect communities is important in understanding, predicting, and managing plant invasions (Vermeij 1996). From these results, it is clear that a very large number of native pollinators utilise the resources provided by *L. camara*, which likely contributes to its success in South Africa.

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Chapter 3

How *Lantana camara* influences the choices made by native pollinators at species and community levels.

Abstract

One way in which invasive plants interact with sympatric, co-flowering native species is through shared pollinators. Pollinators may prefer invasive plants over natives, influencing the pollination success of natives. This study aimed to determine the influence of one of South Africa's worst invasive plants, *Lantana camara* on the quantitative component of the pollination of three native plant species. Two experiments were conducted to investigate the preferences of native pollinators at two levels, species, and community levels. At the species level, the foraging preference of African honey bees (*Apis mellifera scutellata*) between lantana and two indigenous bee plants, *Aloe greatheadii* and *Aloe marlothii* were determined with choice tests. Honey bee colonies at the apiaries of the University of Pretoria were presented with a choice between lantana and aloe test branches. We show that both aloe species were chosen more times than lantana. In addition, honey bees choose aloes quicker than when choosing lantana (significantly quicker for *A. marlothii)*. At a community level, pollinator communities on the indigenous *Kalanchoe rotundifolia* inflorescences were compared between plots invaded with *Lantana camara* and those where the lantana had been removed. These pollinator observations were conducted in Faerie Glen Nature Reserve, an urban reserve in Pretoria heavily infested with lantana. These data indicate that there were no differences in the pollinating communities between kalanchoe plants growing in invaded plots compared to those in plots cleared of lantana. However, post hoc analysis revealed that the power of the statistical analysis is not high enough to confidently reject the alternative hypothesis that lantana influences the number of pollinators per observation bout between the cleared and invaded sites. These results indicate that honey bees prefer foraging on native aloe species. There is still a gap in the understanding of how lantana influences co-flowering natives on a community level and further studies are recommended.

Keywords

Lantana camara, Aloe greatheadii, Aloe marlothii, Kalanchoe rotundifolia, co-flowering, pollination, choice-test, pollinator observations

Introduction

More than 80% of plant species, either partly or completely, rely on animals for their pollination services (Ollerton *et al.* 2011). For invasive alien plants, successful pollination is a keystone of the invasion process, enabling the invaders to spread and increase their genetic diversity (Carrión-Tacuri *et al.* 2014). Alien species have to establish new mutualistic relationships with pollinators in their new habitats, to first become naturalised, and then invasive (Richardson *et al.* 2000).

Shared pollinators are one way that sympatric plant species interact with other co-flowering species (Albrecht *et al.* 2016). Invasive plants may influence the pollination of natives by altering the quantitative or qualitative aspects of their pollination (Moragues and Traveset 2005). The quantitative aspect of pollination relates to the number of pollinators received by a plant, while the qualitative aspects involve the source of the pollen that a plant receives. Competition for pollination services includes co-flowering invasives usurping pollinator visitors, thereby reducing the number of visits received by natives (quantitative effects), and the transfer of heterospecific pollen (qualitative effects) (Albrecht *et al.* 2016). These factors can influence the reproductive success of native plants.

Soto *et al.* (2015) compare pollination systems to markets. In these markets, pollinators are the “customers” who choose between “products” (flowering plant species). The choices made by pollinators have important implications for the reproductive success of flowering plants. Measuring the responses of native plant populations to the presence of invasive plants can be challenging (Bartomeus *et al.* 2016). Investigating pollinator preferences between invasive and native species instead can provide indirect evidence of these responses (Bartomeus *et al.* 2016). To understand the impact invasive species might have on the reproductive success and population dynamics of native co-flowering plants, it is important to explore and compare pollinator visits between native and invasive species.

Floral phenotypes function to advertise available rewards to potential animal pollinators (Soto *et al.* 2015). Suites of flower characteristics, like colour, structure, and scent, have been correlated to specific pollinator visitors, and these associations are called pollination syndromes (Faegri and van der Pijl 1979). Even though this concept has been useful in understanding some plant-pollinator interactions, especially in the absence of direct observations (Fenster *et al.* 2004, Pérez *et al.* 2006, Whittall and Hodges 2007), pollinators have repeatedly been shown not to be confined by the rigid expectations of pollination syndromes (Ollerton *et al.* 2009). For example, regardless of the floral features of aloe species pointing to ornithophily as the pollination syndrome (Reynolds 2004), and birds readily visiting aloe flowers (Wilson *et al.* 2009), it is well known that insects, especially bees, are important pollinators to aloes (Symes *et al.* 2009). *Lantana camara* L. (*sensu lato*) (hereafter *L. camara* or lantana) is another example, where the pollination syndrome suggests pollination by butterflies (Carrión-Tacuri *et al.* 2014), but several other pollinators have been found visiting lantana ranging from honey bees (Goulson and Derwent 2004) to thrips (Mathur and Mohan Ram 1978, Sinha and Sharma 1985). Several characteristics of the flowers of *Kalanchoe rotundifolia* point pollination by butterflies, i.e., grouped inflorescences, nectar accumulation, and nyctinasty of flower (Faegri and van der Pijl 1979, Smith *et al.* 2022). Butterflies have also been observed to visit the flowers of *Kalanchoe rotundifolia* (Smith *et al.* 2022). These two native flowering plants, aloes, and kalanchoes, in South Africa, therefore, share pollinators with the invasive *L. camara.*

Considerable amounts of research have been dedicated to comparing the pollinator visitation between invasive and native plants, but with ambiguous results (Chrobock *et al.* 2013). The review by Morales and Traveset (2009) provides evidence that pollinators visit invasives more than natives, which may in turn negatively affect the reproductive success of natives. Brown *et al.* (2002) found similar results, with the invasive purple loosestrife in their study sites affecting the pollinator visitation of native winged loosestrife. Still, other studies found that pollinators show a preference for natives over their invasive neighbours (Chrobock *et al.* 2013, Morandin and Kremen 2013).

A topic rarely explored is the effect that invasive plants might have on the native pollinator community of invaded ranges. It is often assumed that this impact would be negative. Stout and Tiedeken (2017) postulate that it might be because invasive plants tend to occupy large sections of land, often excluding native plants of which the resources may have been more suitable for the native pollinator community. Previous studies on the effects of invasive plants on native pollinators have focussed on the abundance, species diversity, and community composition of taxa in invaded areas. Very few studies have emphasised the impacts at individual levels (Stout and Tiedeken 2017). Since it is impossible to study every invasive plant in all its ecological contexts, it becomes relevant to consider relevant impacts on individual levels to predict interactions between invasives and invaded environments.

Honey bees (*Apis mellifera*) have garnered a reputation for facilitating the spread of invasive plants via pollination. In previous studies, honey bees have been directly linked to the spread of invasive plants (Barthell *et al.* 2001, Gross *et al.* 2010), and are often found to be the dominant pollinator visiting invasive plants (Aizen *et al.* 2008, Wood *et al.* 2018). Goulson and Derwent (2004) found that the seed and fruit set of lantana correlated strongly to the presence of honey bees in their study sites. However, they also found similar correlations in the seed and fruit set of lantana with the abundance of butterflies in their study sites (Goulson and Derwent 2004). Urbanowicz *et al.* (2020) studied pollinator visits across native and introduced plant species in North America and found that the top three plants most visited by honey bees were invasives in their study region. Honey bees visit around 1000 plant species in South Africa, half of which are invasive, making it likely that they have facilitated the introduction and spread of some invasive plants (Illgner 2002). Illgner (2002) also mentions *L. camara* as a bee forage plant. It follows that in areas with lantana infestations, honey bees may show a preference for *L. camara,* with its attractive and abundant flower displays. Lantana grows and flowers prolifically throughout the year given adequate moisture and light (Day *et al.* 2003, Lonare *et al.* 2012, Vardien *et al.* 2012), and in invaded areas, lantana tend to form large, monospecific thickets (Sharma *et al.* 2005), which would theoretically provide ample, year-round resources for pollinators.

The aim is to determine the influence of lantana plants on the quantitative component of the pollination of three native plant species in South Africa. Two experiments were conducted to investigate the preferences of native pollinators at two different levels. At the species level, the present study aimed to study the preference of an important native pollinator, the African honey bee (*Apis mellifera scutellata*), between one of South Africa's worst invaders, *L. camara,* and two important, indigenous bee plants, *Aloe greatheadii* and *Aloe marlothii*. In these experiments, the factor of floral abundance in the determination of honey bee preference was eliminated to determine which species honey bees would favour. Based on previous studies showing honey bees' affinity for invasive species and the fact that lantana is a bee forage plant, we hypothesised that honey bees might show a preference for lantana. At a community level, the differences in pollinator communities visiting the indigenous *Kalanchoe rotundifolia* flowers in the presence and absence of *L. camara* were determined in a heavily infested urban nature reserve. Here we hypothesised that the abundance of lantana flowers in the invaded site may act as a magnet, drawing pollinators away from the native flowers, thereby negatively affecting the pollination services they receive.

Invasive plants can act as novel and abundant food sources for insects (Bartomeus *et al.* 2016). In areas where lantana occurs, it might be an important foraging resource for honey bee workers, especially in flower-scarce winter months. If honey bees show a preference toward lantana, this may be indicative of possible negative effects of lantana on the aloes in their shared environments. It would also present a conservation conundrum. Given the ever-growing need to ensure access to adequate resources for declining pollinator populations, it may become necessary to consider alternative sources, even if they include invasive plants (Salisbury *et al.* 2015). This may cause the goals of conservation to become disparate, i.e., management of an invasive species at the expense of possible important alternative pollinator resources, or leaving invasive plants intact, but with negative effects on the pollination services received by co-flowering native plant species. It is important to understand all aspects of the influences of invasive plant species on the native fauna and flora that it interacts with to make informed management decisions.

Materials and methods

Preference experiments

Study species

Aloe (Linnaeus) is the largest genus of the family Asphodelaceae (Cousins and Witkowski 2012). South Africa is a hotspot for aloe diversity and boasts the largest number of aloes (140 taxa of 548 accepted species) on the continent (Grace *et al.* 2009, Klopper *et al.* 2009). *A. greatheadii* and *A. marlothii* are two of the most recognisable aloe species occurring in South Africa.

Spotted aloes (*Aloe greatheadii*) are one of the most important indigenous bee plants (Human and Nicolson 2006). It flowers abundantly in the South African winter. Migratory beekeepers in the North Western parts of South Africa often make use of the winter flowering fields of the spotted aloe when other floral resources are scarce (Fletcher and Johannsmeier 1978, Nicolson and Human 2008). Mountain aloes (*Aloe marlothii*) have very large and bright floral displays that attract many nectarivorous bird species (Symes *et al.* 2009, Cousins and Witkowski 2012). *Aloe greatheadii* and *A. marlothii* are both widely distributed in South Africa. *A. greatheadii* throughout the northern summer rainfall area of South Africa (Human and Nicolson 2006), and *A. marlothii* similar, but also extending into the more tropical coastal regions of Kwa-Zulu Natal (Glen *et al.* 2000), a distribution that both aloe species share with the invasive weed, *L. camara* (Simelane *et al.* 2021).

Study sites

Preference tests were conducted at the apiaries on the experimental farm grounds of the University of Pretoria, Pretoria, Gauteng. Apiaries are maintained at three sites on the farm grounds (Site 1: 25°44’57.4’’S, 28°15’56.1’’E; Site 2: 25°45’10.9’’S, 28°15’30.1’’E; and Site 3: 25°45’14.0’’S, 28°15’24.7’’E), each experiment was repeated at each of the three sites.

Two of the three sites are heavily infested with *L. camara*, and the third site is well within the honey bee worker foraging range of areas infested with lantana (Winston 1987). Management of lantana on the university grounds involves mechanical and chemical control but mainly consists of the occasional cutting of lantana closest to the hives. The main purpose of these cuttings seem to be to maintain access to the hives, rather than for the sake of controlling the lantana infestation. At the time of the experiments, the lantana in the area was not flowering.

Collection of test plants

Two preference experiments were conducted to determine whether honey bees show a foraging preference between the invasive weed, *L. camara*, and two indigenous, bee plants in the genus *Aloe*. One experiment compared the preference between lantana and *A. greatheadii*, and the other between lantana and *Aloe marlothii*. Branches with open florets for the *L. camara* versus *A. marlothii* experiment were collected from locations on the University of Pretoria campuses (branches from ten lantana plants: 25°45’12.6’’S, 18°15’33.7’’E, and ten *A. marlothii* plants: 25°45’17.9’’S, 28°14’06.8’’E). For the *L. camara* versus *A. greatheadii* experiment, lantana branches were collected at roadside locations in an urban area in Pretoria East (branches from ten lantana plants were collected in a 1.5 km radius from: 25°48’13.944’’S, 28°19’47.625’’E). Ten *A. greatheadii* plants were provided from a local succulent nursery, Gariep Plants in Pretoria East, from which branches were collected. Once cut, each branch was kept in an individual glass container with 1% (%v/v) sugar solution, with the addition of 5ml household bleach (Regular Jik household bleach) per litre of the solution to prevent bacterial growth on the cut branches. The sugar-bleach solution was replaced every day for the duration of the experiment.

