

**THE BREEDING SYSTEM AND DEMOGRAPHY OF  
THE TRANSVAAL SESAME-BUSH,  
*SESAMOTHAMNUS LUGARDII* (PEDALIACEAE)**

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## ABSTRACT

The aim of this research was to assess the breeding system, pollination relationship and demography of the Transvaal Sesame-bush, *Sesamothamnus lugardii* N. E. Br. Ex Stapf. (Pedaliaceae). *S. lugardii* is an arid savanna succulent shrub which can be found throughout Zimbabwe, southern Botswana and in northern South Africa, where it is anecdotally thought to be rare. Two populations of *S. lugardii* were assessed in Limpopo, South Africa. The floral traits of *S. lugardii* suggest that it is specialised for pollination by long-tongued hawkmoths. The flowers bloom in the evening, are large, sweetly scented and pale in colour. The corolla tubes are very long (ca. 10 cm) and narrow. Very long-tongued hawkmoths (*Agrius convolvuli*) were found to be the only visitors capable of accessing the nectar at the base of the *S. lugardii* corolla tubes while foraging and simultaneously interacting with the reproductive structures, successfully pollinating the flowers. *S. lugardii* is an obligate outcrosser, dependent on *A. convolvuli* hawkmoths for sexual reproduction. The high-risk traits of the *S. lugardii* breeding system, namely pollinator specialisation and obligate out-crossing, could render *S. lugardii* vulnerable to extinction. Predation of flowers by scarab beetles in the study population at Mapungubwe National Park resulted in low fruit set. At Morongwa Private Safari Lodge florivory was negligible and fruit set was higher. In both study populations, *S. lugardii* was dominant in patches across the landscape, but only a small proportion of the numerous plants observed were seedlings. Little is known about the population dynamics of *S. lugardii*, but the absence of seedlings suggests a potentially vulnerable demography. The large shrubs were heavily impacted by meso- and mega-herbivores, but are resilient to herbivory, as they are capable of bark recovery and epicormic resprouting. However, the ever-increasing numbers of large herbivores at Mapungubwe could reach a critical threshold beyond which the populations of *S. lugardii* lose their resilience, putting them at risk of local extirpation. The number of seedlings in the populations at Mapungubwe decreased between an initial study conducted in 2005 and the recent assessment in 2014. This may be due to sporadic recruitment, but could also indicate a demographic bottleneck limiting seedling establishment. The low-risk traits associated with demography, namely resilience through longevity and vigorous resprouting, offset the high-risk traits by buffering the populations from decline during periods of reproductive failure. The environment in the study populations at present favours the continued survival of *S. lugardii*, despite the slow population turnover and low rate of reproductive success. Should the environmental conditions become less favourable for *S. lugardii*, then the extinction risk could greatly increase over a short period of time, and could even result in local extirpation.

## PREFACE

The research described in this thesis was carried out at the School of Life Sciences, University of KwaZulu-Natal (Pietermaritzburg), from March 2015 to September 2019. Data were collected in 2014 and 2015, and additional data from 2005 and 2008 were used, under the supervision of Professor Steven Johnson (University of KwaZulu-Natal), Professor Jeremy Midgley (University of Cape Town) and Dr Laurence Kruger (Organization for Tropical Studies).

The work presented in this thesis represents the original work of the author, and has not been otherwise submitted in any other form for any degree or diploma to any other university. Where use has been made of the work of others, this has been duly acknowledged in the text.

Signed:



Alison Bijl (Candidate)



Professor Steven D. Johnson (Supervisor)

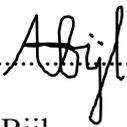


Professor Jeremy J. Midgley (Co-supervisor)

## DECLARATION 1 – PLAGIARISM

I, Alison Bijl, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.
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## DECLARATION 2 – PUBLICATIONS

Details of contribution to publications that form part of and/or include research presented in this thesis

### **Publication 1: Appendix B**

Johnson, S.D., Balducci, M.G., Bijl, A., Castañeda-Zárate, M., Cozien, R., Ortmann, C.R. and van der Niet, T. From dusk till dawn: camera traps reveal the diel patterns of flower-feeding by hawkmoths. *Ecological Entomology*. Accepted.

Authors' contributions: All authors contributed to data collection. SDJ performed analyses and the literature survey and wrote the manuscript with inputs from the other authors. All authors approved publication and agree to be accountable for all aspects of the work.