Preference tests

Tests were conducted between 09h00 and 16h00 and restricted to sufficiently warm days (>20°C) for honey bee activity. The number of racemes, inflorescences, and florets on each of the branches was counted every day for the duration of the experiments. The preference tests were conducted over three days for the *L. camara* vs *A. marlothii* tests, and five days for the *L. camara* vs *A. greatheadii* tests.

Racemes are a type of inflorescence that is often used to describe the flowers of aloe species. In this study, we will refer to the inflorescences of aloes as racemes. For lantana, the term inflorescence will be used. The small flowers making up the racemes and inflorescences respectively will be referred to as florets.

Each test involves placing one branch (containing multiple racemes or inflorescences) of each of the two species (either lantana vs. *A. marlothii*, or lantana vs. *A. greatheadii*, depending on the experiment) in front of a hive, with roughly a meter separating the hive from the plants and the two branches from each other (Figure 3.1, A and B). A consistent distance between tests was maintained by using two footstools, on which the branches were placed in front of the hives, and only moving the branches between tests and not the stools. The position of the two species, relative to the hive, was alternated between tests.

Figure 3.1 The experimental set-up of the preference experiments. Picture A shows a *Lantana camara* test branch (left) and an *Aloe greatheadii* test branch (right). Picture B shows a *Lantana camara* test branch (left) and an *Aloe marlothii* test branch (right). The hives are located in the apiaries of the

University of Pretoria experimental farm. In both set-ups the test plants are placed on stools approximately a meter from the hive, keeping the distances constant between tests. Positions of the species with respect to the hives were alternated between tests.

Once placed, the plants were observed by two people, each observing one of the two plants, to determine which of the two species (either lantana or *A. marlothii*, or lantana or *A. greatheadii*, depending on the experiment) a honey bee first landed on. The first species to receive a honey bee visitor was recorded as the “choice”-species. A visit was only counted if the bee could be seen foraging on the chosen flower. Just landing and leaving or resting on leaves and flowers was not considered a visit. When a honey bee chooses one of the two species that were presented, that was considered a “successful” test for that chosen species. The time it took for the first bee to land was noted. If no choice was made after 15 minutes, the test was abandoned and returned to at the end of that round of observations. For each non-choice, this process was repeated three times to allow sufficient time for bees to decide, however, if, after three times, no choice was made, the data reflect it as such.

The ten branches of each of the two species in the test were paired in 100 unique combinations. Each combination was tested once, resulting in a total of 100 tests conducted, never with the same combination of individual branches tested twice. For each of the two experimental pairings (*L. camara* vs. A. *marlothii*, and *L. camara* vs. *A. greatheadii*), 30 tests were conducted at apiary sites one and three, and the remaining 40 tests were conducted at apiary site two.

Data analyses

The preference experiments were conducted under the assumption that, if the honey bees show no preference for either lantana or aloe plants, the number of successes – i.e., being chosen by a bee – for either species would be 50%. A one-sample test of proportions was conducted, with the null hypothesis stating that the number of times lantana (or aloe) is picked would be equal to 50%. The test was conducted in RStudio (Version 2022.12.0.353), with the function prop.test, without continuity correction.

The time it took a honey bee to choose a species during each test was recorded. Mann-Whitney U-tests with continuity correction were used to determine whether there was a significant difference in the time it took the honey bees to decide between either lantana or aloe species, as well as if there is a significant difference in the time it took honey bees to choose either of the two lantana species in the different experiments. Analyses were performed in Statistica Version 13 (TIBCO Software Inc.).

Pollinator observations in cleared versus invaded sites

Study species

Kalanchoe rotundifolia is widespread in southern Africa (Van Jaarsveld 2017), and its range overlaps with that of lantana (Smith *et al.* 2019). The two species have overlapping flowering periods and similar floral structures (Smith *et al.* 2019, Smith *et al.* 2022). Flowers are tubular and grouped in inflorescences of brightly coloured florets. Pollinators that have been observed on the flowers of kalanchoe include birds and flying insects, often butterflies (Smith *et al.* 2022). Kalanchoe flowers remain open for several days, ensuring multiple visits from pollinating species, and increasing the chance of successful pollination (Smith *et al.* 2022). In October 2021, a large area within the range of *Kalanchoe rotundifolia* (Family: Crassulaceae) was cleared of lantana plants by a group of volunteers (The Friends of Faerie Glen) working in the reserve as part of a larger effort to eradicate and manage the lantana invasion in the reserve. This provided a unique opportunity to investigate the effects of the presence of *L. camara* on the pollinator visitations to this indigenous plant growing in a natural environment alongside it.

Pollinator observations

Two observational blocks (25 m x 25 m) were established in March 2022 in an area of the Faerie Glen Nature Reserve (FGNR) where *Kalanchoe rotundifolia* occurs in large numbers alongside the invasive *L. camara.* The two observational blocks were separated by 100 meters. One block was situated in an area where *L. camara* had been removed the year before (cleared), while the other block was heavily infested with the invasive (invaded). Before starting the observations in March, the regrowth of lantana was removed from the cleared plot. In cases where the lantana was too extensive to remove, care was taken to remove any inflorescences throughout the observation period. In both observational blocks, patches of Kalanchoe plants were counted and numbered. A patch was defined as the area within which pollinator observations could reasonably be conducted on the plants occurring in that patch, i.e., for a single observer, a roughly 1 m x 1 m area. Kalanchoe plants were only counted as patches if there were open florets on the inflorescences (Figure 3.2, A). The number of kalanchoe plants making up a patch was counted, and the number of inflorescences and open florets contained on the flowers in every patch was noted. In each of the two observation blocks, 10 patches of Kalanchoe plants were randomly selected on which to observe pollinator visitors.

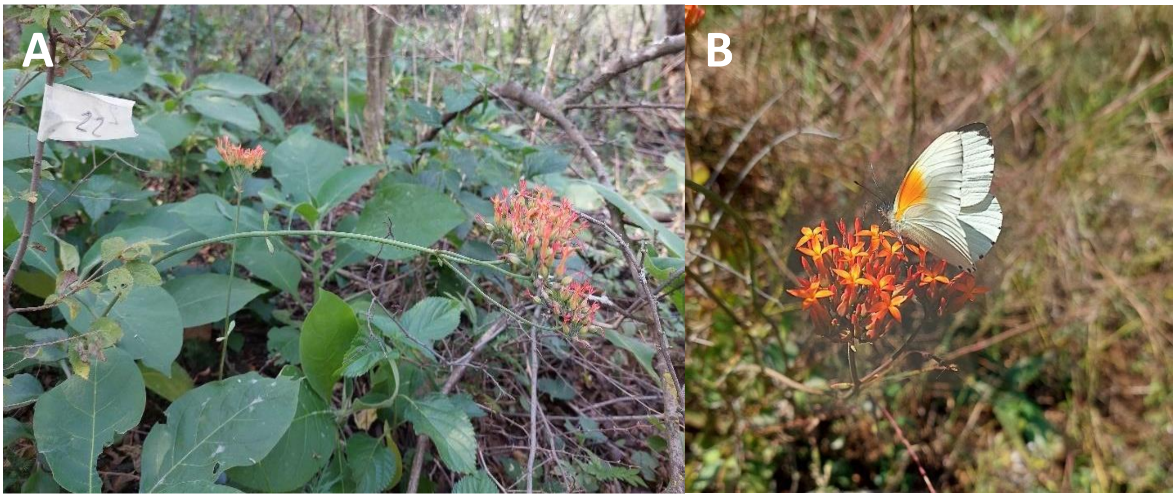

Figure 3.2 Picture A shows a patch of *Kalanchoe rotundifolia* plants marked for observation in an area where the invasive co-flowering *Lantana camara* was removed. Picture B shows a common dotted border butterfly (*Mylothris agathina*) foraging on *Kalanchoe rotundifolia*, captured during the pollinator observations in one of the kalanchoe patches.

Kalanchoe flowers are nyctinastic (Smith *et al.* 2022), and the flowers in the reserve only opened after 10h00. Pollinator observations were, thus, conducted during times of peak insect activity while kalanchoe flowers are open (between 10h00 and 15h00). Observations were restricted to days on which weather favoured insect activity. Observations at the 20 selected kalanchoe patches (10 in the cleared site and 10 in the invaded site) were conducted randomly in 15-minute observation bouts until an accumulated time of one hour was reached for each of the 20 kalanchoe patches. The following was noted during the observations: 1. Pollinating insects visiting kalanchoe inflorescences in the patch. An insect visitor was considered a pollinator if the insect could be observed inserting itself or its mouthparts into the corolla of the flower (Larson *et al.* 2006, Carrión-Tacuri *et al.* 2014), 2. A count of the inflorescences containing open florets, as well as the number of open florets at the time of observation, and 3. The weather conditions at the time of observation as measured by a Kestrel 3000 weather meter. Photographs were taken to aid in the identification of the visiting insects (Figure 3.2, B). In addition to photographs, whenever it was necessary and possible insects were caught, either with a net or an aspirator, and stored in 70% alcohol or pinned when appropriate, to be identified later.

Data analyses

Mann-Whitney U-tests were conducted to determine whether the differences in the numbers of pollinators observed per observation bout between the cleared and invaded sites were significant, as well as whether there were differences in the orders of insects observed per observation bout between the two sites. These analyses were conducted in Statistica Version 13 (TIBCO Software Inc.). G*Power (Version 3.1.9.7) was used to perform a post hoc test to determine the statistical power of the Mann-Whitney U-test on the differences in insect observations per bout.

Results

Preference experiments

Numbers of inflorescences, racemes, and florets

There was a decrease in the number of florets on the lantana test plants over the three experiment days, while the number of florets on the *A. marlothii* plants increased (Table 3.1). In the tests between lantana and *A. greatheadii*, there was a decrease in the number of florets on both the lantana test plants and the *A. greatheadii* test plants over the three experiment days (Table 3.2).

Table 3.1 The mean numbers of inflorescences, racemes, and florets for the *Lantana camara* vs. *Aloe marlothii* preference experiments.

	Lantana camara		*Aloe marlothii*	
Day	Number of inflorescences	Number of florets	Number of racemes	Number of florets
1	11.7 (3)	43.3 (12.1)	4.8 (2)	45.5 (28)
2	-	-	4.7 (1.8)	38.6 (20.9)
3	8.375* (2.8) (n=8)	35.5* (10.6) (n=8)	4.6 (1.6)	61.2 (61.4)

* Means calculated from fewer than the ten original plants used in the experiment due to loss of plants. Standard deviations are shown in parentheses. On aloe test plants, only the open florets were counted. On lantana test plants, only yellow florets were counted. Sample sizes were n=10 unless stated otherwise. The symbol '-' represents values that were lost or not recorded.

Table 3.2 The mean numbers of inflorescences, racemes, and florets for the *Lantana camara* vs. *Aloe greatheadii* preference experiments**.**

	Lantana camara		*Aloe greatheadii*	
Day	Number of inflorescences	Number of florets	Number of racemes	Number of florets
1	14.2 (5.1)	45.3 (12.7)	4.9 (1)	25.7 (5.7)
2	13.4 (3.2)	-	4.9 (1)	-
3	14.2 (3.5)	54.8 (13.7)	4.9 (1)	25.6 (7.2)
4	12.4 (2.6)	45 (18)	4.9 (1)	23.5 (6.4)
5	10.75* (2.2) (n=8)	32.75* (13.9) (n=8)	5.2* (1.2) (n=6)	18.8* (5.5) (n=6)

* Means calculated from fewer than the ten original plants used in the experiment due to loss of plants. Standard deviations are shown in parentheses. On aloe test plants, only the open florets were counted. On lantana test plants, only yellow florets were counted. Sample sizes were n=10 unless stated otherwise. The symbol '-' represents values that were lost or not recorded.

Preference tests

Out of the 100 tests that were performed for each of the two preference experiments, 87 and 94 of the tests were completed for lantana vs. *A greatheadii*, and lantana vs. *A. marlothii*, respectively. The lantana vs. *A greatheadii* were conducted over five days, while the lantana vs*. A. marlothii* preference tests were conducted over three days. Some tests were not completed due to time and weather restraints, or because of the loss of some of the test branches. Loss of tests branches between tests days were caused by accidental wilting of the branches. No choice was recorded for six tests between lantana and *A. greatheadii*, and four tests between lantana and *A. marlothii*. For one test between lantana and *A. marlothii*, two bees landed on the two plants simultaneously. This was recorded as the same choice. These tests were omitted from the data analyses.

In both preference tests conducted, *A. marlothii* vs. *L. camara*, and *A. greatheadii* vs. *L. camara*, the aloes had higher numbers of successful tests (Figure 3.3 and Figure 3.4). The null hypothesis, stating that the number of times lantana would be successful is equal to 50% was rejected for both experiments (*L. camara* vs. *A. marlothii*: $X^2 = 13.764$, p = 0.0002 and *L. camara* vs. *A. greatheadii*: $X^2 = 4$, p = 0.0455). In both experiments, the aloe species were preferred over lantana.

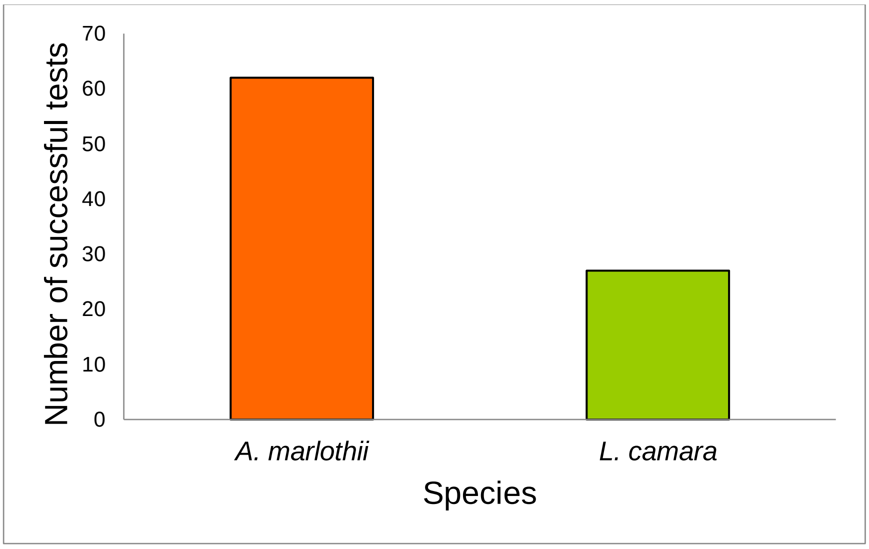

Figure 3.3 The number of times either *L. camara* or *A. marlothii* was successfully “chosen” by an African honey bee (*Apis mellifera scutellata*). The term “choice” in this context, refers to the plant that was first foraged on by a honey bee in a preference test presenting two species to a colony.

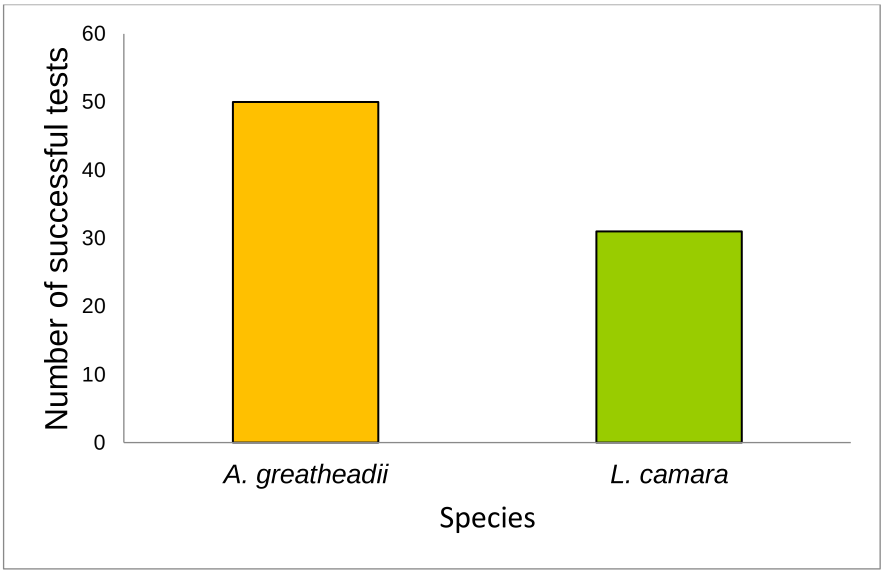

Figure 3.4 The number of times either *L. camara* or *A. greatheadii* was successfully “chosen” by an African honey bee (*Apis mellifera scutellata*). The term “choice” in this context, refers to the plant that was first foraged on in a preference test presenting two species to a hive. The test is considered successful for each species when that species was chosen.

Honey bees took an average of 144 (SD, 144.6) seconds to choose *A. marlothii* and 180 (184.6) seconds to choose *A. greatheadii* (Figure 3.5 and Figure 3.6). It took the bees 247 (216.5) seconds (lantana vs. *A. marlothii)* and 257 (243.9) seconds (lantana vs. *A. greatheadii*) to choose lantana. In the test between *A. marlothii* and lantana, the bees took significantly longer to choose lantana (Mann-Whitney U-test with continuity correction: z = -2.11961 0, p = 0.034040). When presented with a choice between lantana and *A. greatheadii*, there was no significant difference in the time it took the bees to choose either of the two species (Mann-Whitney U-test with continuity correction: z = 1.6616, p = 0.0966).

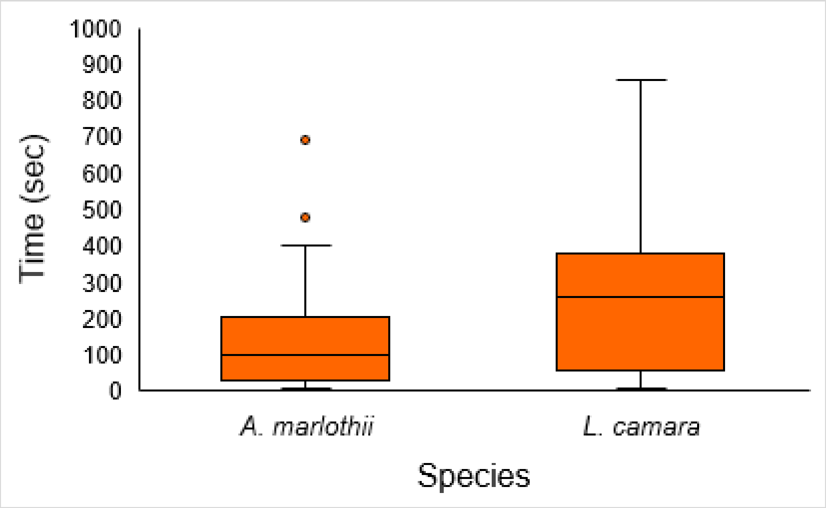

Figure 3.5 Box plots of the time it took African honey bees (*Apis mellifera scutellata*) to choose *Aloe marlothii* and *Lantana camara* in preference experiments. The difference between the two species is significant (Mann-Whitney U-test with continuity correction: z = -2.11961 0, p = 0.034040). Boxes show the exclusive median, 25th and 75th percentiles; the whiskers show the minimum and maximum observation.

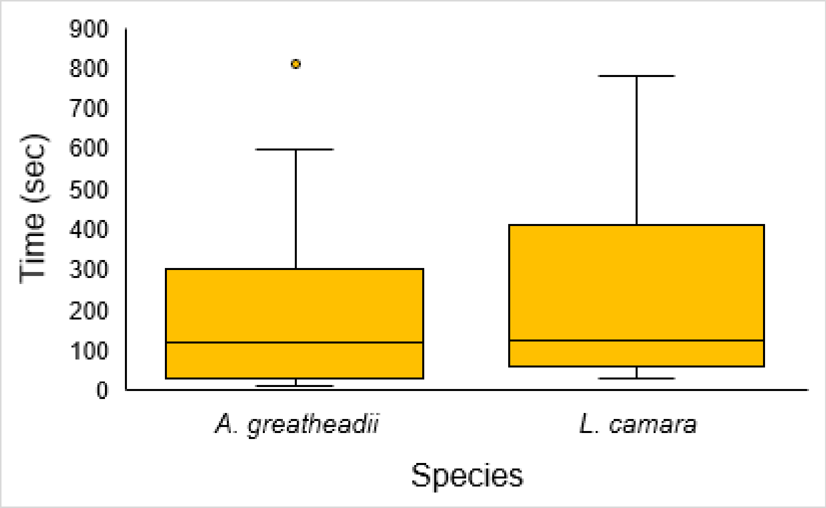

Figure 3.6 Box plots of the time it took African honey bees (*Apis mellifera scutellata*) to choose *Aloe greatheadii* and *Lantana camara* in preference experiments. The difference between the two species is not significant (Mann-Whitney U-test with continuity correction: z = 1.6616, p = 0.0966). Boxes show the exclusive median, 25th and 75th percentiles; the whiskers show the minimum and maximum observation.

Pollinator observations in cleared vs. invaded plots.

There were 54 patches of flowering *Kalanchoe rotundifolia* in the cleared observation block and 35 patches in the invaded observation block. Kalanchoe flowers in both patches were visited by pollinators from Lepidoptera and Hymenoptera (Table 3.3).

Table 3.3 The species of insect orders that were observed pollinating flowers of *Kalanchoe rotundifolia* in patches cleared of or invaded by *Lantana camara*.

Site	Lepidoptera	Hymenoptera
Invaded	*Belenois aurota*	*Allodapula variegata*
	Belenois java	Formicidae
	Catopsilia florella	
	Eurema brigitta	
	Mylothris agathina	
Cleared	*Catopsilia florella*	*Allodapula variegata*
	Mylothris agathina	Anthoporidae

A Mann-Whitney U-test showed no significant difference in the numbers of insects observed per observation bout between the two sites, one cleared of lantana, and one invaded by lantana (with continuity correction: z = 0.1056, p = 0.916) (Figure 3.7).

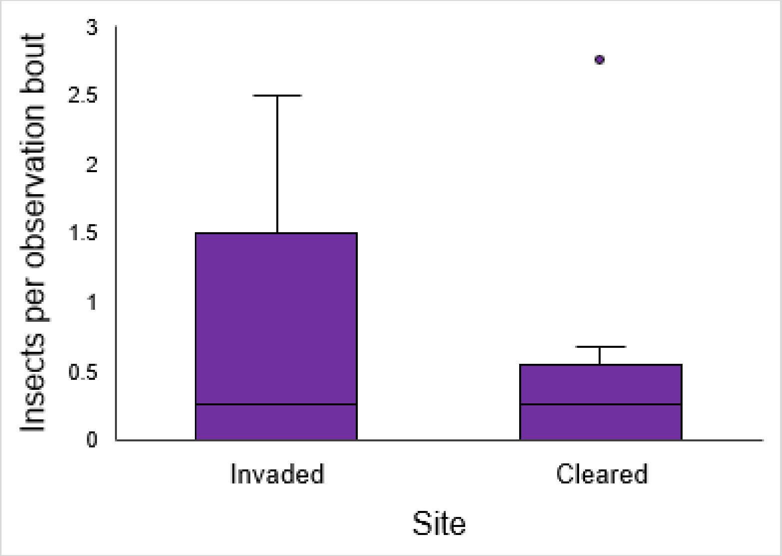

Figure 3.7 Box plots of the numbers of insects observed per observation bout in two plots, on invaded by and cleared of *Lantana camara*. The difference in the number of insects observed in the two different sites, invaded and cleared, is not statistically significant (Mann-Whitney U-test with continuity correction:

z = 0.1056, p = 0.916). Boxes show the exclusive median, 25th and 75th percentiles; the whiskers show the minimum and maximum observations.

Pollinators from Hymenoptera and Lepidoptera were observed foraging on Kalanchoe inflorescences. Hymenoptera was observed in three of the 10 kalanchoe patches in both invaded and cleared sites, while Lepidoptera was observed in four of the 10 kalanchoe patches in both sites. Mann-Whitney U-tests revealed no differences in the number of either Hymenoptera or Lepidoptera observed per bout between invaded and cleared plots (with continuity correction: Hymenoptera, z = -0.1056, p = 0.9159; Lepidoptera, z = 0.7746, p = 0.4586) (Figure 3.8).

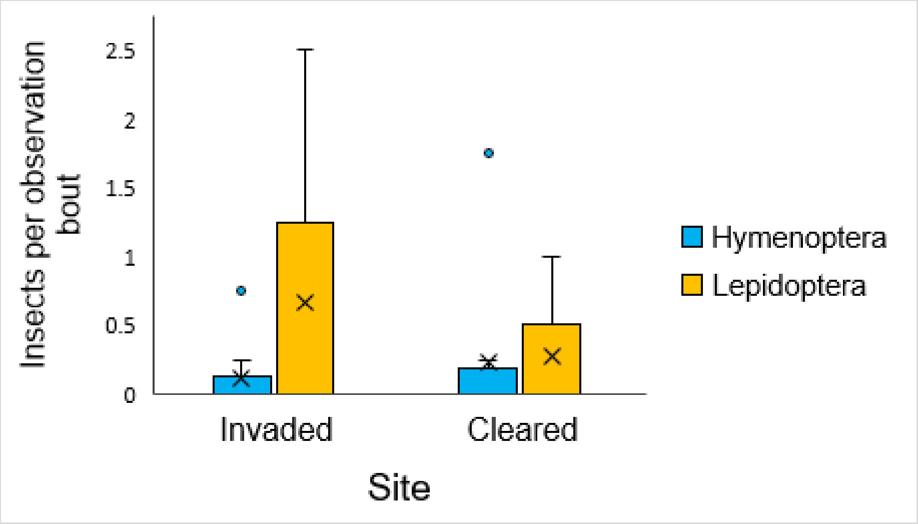

Figure 3.8 Box plots of the numbers of insects observed per observation bout in two plots, one invaded by and one cleared of *Lantana camara*. The differences in insects observed in the two orders between the two plots, invaded and cleared, were not statistically significant (Mann-Whitney U-tests with continuity correction: Hymenoptera, z = -0.1056, p = 0.9159; Lepidoptera, z = 0.7746, p = 0.4586). Boxes show the 75th percentiles, crosses represent the means; the whiskers show the maximum observed insects per bout.

Post hoc analysis reveals that the power of the statistical analysis is not high enough to confidently reject the alternative hypothesis that lantana influences the number of pollinators per observation bout between the cleared and invaded sites (1 – β error probability = 0.05).

To increase the power of the statistical analysis the experiment should be repeated with more observation plots and increased observation in the plots.

Discussion

The present chapter investigated the effects of *L. camara* on three different native flowering plants via their pollinator visitors. Preference experiments revealed that African honey bees (*Apis mellifera scutellata*) do not show a preference for the invasive *L. camara* over two abundant and indigenous aloe species, *A. greatheadii* and *A. marlothii.*

Most alien invasive plants rely on establishing new biotic relationships in invaded habitats to become successful. Honey bees are often suggested to be at the forefront of the facilitation of invasive species, either directly linked to the spread of invasive plants (Barthell *et al.* 2001, Gross *et al.* 2010) or being the dominant pollinators observed on invasive plants (Aizen *et al.* 2008, Wood *et al.* 2018). Recently, however, Urbanowicz *et al.* (2020) found that, at a community level, honey bees do not show a preference for either native or invasives, regardless of floral abundance. In the preference experiments, the factor of floral abundance was eliminated by using single test branches. These test branches were presented to honey bees in a choice test. Honey bees visited aloe branches more often than lantana branches, indicating that the bees prefer foraging on aloes, rather than lantana, in the absence of floral abundance. These results indicate that, apart from the relative abundances of these two species in their habitats, aloe flowers likely possess traits that honey bees prefer. *Aloe greatheadii* is important bee plants in South Africa. They have high pollen protein content (Human and Nicolson 2006) and concentrated nectar (Human 2006, Human and Nicolson 2008), making them a high-value resource. This may be the driving factor in honey bee decisions in this experiment. These results are surprising for *A. marlothii*, however, because it is primarily a bird-pollinated aloe species (Symes *et al.* 2009, Cousins and Witkowski 2012).

The preference of honey bees is also reflected in the time it took them to choose the respective species. In both experiments, honey bees took longer to choose lantana than aloes. In the case of *A. marlothii*, the difference in the time it took bees to choose was significant. This might be attributed to the fact that *A. marlothii* are very big plants, with racemes much larger than the branches of lantana that were used in the choice tests. Even though the numbers of florets of both the *L. camara* test branches and *Aloe marlothii* test branches were similar at the start of the experiments, the number of florets of *A. marlothii* increased over the test period, while that of lantana decreased. The difference in floret abundance by the last day of the experiments could very well be the cause of increased honey bee choices for the aloe. Previous studies show higher visitations to inflorescences with higher numbers of open florets (Schmid-Hempel and Speiser 1988, Brody and Mitchell 1997).

The question remains whether competition exists between these two honey bee food sources in shared habitats. In the absence of floral abundance, aloes are the clear winners. However, in invaded areas, where lantana tend to form large, monospecific thickets (Sharma *et al.* 2005), producing extremely high numbers of inflorescences, it may be a numbers game and honey bees are going for the mass flowering source and hence entering a positive feedback loop. Indeed, Hung *et al.* (2019) found that floral abundance was a driving factor for honey bee visitation preference.

Research dedicated to comparing pollinator visitation between invasive and co-flowering native plants has been contradicting. Some studies found negative effects on the visitation to natives in the presence of alien species, as reviewed by Morales and Traveset (2009). Still, other studies show pollinators prefer natives over invasives (Chrobock *et al.* 2013, Morandin and Kremen 2013). The findings of Dietzsch *et al.* (2011) show the abundance of invasive plants in their studies negatively affected pollinator diversity and pollination of native co-flowering plants. In their studies, this, ultimately, did not affect the reproductive success of the native species.

An attempt was made to investigate the influence of lantana on the pollinating visitors of a co-flowering native plant, *Kalanchoe rotundifolia*. Pollinator communities of kalanchoe were compared between a site invaded with lantana and a site cleared of lantana. Although no effect was found, post hoc tests revealed that the statistical power of the analysis was not sufficient to confidently reject the alternative hypothesis. Further observations are recommended, with increased observation plots in the two sites and longer observation times for each plot to truly determine whether lantana influences the pollinating visitors of kalanchoe.

In summary, our results suggest that honey bees do not have a preference for *L. camara* when single test branches are presented to honey bee colonies. Data collected in this study cannot be used to confidently determine whether lantana influenced the pollinator communities of the indigenous *Kalanchoe rotundifolia*. The presence of invasive plants in habitats is increasing, and understanding the interactions between invasive plants, native plants, and native pollinators is important to ultimately making appropriate management decisions.

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Chapter 4

Concluding remarks

Most of the research on *Lantana camara* L. (*sensu lato*) in South Africa has been concerned with the biological control of the weed. Very little research has been dedicated to studying the interactions between lantana and native fauna and flora. With this study, the first aim was to investigate the pollinating communities visiting lantana in an urban nature reserve in South Africa, and which pollinating insects are important for the successful pollination of lantana.

Lantana is established in more than 60 countries worldwide (Day *et al.* 2003). In several other countries, the main pollinators of lantana have been determined (Mathur and Mohan Ram 1978, Goulson and Derwent 2004, Carrión-Tacuri *et al.* 2014, Kritasampan *et al.* 2016). To understand the influence of pollinators on lantana, it is important to know which pollinators utilise its floral resources. This study revealed the diverse community of insects that utilise lantana inflorescences. The most abundant pollinator on lantana was thrips. This was consistent in all observation seasons. Visitation frequency and abundance of pollen transfer determine the effectiveness of pollinators (Aslan *et al.* 2016). While our observations did not include data on the amount of pollen transferred, the sheer abundance of thrips sampled in this study indicates that they are likely the most effective pollinators of lantana in these study sites. Previous studies in India found that thrips are regular and effective pollinators of lantana (Mathur and Mohan Ram 1986). Honey bees did utilise lantana, but to a much lesser extent than was expected. The diversity of pollinators sampled on lantana inflorescences shows that it can be considered a pollinator-generalist plant, in an ecological and functional sense, according to the descriptions by Ollerton *et al.* (2007).

Variation in the numbers of honey bees observed on lantana was only explained by the season in which observations were conducted. The fact that the abundance of lantana inflorescences in observation plots did not explain the variation in honey bee numbers may be an indication that honey bee visits to lantana are not driven by the abundance of resources provided by the plant at a given time. This is even though lantana grows in large, monospecific thickets in the study sites. These results are contrary to those of Hung *et al.* (2019), who found that honey bee visits increase with flower abundance, and in line with those of Urbanowics *et al.* (2020), who showed that the visitation rates of honey bees do not increase with increases in floral abundances.

Similar to previous studies (Goulson and Derwent 2004), pollinator exclusion experiments showed that the fruit set of lantana was the highest when no pollinators were excluded from the inflorescences. The exclusion of large pollinators, such as large bees and butterflies did not significantly affect the fruit set, and neither did the limitation of pollination to a single pollinator visit. Only when smaller pollinators, such as honey bees, drone flies, bee flies, and medium-sized beetles, were excluded, was there a significant decline in the number of fruits set by the lantana plants. Collected fruits were subjected to seed viability tests, and despite the results for the fruit set between treatments, there were no differences in the number of viable fruits set by the lantana between any of the four exclusion treatments and the control. These results show that the type of pollinator likely does not influence the proportion of viable seeds that lantana produces. It also shows the importance of considering the viability of seeds as a measure of pollination success, and not fruit and seed set alone.

The effects lantana might have on native co-flowering plant species, via its interactions with shared native pollinators, were considered. Different invasive plants elicit different responses from pollinators (Stout and Tiedeken 2017). There is a plethora of plant traits that determine pollinator decisions for host plants, and generalisations are often difficult to make. It, therefore, becomes important to understand the responses of pollinators on species and population levels to better understand impacts on community levels (Bartomeus *et al.* 2016, Stout and Tiedeken 2017). At a species level, this study aimed to investigate the preference of honey bees between lantana and two native aloe plants. As highly generalist pollinators (Stokes *et al.* 2006, Aslan *et al.* 2016), honey bees have a reputation for facilitating the spread of invasive species (Barthell *et al.* 2001, Illgner 2002, Gross *et al.* 2010). Investigating the preference of honey bees may inform on both the effect of lantana on honey bees (as a possible preferred alternative foraging resource), as well as the effect of lantana on native co-flowering plants species which could potentially draw away important pollinators. At a community level, the pollinating communities on an indigenous kalanchoe were compared between patches in the presence and patches in the absence of lantana. In infested areas lantana grows in large monospecific thickets with an abundance of floral resources (Sharma *et al.* 2005), which may act to draw pollinators away from native plants.

Results from this study show that honey bees preferred aloes in preference tests conducted between lantana and two indigenous aloe species, *Aloe greatheadii* and *Aloe marlothii*. These tests were conducted with single branches of each of the species, eliminating the effects of abundance on the preference of the honey bees. These results are unsurprising for *A. greatheadii*. It is described as a major bee plant in South Africa and beekeepers in the northern parts of the country often move their hives into aloe fields in the winter (Fletcher and Johannsmeier 1978). These results were, however, more surprising for *A. marlothii*, since this plant is predominantly bird-pollinated (Symes *et al.* 2009). The preference of honey bees for the two aloe species may be explained by the high-value resources produced by *A. greatheadii (*Human and Nicolson 2006, Human and Nicolson 2008), and the higher number of florets and larger inflorescences of *A. marlothii* branches (Schmid-Hempel and Speiser 1988).

The communities of insects visiting the flowers of the indigenous *Kalanchoe rotundifolia* were observed in the presence and absence of a lantana infestation. Similar species were observed on the kalanchoe flowers in the infested site and in the cleared site. There was no effect of the presence of lantana observed in communities of pollinators on kalanchoe flowers, however, there are limitations to the conclusions that can be made from these experiments due to the statistical power of these analyses.

How pollinating insects use invasive plant species has received considerable attention in the literature, however, with contrasting conclusions. In a review paper, Morales and Traveset (2009) concluded that pollinators show a preference for invasive plants over natives. Still, other studies found that pollinators prefer natives (Chrobock *et al.* 2013, Morandin and Kremen 2013). The effect of invasive plants on native pollinators is less explored. This effect is often assumed to be negative, however, the presence of invasive plant species may have a positive influence on native pollinators in the form of resource provision (Rodriguez 2006). In light of increasing urbanisation, there is a need to ensure adequate access to resources for declining pollinator populations. It has been suggested that these resources may need to include invasive plants in certain contexts (Salisbury *et al.* 2015, Bartomeus *et al.* 2016, Wood *et al.* 2018). Invasive plants may provide novel and abundant resources to pollinating species, especially in highly urbanised environments which provide many challenges to pollinators (Bartomeus *et al.* 2016, Baldock 2020). However, a recent paper from Breed *et al.* (2022) highlights how the intentional design of native gardens can increase the number of insects supported in even small patches of green space in an urban environment. Our results showing the preference of honey bees for indigenous aloes, which are popular garden ornamentals in South Africa, reinforce these findings.

My results present important insights about *L. camara* and its interactions with natives around it, which places its invasion in an ecological context. Many native species utilise lantana flowers, whether for resources or shelter, the most abundant of which was thrips. Lantana can be considered a pollinator-generalist plant. The fact that lantana is utilised by such a wide array of pollinators, is likely the cause of its immense success in South Africa. Similar conclusions were reached in other studies as well (Sharma *et al.* 2005). Honey bees are not an important pollinator of lantana in our study sites and are likely not significantly contributing to its spread. This is despite the reputation of honey bees as facilitators of invasive plants through pollination (Gross *et al.* 2010). Honey bees show a preference for aloes over lantana. These results indicate that lantana is likely not a threat to aloes, at least where pollinators and pollination are concerned. The interaction of lantana with co-flowering natives at a community level remains to be explored. There is a need to continue the study of lantana and its interaction with native fauna and flora since these factors may influence the propagation success of lantana. An improved understanding of these factors could be of great value in enabling the design of appropriate and effective control programmes for this weed. This study aimed to start filling these research gaps for lantana in Southern Africa.

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Appendices

Appendix A

Table A-1 The distances between all observational plots, and the three colonies of African honey bee that were used in observation of honey bee activity. The centre plots of each observation site, are highlighted in green.

Location	Bee hive 1	Bee nest	Bee hive 2	Average distance	Standard deviation
Site1 Plot 1	125,40	473,54	1387,67	662,2	651,9
Site 1 Plot 2	119,33	454,27	1353,44	642,3	638,2
Site 1 Plot 3	68,92	418,94	1399,79	629,2	689,9
Site 1 Plot 4	111,23	464,74	1352,50	642,8	639,5
Site 1 Plot 5	160,81	513,57	1431,94	702,1	656,2
Site 1 Plot 6	212,40	561,07	1395,80	723,1	608,1
Site 1 Plot 7	175,65	514,57	1378,38	689,5	620,2
Site 1 Plots 8	171,74	504,54	1333,16	669,8	598,1
Site 1 Plot 9	65,33	375,01	1353,81	598,1	672,6
Site 2 Plot 1	500,00	561,70	918,51	660,1	225,9
Site 2 Plot 2	597,20	632,17	824,37	684,6	122,3
Site 2 Plot 3	649,96	678,63	773,16	700,6	64,5
Site 2 Plot 4	636,15	696,53	778,62	703,8	71,5
Site 2 Plot 5	587,83	656,98	827,07	690,6	123,1
Site 2 Plot 6	534,98	638,57	882,03	685,2	178,2
Site 2 Plot 7	523,26	659,72	904,87	696,0	193,4
Site 2 Plot 8	582,54	692,80	838,58	704,6	128,4
Site 2 Plot 9	624,49	718,84	793,48	712,3	84,7
Site 3 Plot 1	448,91	763,83	1238,27	817,0	397,4
Site 3 Plot 2	491,17	797,70	1202,13	830,3	356,6
Site 3 Plot 3	536,54	829,73	1153,83	840,0	308,8
Site 3 Plot 4	570,19	875,02	1194,03	879,7	311,9
Site 3 Plot 5	524,28	838,75	1233,31	865,4	355,3
Site 3 Plot 6	582,81	898,80	1230,11	903,9	323,7
Site 3 Plot 7	570,88	893,98	1274,31	913,1	352,1
Site 3 Plot 8	526,41	855,83	1305,18	895,8	390,9
Site 3 Plot 9	481,49	805,95	1274,21	853,9	398,5

Table A-2 The total number of arthropods recorded, the number of observation bouts performed, and the number of arthropods observed per observation bout in the four sampling seasons. These data do not include the opportunistic samples (n=40) since those observations are not included in the count of the observation bouts

Season	Total number of arthropods observed	Total number of observation bouts performed	Arthropods per bout
Spring	528	36	14.6667
Summer	1289	109	11.8257
Autumn	1231	103	11.9514
Winter	259	77	3.3636
Grand Total	3321	331	

Table A-3 Numbers of insects observed belonging to the five orders of pollinators in the four observation seasons and the overall numbers of insects.

Season	Coleoptera	Diptera	Hymenoptera	Lepidoptera	Thysanoptera	Grand Total
Spring	7	16	22	6	470	521
Summer	24	97	186	37	828	1172
Autumn	41	71	132	121	693	1058
Winter	5	1	23	40	133	202
Not recorded	-	-	1	-	13	14
Overall	77	185	364	204	2137	2967

Not recorded refers to samples for which the observation season were not recorded or lost. These data include the opportunistic samples that were collected.

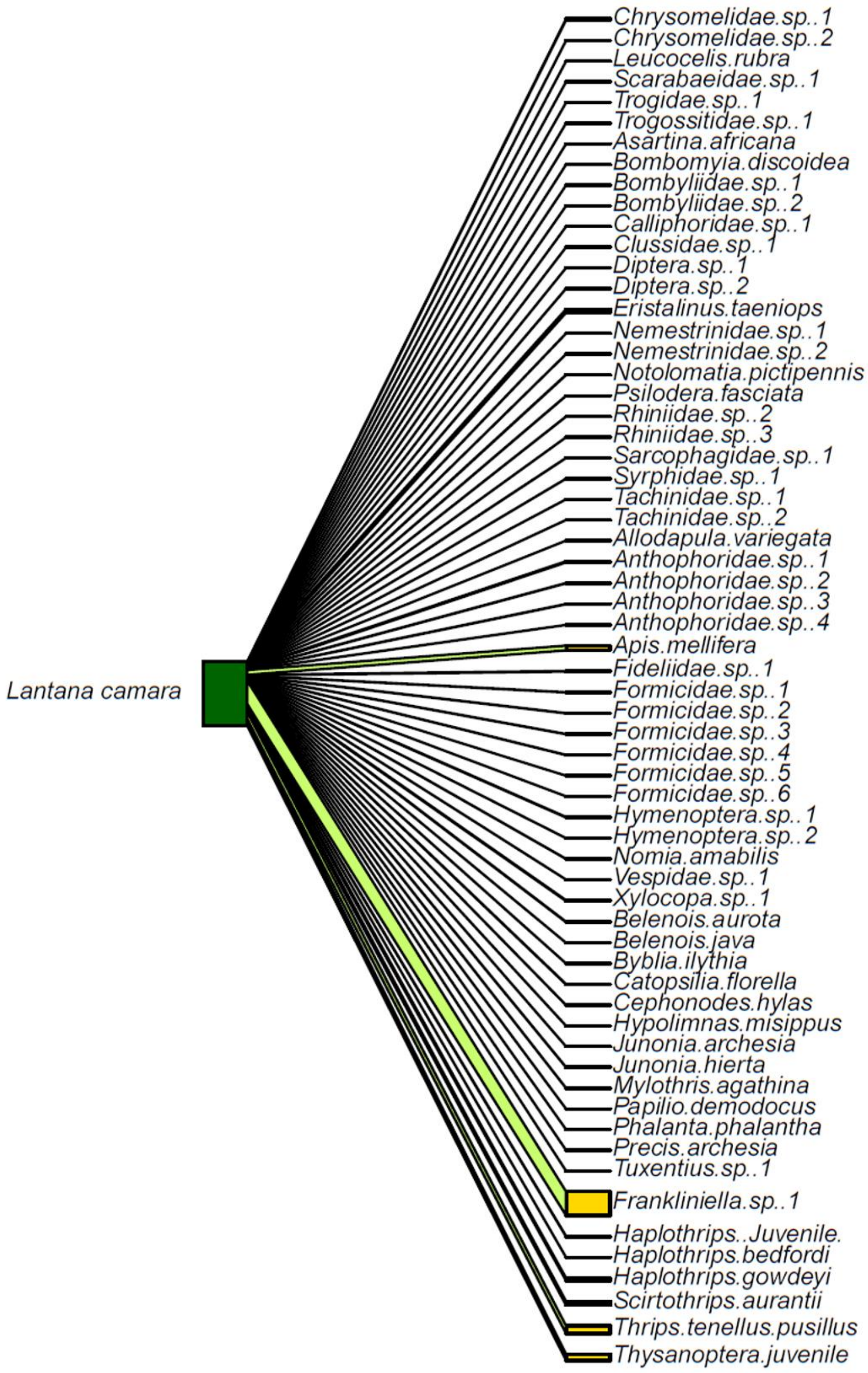

Figure A-1 Pollinating species sampled on *Lantana camara* inflorescences in summer (January) via three collection methods: in-person observations, opportunistic sampling, and inflorescence collections. The thickness of the bars represents the abundance of each species as a proportion of the total number of pollinators collected in the sampling season

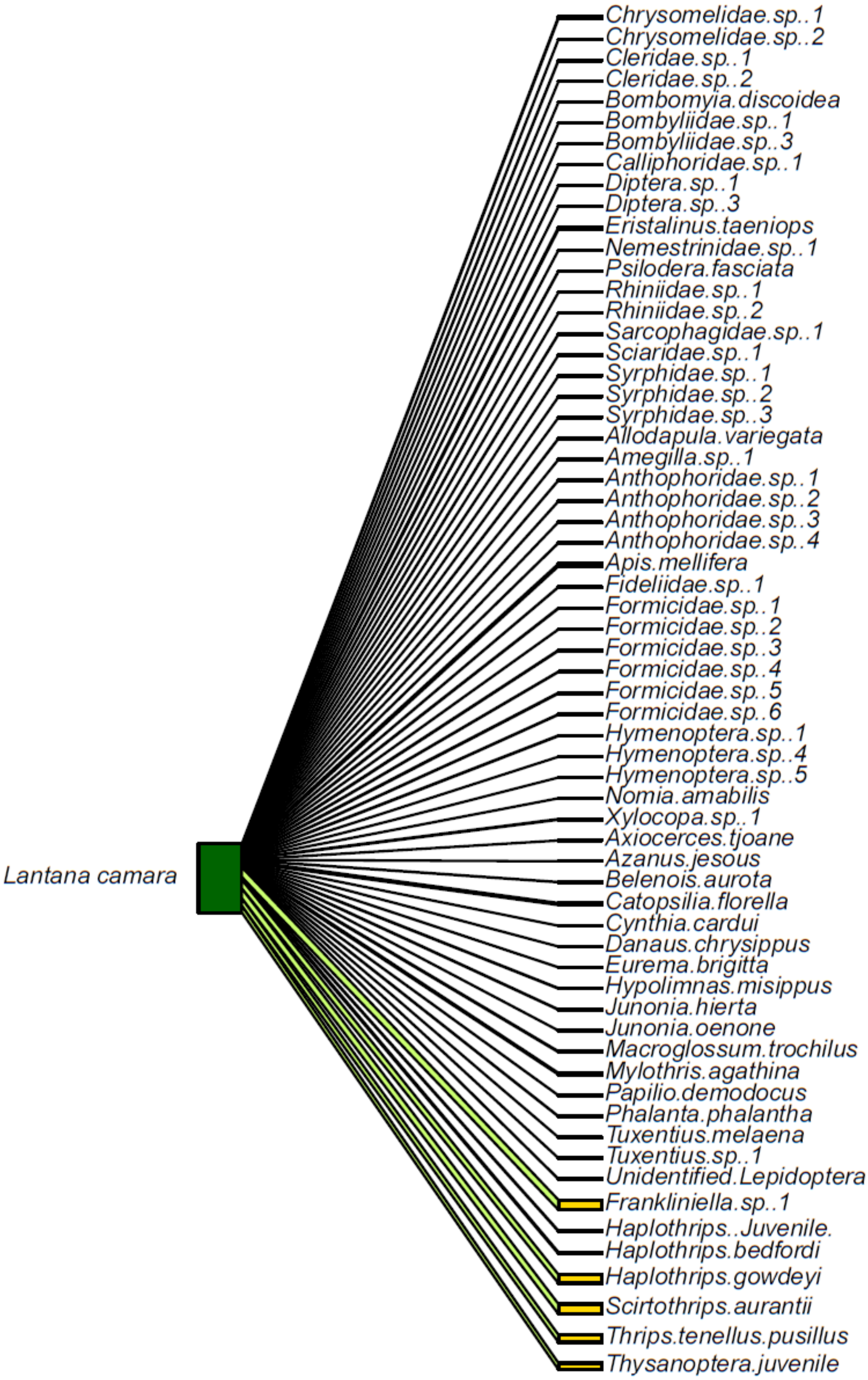

Figure A-2 Pollinating species sampled on *Lantana camara* inflorescences in autumn (March) via three collection methods: in-person observations, opportunistic sampling, and inflorescence collections. The thickness of the bars represents the abundance of each species as a proportion of the total number of pollinators collected in the sampling season.

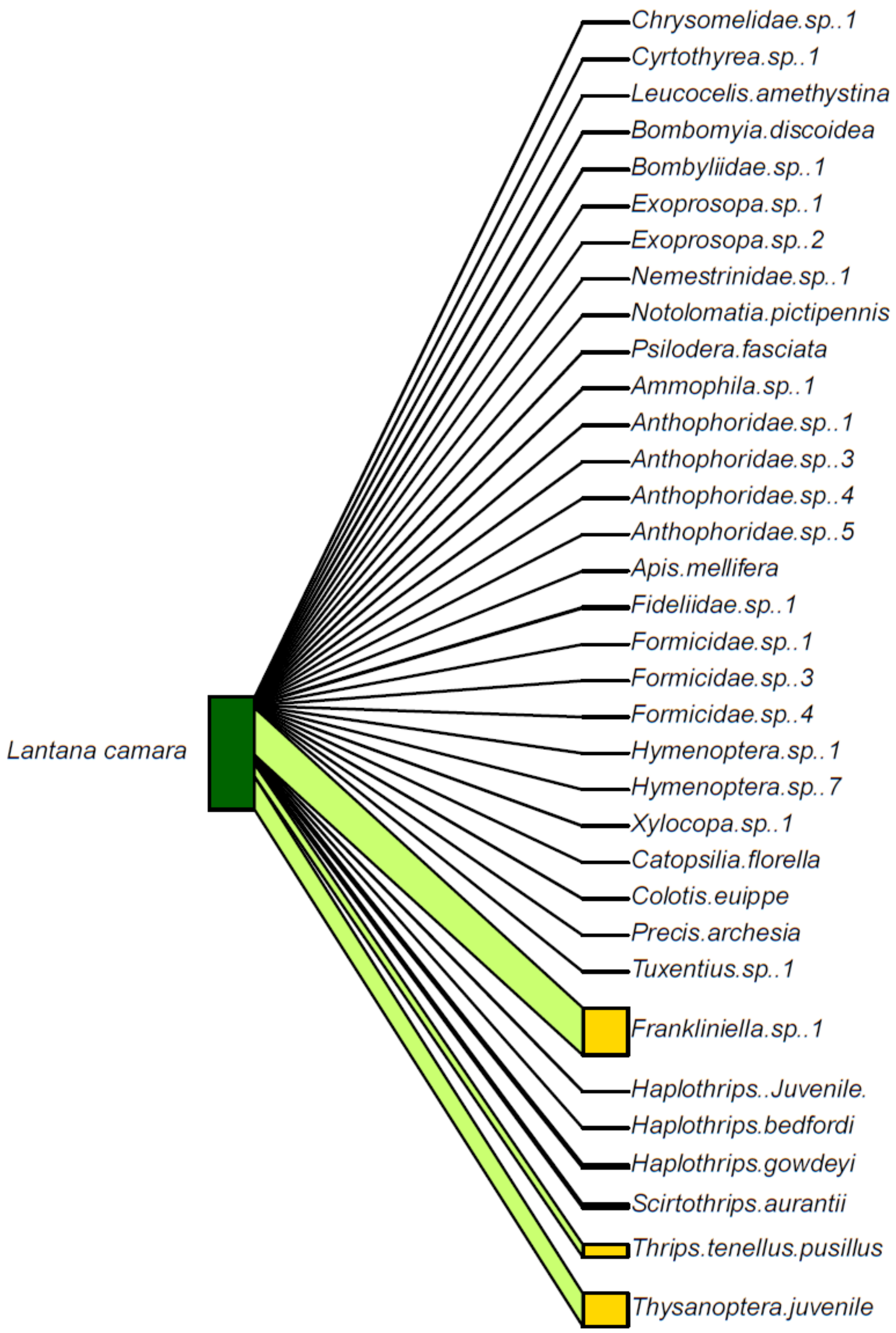

Figure A-3 Pollinating species sampled on *Lantana camara* inflorescences in spring (November) via three collection methods: in-person observations, opportunistic sampling, and inflorescence collections. The thickness of the bars represents the abundance of each species as a proportion of the total number of pollinators collected in the sampling season.

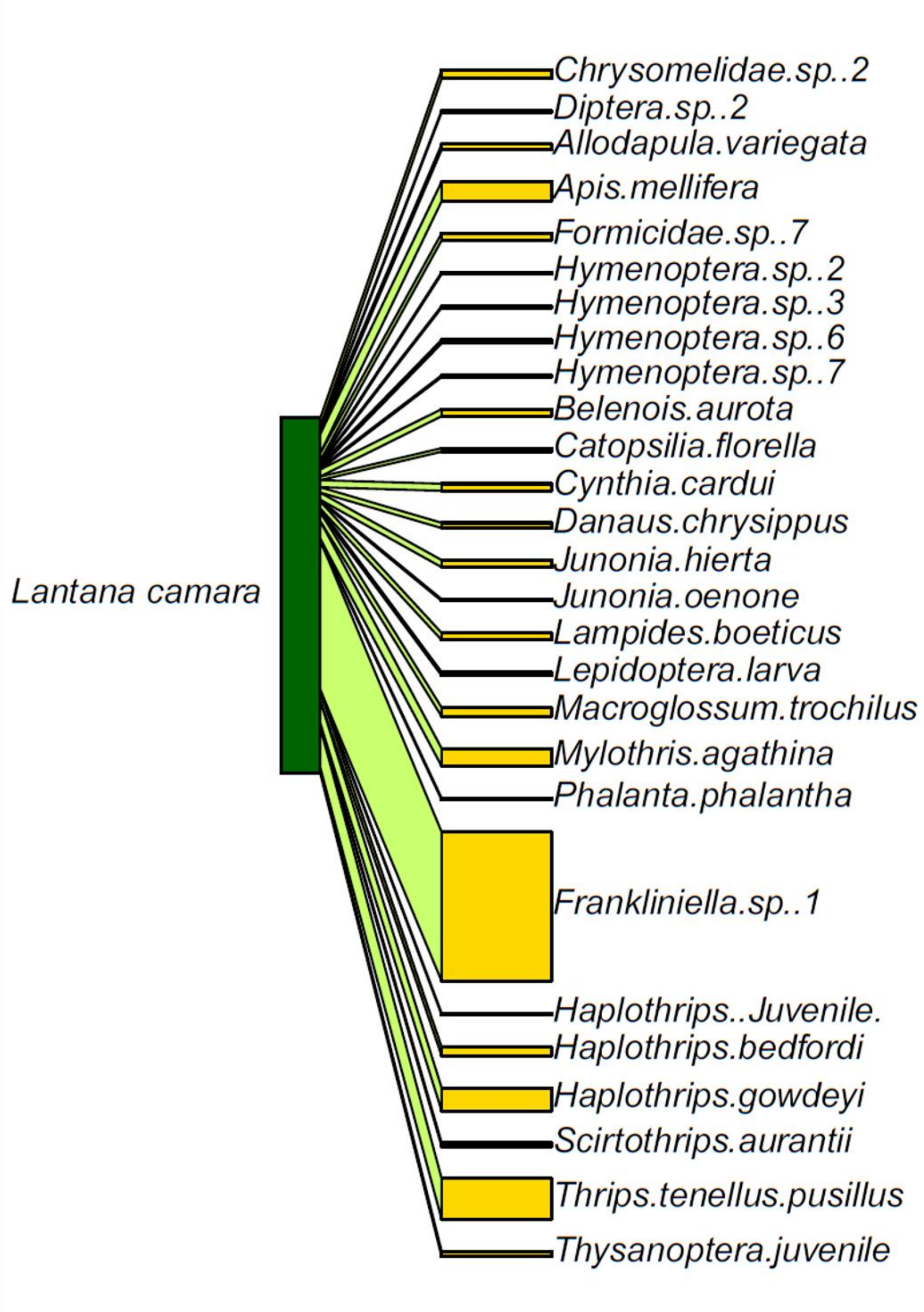

Figure A-4 Pollinating species sampled on *Lantana camara* inflorescences in winter (June) via three collection methods: in-person observations, opportunistic sampling, and inflorescence collections. The thickness of the bars represents the abundance of each species as a proportion of the total number of pollinators collected in the sampling season.

Table A-4 Non-pollinating arthropods sampled on *Lantana camara* inflorescences during the four observation seasons.

			Sampling time				
Order	Family	Scientific name	Summer	Autumn	Spring	Winter	Total
Araneae	Aranaeidae (Juvenile)	*Aranaeidae sp. 1*	-	2	-	-	2
	Cheiracanthiidae (Juvenile)	*Cheiracanthiidae sp. 1*	2	2	-	-	4
	Oxyopidae (Juvenile)	*Oxyopidae sp. 1*	-	5	-	-	5
	Salticidae (Juvenile)	*Salticidae sp. 1*	-	1	-	-	1
		Salticidae sp. 2	-	1	-	-	1
		Salticidae sp. 3	-	1	-	-	1
	Selenopidae (Juvenile)	*Selenopidae sp. 1*	-	1	-	-	1
	Theridiidae (Juvenile)	*Theridiidae sp. 1*	-	1	-	-	1
	Tomicidae (Juvenile)	*Parabomus sp. 1*	-	2	-	-	2
		Tomicidae sp. 1	-	6	1	1	8
		Tomicidae sp. 2	-	1	-	-	1
		Tomicidae sp. 3	-	3	-	-	3
		Tomicidae sp. 5	2	-	-	1	3
		Tomicidae sp. 6	-	2	-	-	2
		Tomicidae sp. 7	-	-	-	2	2
	Tomicidae (Male)	*Tomicidae sp. 4*	-	2	-	-	2
		Tomicidae sp. 6	1	-	-	-	1
Blattodea	Unidentified Blattodea	*Blattodea sp. 1*	-	1	-	-	1
Collembola	Unidentified Collembola	*Collembola sp. 1*	1	-	-	-	1
Hemiptera	Anthocoridae	*Anthocoridae sp. 1*	6	10	2	27	45
		Anthocoridae sp. 2	10	31	-	5	46
		Anthocoridae sp. 3	-	1	-	-	1
		Anthocoridae sp. 4	1	-	-	-	1
	Aphididae	*Aphus nerii*	1	2	1		4
		Aphus sp. 1	2	-	-	2	4
		Metopolophium dirhodum	6	-	-	-	6
		Sitobion sp. 1	1	-	-	-	1
	Cicadellidae	*Cicadellidae nymph*	1	1	-	-	2
		Cicadellidae sp. 1	-	1	-	-	1
		Cicadellidae sp. 2	-	1	-	-	1
	Flugoridae	*Flugoridae sp. 1*	-	1	-	-	1

			Sampling time				
Order	Family	Scientific name	Summer	Autumn	Spring	Winter	Total
	Hemiptera nymph	*Hemiptera nymph*	46	35	5	22	108
	Tingidae	*Teleonemia scrupulosa*	65	76	1	29	171
Trombidiformes	Trombidiformes	Trombidiformes	-	1	1	1	3
Unknown	Unknown	Unknown	3	2	-	-	5
Grand Total			148	193	11	90	442

Table A-5 Pollinating insects sampled on *Lantana camara* inflorescences during the four observation seasons.

			Sampling time					
Order	Family	Scientific name	Summer	Autumn	Spring	Winter	Not recorded	Total
Coleoptera	Chrysomelidae	*Chrysomelidae sp. 1*	19	27	4	-	-	50
		Chrysomelidae sp. 2	1	2	-	5	-	8
	Cleridae	*Cleridae sp. 1*	-	11	-	-	-	11
		Cleridae sp. 2	-	1	-	-	-	1
	Scarabaeidae	*Cyrtothyrea sp. 1*	-	-	2	-	-	2
		Leucocelis amethystina	-	-	1	-	-	1
		Leucocelis rubra	1	-	-	-	-	1
		Scarabaeidae sp. 1	1	-	-	-	-	1
	Trogidae	*Trogidae sp. 1*	1	-	-	-	-	1
	Trogossitidae	*Trogossitidae sp. 1*	1	-	-	-	-	1
Diptera	Acroceridae	*Psilodera fasciata*	4	10	3	-	-	17
	Bombyliidae	*Bombomyia discoidea*	1	1	4	-	-	6
		Bombyliidae sp. 1	1	1	5	-	-	7
		Bombyliidae sp. 2	1	-	-	-	-	1
		Bombyliidae sp. 3	-	3	-	-	-	3
		Exoprosopa sp. 1	-	-	1	-	-	1
		Exoprosopa sp. 2	-	-	1	-	-	1
		Notolomatia pictipennis	7	-	1	-	-	8
	Calliphoridae	*Calliphoridae sp. 1*	1	1	-	-	-	2
	Clussidae	*Clussidae sp. 1*	1	-	-	-	-	1
	Nemestrinidae	*Nemestrinidae sp. 1*	2	1	1	-	-	4
		Nemestrinidae sp. 2	1	-	-	-	-	1
	Rhiniidae	*Rhiniidae sp. 1*	-	1	-	-	-	1
		Rhiniidae sp. 2	2	1	-	-	-	3
		Rhiniidae sp. 3	1	-	-	-	-	1
	Sarcophagidae	*Sarcophagidae sp. 1*	3	8	-	-	-	11
	Sciaridae	*Sciaridae sp. 1*	-	1	-	-	-	1
	Syrphidae	*Asartina africana*	2	-	-	-	-	2
		Eristalinus taeniops	64	34	-	-	-	98
		Syrphidae sp. 1	1	2	-	-	-	3
		Syrphidae sp. 2	-	2	-	-	-	2
		Syrphidae sp. 3	-	1	-	-	-	1

			Sampling time					
Order	Family	Scientific name	Summer	Autumn	Spring	Winter	Not recorded	Total
	Tachinidae	*Tachinidae sp. 1*	1	-	-	-	-	1
		Tachinidae sp. 2	1	-	-	-	-	1
	Unidentified Diptera	*Diptera sp. 1*	1	3	-	-	-	4
		Diptera sp. 2	2	-	-	1	-	3
		Diptera sp. 3	-	1	-	-	-	1
Hymenoptera	Anthophoridae	*Allodapula variegata*	1	7	-	3	-	11
		Amegilla sp. 1	-	1	-	-	-	1
		Anthophoridae sp. 1	21	3	2	-	-	26
		Anthophoridae sp. 2	6	3	-	-	-	9
		Anthophoridae sp. 3	6	1	2	-	-	9
		Anthophoridae sp. 4	2	3	1	-	-	6
		Anthophoridae sp. 5	-	-	1	-	-	1
		Xylocopa sp. 1	16	11	2	-	-	29
	Apidae	*Apis mellifera*	86	38	1	11	-	136
	Fideliidae	*Fideliidae sp. 1*	11	4	5	-	-	20
	Formicidae	*Formicidae sp. 1*	6	10	1	-	-	17
		Formicidae sp. 2	5	2	-	-	-	7
		Formicidae sp. 3	5	11	1	-	-	17
		Formicidae sp. 4	4	7	1	-	-	12
		Formicidae sp. 5	1	12	-	-	-	13
		Formicidae sp. 6	1	7	-	-	-	8
		Formicidae sp. 7	-	-	-	4	-	4
	Halictidae	*Nomia amabilis*	3	1	-	-	-	4
	Sphecidae	*Ammophila sp. 1*	-	-	3	-	-	3
	Unidentified Hymenoptera	*Hymenoptera sp. 1*	10	9	1	-	-	20
		Hymenoptera sp. 2	1	-	-	1	-	2
		Hymenoptera sp. 3	-	-	-	1	-	1
		Hymenoptera sp. 4	-	1	-	-	-	1
		Hymenoptera sp. 5	-	1	-	-	-	1
		Hymenoptera sp. 6	-	-	-	2	-	2
		Hymenoptera sp. 7	-	-	1	1	1	3
	Vespidae	*Vespidae sp. 1*	1	-	-	-	-	1
Lepidoptera	Danainae	*Danaus chrysippus*	-	1	-	3	-	4

			Sampling time					
Order	Family	Scientific name	Summer	Autumn	Spring	Winter	Not recorded	Total
	Lepidoptera larva	*Lepidoptera larva*	1	8	1	2	-	12
	Lycaenidae	*Axiocerces tjoane*	-	1	-	-	-	1
		Azanus jesous	-	1	-	-	-	1
		Lampides boeticus	-	-	-	4	-	4
		Tuxentius melaena	-	1	-	-	-	1
		Tuxentius sp. 1	1	2	1	-	-	4
	Nymphalidae	*Byblia ilythia*	1	-	-	-	-	1
		Cynthia cardui	-	1	-	4	-	5
		Hypolimnas misippus	1	8	-	-	-	9
		Junonia archesia	1	-	-	-	-	1
		Junonia hierta	5	7	-	4	-	16
		Junonia oenone	-	1	-	1	-	2
		Phalanta phalantha	1	6	-	1	-	8
		Precis archesia	2	-	1	-	-	3
	Papilionidae	*Papilio demodocus*	1	2	-	-	-	3
	Pieridae	*Belenois aurota*	9	4	-	4	-	17
		Belenois java	1	-	-	-	-	1
		Catopsilia florella	7	31	1	2	-	41
		Colotis euippe	-	-	2	-	-	2
		Eurema brigitta	-	2	-	-	-	2
		Mylothris agathina	4	26	-	10	-	40
	Sphingidae	*Cephonodes hylas*	2	-	-	-	-	2
		Macroglossum trochilus	-	14	-	5	-	19
	Unidentified Lepidoptera	*Unidentified Lepidoptera*	-	5	-	-	-	5
							-	
Thysanoptera	Phlaeothripidae	*Haplothrips (Juvenile)*	11	12	3	1	-	27
		Haplothrips bedfordi	1	26	1	5	1	34
		Haplothrips gowdeyi	49	132	18	13	7	219
		Unidentified Phlaeothripidae	1	-	-	-	-	1
	Thripidae	*Frankliniella sp. 1*	432	151	218	84	2	887
		Scirtothrips aurantii	71	152	18	2	1	244
		Thrips tenellus/pusillus	138	114	59	23	-	334
	Juvenile Thysanoptera	*Juvenile Thysanoptera*	114	99	153	3	2	371
	Unidentified Thysanoptera	*Unidentified Thysanoptera*	12	7	-	2	-	20
Grand Total			1172	1058	521	202	14	2967

Table A-6 The numbers of honey bees (*Apis mellifera scutellata*) recorded on *Lantana camara* inflorescences, and the activity of honey bee colonies measured during seasonal pollinator observations.

Date	Honey bees recorded	Season	Observation 1	Observation 2	Additional observation	Daily Average
1-Mar-21	4	Autumn	334	251	-	292.5
5-Mar-21	8	Autumn	659	637	-	648
9-Mar-21	4	Autumn	671	586	-	628.5
10-Mar-21	1	Autumn	716	1028	-	872
11-Mar-21	0	Autumn	1040	739	-	889.5
12-Mar-21	1	Autumn	626	744	-	685
15-Mar-21	1	Autumn	598	451	-	524.5
16-Mar-21	1	Autumn	472	760	-	616
17-Mar-21	0	Autumn	-	331	-	331
18-Mar-21	1	Autumn	462	1264	-	863
19-Mar-21	1	Autumn	492	340	-	416
12-Nov-21	0	Spring	63	-	-	63
14-Nov-21	0	Spring	413	96	-	254.5
15-Nov-21	1	Spring	74	-	-	74
16-Nov-21	0	Spring	71	309	-	190
17-Nov-21	0	Spring	502	78	-	290
18-Nov-21	0	Spring	154	515	-	334.5
10-Jan-22	14	Summer	448	-	-	448
11-Jan-22	11	Summer	-	142	-	142
12-Jan-22	4	Summer	335	-	-	335
14-Jan-22	8	Summer	-	322	-	322
22-Jan-22	5	Summer	332	-	-	332
23-Jan-22	3	Summer	-	625	-	625
25-Jan-22	7	Summer	366	-	-	366
27-Jan-22	8	Summer	127	91	-	109
1-Feb-22	1	Summer	116	101	-	108.5
2-Feb-22	4	Summer	293	-	-	293
2-Jun-22	1	Winter	137	449	-	293
7-Jun-22	0	Winter	428	233	-	330.5
9-Jun-22	0	Winter	427	252	-	339.5
13-Jun-22	0	Winter	118	102	430	216.6666667
14-Jun-22	1	Winter	305	320	-	312.5

Table A-7 The model iterations that were conducted for the generalised linear mixed model selection procedure that was performed on the numbers of African honey bees that were observed on lantana inflorescences. The cells highlighted in green show the two most parsimonious models that were chosen for further analysis.

Response variable: Number of African honey bees										
	Explanatory variables				Random effects					
Model iterations	S	T	AD	AB	L	O	AICc	Fit	Overdispersion ratio	Overdispersion p-value
Full model	+	+	+	+	+	-	461.0410	Model is overfit	0.86556688	0.95643603
Reduced model 1	+	+	+	-	+	-	469.2716	-	0.86126710	0.96255970
Reduced model 2	+	-	+	-	+	-	467.1890	-	0.85799402	0.96631460
Reduced model 3	+	+	-	-	+	-	467.2142	-	0.85832940	0.96596733
Reduced model 4	-	+	+	-	+	-	522.5414	-	1.05622870	0.23728750
Reduced model 5	+	-	-	-	+	-	465.1459	-	0.85506020	0.96944900
Reduced model 6	-	+	-	-	+	-	520.7420	-	1.05193200	0.25324630
Reduced model 7	-	-	+	-	+	-	531.2965	-	1.07951700	0.15998600
Reduced model 8	+	+	-	+	+	-	459.3299	Model is overfit	0.86516005	0.95843386
Reduced model 9	+	-	+	+	+	-	458.9463	Model is overfit	0.86290234	0.95993470
Reduced model 10	+	-	-	+	+	-	457.2390	Model is overfit	0.81475060	0.96183441
Reduced model 11	-	+	+	+	+	-	474.8664	Model is overfit	0.92299140	0.82962080
Reduced model 12	-	-	+	+	+	-	479.6538	Model is overfit	0.94237520	0.75876200
Reduced model 13	-	+	-	+	+	-	473.6716	Model is overfit	0.92295250	0.83016450
Reduced model 14	-	-	-	+	+	-	478.8703	Model is overfit	0.94356020	0.75434790

Explanatory variables: Season (S); Temperature (T); Average distance to honey bee colonies (AD); Abundance of lantana inflorescences (AB). Random effect: Location (L); Observation (O). The ‘+’ represents the inclusion of an explanatory variable in the model iteration.

Table A-8 The model iterations that were conducted for the generalised linear mixed model selection procedure that was performed on the numbers of all pollinators that were observed on lantana inflorescences. The cells highlighted in green show the two most parsimonious models that were chosen for further analysis.

Response variable: All pollinators										
	Explanatory variables				Random effects					
Model iterations	S	T	AD	AB	L	O	AICc	Fit	Overdispersion ratio	Overdispersion p-value
Full model	+	+	+ *	+	+	+	1919.229	-	0.52394	1.00
Reduced model 1	+	+ *	+ *	-	+	+	1912.736	-	0.51863	1.00
Reduced model 2	+	-	+ *	-	+	+	1911.761	-	0.51312	1.00
Reduced model 3	+	+	-	-	+	+	1911.909	-	0.51461	1.00
Reduced model 4	-	+ *	+ *	-	+	+	1976.354	-	0.49489	1.00
Reduced model 5	+	-	-	-	+	+	1910.923	-	0.50926	1.00
Reduced model 6	-	+	-	-	+	+	1975.983	-	0.49205	1.00
Reduced model 7	-	-	+ *	-	+	+	2077.822	Model is overfit	0.46297	1.00
Reduced model 8	+	+ *	-	+	+	+	1918.378	-	0.51959	1.00
Reduced model 9	+	-	+ *	+	+	+	1918.308	-	0.51859	1.00
Reduced model 10	+	-	-	+	+	+	1917.421	-	0.51448	1.00
Reduced model 11	-	+ **	+ **	+	+	+	1975.744	-	0.49310	1.00
Reduced model 12	-	-	+	+	+	+	2073.546	Model is overfit	0.46169	1.00
Reduced model 13	-	+ *	-	+	+	+	1975.73	-	0.49037	1.00
Reduced model 14	-	-	-	+	+	+	2073.547	-	0.45913	1.00

Explanatory variables: Season (S); Temperature (T); Average distance to honey bee colonies (AD); Abundance of lantana inflorescences (AB). Random effect: Location (L); Observation (O). The '+' represents the inclusion of an explanatory variable in the model iteration. The '*' represents models for which the variable in question had to be rescaled from 0-20, and '**' form 0-10.

Table A-9 The model iterations that were conducted for the generalised linear mixed model selection procedure that was performed on the numbers of all pollinators that were observed on lantana inflorescences. The cells highlighted in green show the two most parsimonious models that were chosen for further analysis.

Response variable: Non-pollinating insects									
	Explanatory variables				Random effects				
Model iterations	S	T	AD	AB	L	O	AICc	Overdispersion ratio	Overdispersion p-value
Full model	+	+	+ *	+	+	+	993.8235	0.8031008	0.9950062
Reduced model 1	+	+	+ *	-	+	+	987.2308	0.7890029	0.9974225
Reduced model 2	+	-	+ *	-	+	+	985.3221	0.7862333	0.9977597
Reduced model 3	+	+	-	-	+	+	987.9023	0.7821993	0.9981409
Reduced model 4	+	-	-	-	+	+	985.9865	0.779489	0.998389
Reduced model 5	-	+	-	-	+	+	1021.924	0.7205854	1.00
Reduced model 6	-	-	+ *	-	+	+	1019.5	0.7237907	1.00
Reduced model 7	-	-	-	+	+	+	1019.948	0.7456244	1.00
Reduced model 8	+	-	-	+	+	+	991.8808	0.7936191	0.9967473
Reduced model 9	-	+	+ *	-	+	+	1021.558	0.7265893	1.00
Reduced model 10	-	+	+ *	+	+	+	1022.454	0.7533731	1.00
Reduced model 11	-	-	+ *	+	+	+	1020.353	0.751654	1.00
Reduced model 12	-	+	-	+	+	+	1022.033	0.7472782	1.00
Reduced model 13	+	+	-	+	+	+	993.9651	0.7963915	0.9962827
Reduced model 14	+	-	+ *	+	+	+	991.729	0.800301	0.9956118

Explanatory variables: Season (S); Temperature (T); Average distance to honey bee colonies (AD); Abundance of lantana inflorescences (AB). Random effect: Location (L); Observation (O). The '+' represents the inclusion of an explanatory variable in the model iteration. The '*' represents models for which the variable in question had to be rescaled from 0-20.

Table A-10 Crude calculations of the Shannon diversity index (H') of pollinators observed on inflorescences of *Lantana camara* from data published in two papers, Goulson and Derwent (2004) and Carrión-Tacuri et al. (2014).

Goulson and Derwent (2004)						
Order	Family	Species	Number of species	Proportion of total sample (p)	ln(p)	p.ln(p)
Hymenoptera	Apidae	*Apis mellifera*	171	0.6552	-0.4229	-0.2770
	Anthophoridae	*Amegilla* spp.	11	0.0421	-3.1666	-0.1335
Lepidoptera	Papilionidae	*Graphium macleayanum* (Leach)	1	0.0038	-5.5645	-0.0213
		Graphium sarpedon choredon (C. and R. Felder)	2	0.0077	-4.8714	-0.0373
		Papilio ulysses joesa Butler	3	0.0115	-4.4659	-0.0513
		Papilio aegeus aegeus Donovan	6	0.0230	-3.7728	-0.0867

Goulson and Derwent (2004)

Order	Family	Species	Number of species	Proportion of total sample (p)	ln(p)	p.ln(p)
		Ornithoptera priamus euphorion (Gray)	1	0.0038	-5.5645	-0.0213
		Cressida cressida cressida (Fabricius)	1	0.0038	-5.5645	-0.0213
	Nymphalidae	*Cethosia cydippe chrysippe* (Fabricius)	4	0.0153	-4.1782	-0.0640
		Hypolimnas bolina nerina (Fabricius)	1	0.0038	-5.5645	-0.0213
		Pantoporia consimilis consimilis (Boisduval)	2	0.0077	-4.8714	-0.0373
		Euploea sp.	1	0.0038	-5.5645	-0.0213
		Danaus hamatus hamatus (W.S. Macleay)	5	0.0192	-3.9551	-0.0758
		Danaus affinis affinis (Fabricius)	2	0.0077	-4.8714	-0.0373
		Danaus plexippus plexippus (L.)	2	0.0077	-4.8714	-0.0373
		Hypocysta adiante adiante (Hübner)	1	0.0038	-5.5645	-0.0213
		Junonia orithya albicincta Butler	1	0.0038	-5.5645	-0.0213
	Pieridae	*Eurema brigitta australis* (Wallace)	14	0.0536	-2.9255	-0.1569
		Delias mysis mysis (Fabricius)	5	0.0192	-3.9551	-0.0758
		Catopsilia pyranthe crokera (W.S. Macleay)	1	0.0038	-5.5645	-0.0213
		Appias paulina ega Boisduval	1	0.0038	-5.5645	-0.0213
		Elodina perdita perdita Miskin	2	0.0077	-4.8714	-0.0373
	Lycaenidae	*Hypochrysops digglesii* (Hewitson)	1	0.0038	-5.5645	-0.0213
		Lampides boeticus (L.)	3	0.0115	-4.4659	-0.0513
	Hesperiidae	*Pelopidas agna dingo* Evans	8	0.0307	-3.4851	-0.1068
		Notocrypta waigensis proserpina (Butler)	7	0.0268	-3.6186	-0.0971
		Parnara naso sida (Waterhouse)	1	0.0038	-5.5645	-0.0213
		Telicota mesoptis mesoptis Lower	2	0.0077	-4.8714	-0.0373
		Ocybadistes sp.	1	0.0038	-5.5645	-0.0213
		Total	**261**		**H'**	**1.6561**

Carrión-Tacurri *et al.* (2014)

Order	Family	Species	Number of species	Proportion of total sample (p)	ln(p)	p.ln(p)
Diptera	Syrphidae	Unidentified fly	1	0.0028	-5.8636	-0.0167
Hymenoptera	Apidae	*Xylocopa darwinii*	11	0.0313	-3.4657	-0.1083
Lepidoptera	Hesperiidae	*Urbanus galapagensis*	284	0.8068	-0.2147	-0.1732
	Pieridae	*Phoebis sennae marcellina*	29	0.0824	-2.4963	-0.2057
	Unidentified	Moth 1	13	0.0369	-3.2987	-0.1218
		Moth 2	6	0.0170	-4.0719	-0.0694
		Moth 3	1	0.0028	-5.8636	-0.0167
	Noctuidae	*Melipotis indomita*	4	0.0114	-4.4773	-0.0509
		Anticarsia gemmatalis	2	0.0057	-5.1705	-0.0294
	Crambidae	*Hymenia perspectalis*	1	0.0028	-5.8636	-0.0167
		Total	**352**		**H'**	**0.8086**

References

Carrión-Tacuri, J., R. Berjano, G. Guerrero, E. Figueroa, A. Tye, and J. M. Castillo. 2014. Fruit set and the diurnal pollinators of the invasive *Lantana camara* and the endemic Lantana peduncularis in the Galapagos Islands. Weed Biology and Management 14:209-219.

Goulson, D., and L. C. Derwent. 2004. Synergistic interactions between an exotic honeybee and an exotic weed: pollination of *Lantana camara* in Australia. Weed Research 44:195-202.

Appendix B

Table B-1 Numbers of *Lantana camara* inflorescences, *Aloe marlothii* racemes, and florets on the 10 test plants for each species that were presented to African honey bee colonies in preference experiments.

	04/08/2020		05/08/2020		07/08/2020	
Plant	Inflorescences / Racemes	Yellow / open florets	Inflorescences / Racemes	Yellow / open florets	Inflorescences / Racemes	Yellow / open florets
L1	13	48	-	-	-	-
L2	17	71	-	-	12	45
L3	11	47	-	-	9	20
L4	15	43	-	-	8	46
L5	8	28	-	-	7	42
L6	13	41	-	-	13	30
L7	11	37	-	-	-	-
L8	12	39	-	-	7	47
L9	10	49	-	-	5	26
L10	7	30	-	-	6	28
x	**11.7**	**43.3**	**-**	-	**8.375**	**35.5**
SD	**3.0203**	**12.0651**	**-**	-	**2.8252**	**10.6368**
A1	7	49	7	53	7	67
A2	3	31	3	27	3	38
A3	4	46	4	32	4	48
A4	6	32	6	25	6	74
A5	5	42	5	29	5	19
A6	3	31	3	31	3	35
A7	5	45	5	32	5	54
A8	9	122	8	94	7	228
A9	3	28	3	33	3	18
A10	3	29	3	30	3	31
x	**4.8**	**45.5**	**4.7**	**38.6**	**4.6**	**61.2**
SD	**2.0439**	**28.0049**	**1.8287**	**20.9188**	**1.6465**	**61.4903**

The symbol "-" represents data that were lost or not recorded. Standard deviation (SD) and mean (x) are shown.

Table B-2 Numbers of *Lantana camara* inflorescences, *Aloe greatheadii* racemes, and florets on the 10 test plants for each species that were presented to African honey bee colonies in preference experiments.

	14/07/2021		15/07/2021		16/07/2021		17/07/2021		18/07/2021	
Plant	Inflorescence / Raceme	Yellow / open florets	Inflorescence / Raceme	Yellow / open florets	Inflorescence / Raceme	Yellow / open florets	Inflorescence / Raceme	Yellow / open florets	Inflorescence / Raceme	Yellow / open florets
L1	11	51	11	-	11	55	11	52	10	33
L2	14	31	14	-	14	40	9	31	9	23
L3	14	70	14	-	16	79	15	80	14	55
L4	11	40	13	-	13	62	13	56	10	13
L5	14	47	13	-	14	51	13	34	9	13
L6	15	50	15	-	17	59	16	43	14	19
L7	27	56	20	-	21	63	15	64	9	31
L8	12	30	11	-	11	36	10	28	-	-
L9	8	31	8	-	9	38	8	22	-	-
L10	16	47	15	-	16	65	14	40	10	20
x	**14.2**	**45.3**	**13.4**	**-**	**14.2**	**54.8**	**12.4**	**45**	**10.75**	**32.75**
SD	**5.07280**	**12.7196**	**3.1692**	**-**	**3.4896**	**13.7258**	**2.7568**	**18.0123**	**2.1339**	**13.8712**
A1	5	25	5	-	5	28	5	25	5	11
A2	5	28	5	-	5	31	5	28	5	22
A3	4	17	4	-	4	18	4	18	-	-
A4	5	20	5	-	5	17	5	16	-	-
A5	5	28	5	-	5	28	5	27	-	-
A6	4	24	4	-	4	18	4	17	-	-
A7	6	37	6	-	6	38	6	34	6	24
A8	3	24	3	-	3	19	3	16	3	11
A9	6	31	6	-	6	32	6	30	6	20
A10	6	23	6	-	6	27	6	24	6	17
x	**4.9**	**25.7**	**4.9**	**-**	**4.9**	**25.6**	**4.9**	**23.5**	**5.2**	**18.8**
SD	**0.9944**	**5.6578**	**0.9944**	**-**	**0.9944**	**7.2295**	**0.9944**	**6.4334**	**1.1690**	**5.5407**

The symbol “-” represents data that were lost or not recorded. Standard deviation (SD) and mean (x) are shown.

Table B-3 Summary of the preference tests conducted showing the species “chosen” by honey bees (*Apis mellifera scutellata*) and the mean time it took to choose for each species.

	Number of tests	Honey bee choice	Mean time to choose, min (SD)
L. camara vs. *A. greatheadii*	87		4.29 (4.49)
A. greatheadii		50	3 (3.07)
L. camara		31	4.3 (4.06)
No choice		6	-
L. camara vs. *A. marlothii*	94		3.41 (3.76)
A. marlothii		62	2.41 (2.41)
L. camara		27	4.12 (3.61)
No choice		4	-
Same choice		1	0.27

Table B-4 Summary of the all the arthropod species recorded on *Kalanchoe rotundifolia* inflorescences in sites invaded by and sites cleared of *Lantana camara*. The table includes official and opportunistic observations

Site	Type of sampling	Order	Family	Species	Total
Invaded	Official	Hymenoptera	Anthoporidae	*Allodapula variegata*	3
			Formicidae	*Formicidae sp. 1*	1
		Lepidoptera	Pieridae	*Belenois aurota*	2
				Belenois java	1
				Eurema brigitta	1
				Mylothris agathina	6
	Opportunistic	Hymenoptera	Anthoporidae	*Allodapula variegata*	1
		Lepidoptera	Pieridae	*Belenois aurota*	6
				Catopsilia florella	2
				Eurema brigitta	1
				Mylothris agathina	5
Cleared	Official	Hemiptera	Aphididae	*Aphus sp.*	1
		Hymenoptera	Anthoporidae	*Allodapula variegata*	6
				Anthoporidae sp 1	1
		Lepidoptera	Pieridae	*Catopsilia florella*	1
				Mylothris agathina	2
	Opportunistic	Hymenoptera	Anthoporidae	*Allodapula variegata*	1
				Anthoporidae sp 1	1
		Lepidoptera	Pieridae	*Catopsilia florella*	4
				Mylothris agathina	3
Grand Total					49