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# TABLE OF CONTENTS

<b>Chapter 1: Introduction</b>	<b>1</b>
Outline	1
Thesis aim	20
Chapter outlines	20
References	21
<b>Chapter 2: hawkmoth pollination and florivory in</b>	
<b><i>Sesamothamnus lugardii</i> (Pedaliaceae): are flowers hiding in the dark?</b>	<b>35</b>
Abstract	35
Introduction	36
Methods	46
Results	56
Discussion	64
Conclusion	70
References	72
Tables and figures	80

### **Chapter 3: The demography of *Sesamothamnus lugardii***

<b>(Pedaliaceae): the message in a bottleneck</b>	<b>96</b>
Abstract	96
Introduction	97
Methods	104
Results	109
Discussion	113
Conclusion	120
References	122
Tables and figures	129
<b>Chapter 4: Conclusion</b>	<b>141</b>
References	149

**Appendix A: the breeding system and demography of**

<b><i>Sesamothamnus guerichii</i>: a pilot study</b>	<b>150</b>
Abstract	150
Introduction	151
Methods	155
Results	159
Discussion	162
Conclusion	166
References	167
Tables and figures	168

**Appendix B: From dusk till dawn: camera traps reveal the diel patterns of**

<b>flower-feeding by hawkmoths</b>	<b>180</b>
Abstract	180
Introduction	182
Materials and methods	183
Results	185
Discussion	186
Acknowledgements	188
Supplementary information	189
References	190
Figures and tables	193

# CHAPTER 1

## Introduction

### OUTLINE

In this chapter, I briefly review the role of pollinator-mediated selection in driving floral trait convergence and discuss how these convergent floral traits have been grouped into pollination syndromes. I focus on hawkmoth pollination. The floral traits that define this syndrome, and the characteristics and behaviour of hawkmoths are described. The evolutionary processes that have led to the high degree of specialisation characteristic of this syndrome are summarised, and the prevalence of this syndrome globally and locally is explored. The benefits and vulnerabilities associated with pollination specialisation, as well as the compensation strategies associated with pollination specialisation are considered. I review approaches to determining the vulnerability of a plant species to extinction in the face of a pollination mutualism collapse, and describe a detailed approach that can be used to determine whether the populations of a given plant are at risk of local extirpation. I discuss how the life history strategy and demographic profile of a plant population can be useful in determining extirpation risk. I focus on savanna ecosystems, and review the ecosystem processes that affect plant demography, and particularly the disturbance processes that create demographic bottlenecks in woody plant populations. The life history traits and compensation strategies that buffer woody savanna plant populations from extirpation are also described. I describe the study species, *Sesamothamnus lugardii* and outline the thesis aims.

## **Plant trait convergence and pollination syndromes**

When plant species (even from diverse lineages) are exposed to similar environmental conditions, trait convergence may occur (Darwin, 1859; Cody & Diamond, 1975; Silvertown, *et al.*, 1993; Westoby & Wright, 2006). Pollinators are a key component of the “environmental conditions” to which plants adapt, because the available pollinators across the geographic range of biotically-pollinated plant species represent an ecological niche (Johnson, 2010; Johnson & Raguso, 2016). As a result of convergent adaptation to these ecological niches, many floral traits and combinations of floral traits are common across disparate plant taxa (Stebbins, 1970; Dodd, Silvertown & Chase, 1999; Barrett, 2002; Fenster, *et al.*, 2004; Harder & Johnson, 2009; Ollerton, *et al.*, 2009; Waser, Ollerton & Erhardt, 2011; Anderson, Wiese & Ellis, 2014; Newman, Manning & Anderson, 2014).

The pollination biologist, Federico Delpino (1868–1874), developed a scheme (his second), for grouping floral traits according to pollinator associations, establishing the first iteration of the pollination syndromes theory, and this work sparked much research and debate in the field (Waser, 2006). Since its time of inception in the late 1800s, the syndromes theory has been much adapted and advanced by the works of Van der Pijl (1961), Proctor and Yeo (1973), Proctor, Yeo and Lack (1996) and Faegri and Van der Pijl (1979). A pollination syndrome represents a suite of floral traits associated with a particular pollinator species or functional type. These floral traits include: the timing of floral anthesis, the presence and qualities of floral scent and nectar, as well as flower colour, size and morphology (Faegri & Van der Pijl, 1979). A functional group of pollinators may comprise a variety of different species with features in common such as the shape of their mouthparts or their mode of feeding.

Even though detail is lost in the grouping of diverse plant-pollinator relationships into syndromes, it is useful to have this theoretical framework. Watt (1971) said that without such groupings and simplification, ecologists would be “washed out to sea in an immense tide of unrelated information”, just as an inorganic chemist might be without the periodic table. The pollination syndromes theory helps biologists apply structure to the incredible diversity in floral form and function (Whittall & Hodges, 2007; Johnson

& Wester, 2017).

Although pollinator-mediated selection can result in highly specialised plant-pollinator relationships (Kearns, Inouye & Waser, 1998), this is not the general rule, as there are varying degrees of specialisation, and there is also widespread generalisation in pollination systems (Waser, *et al.*, 1996; Johnson & Steiner, 2000; Fenster, *et al.*, 2004; Valdivia & Niemeyer, 2006; Ollerton, *et al.*, 2009). One must bear in mind that the generalisation-specialisation dichotomy applied to plant-pollinator relationships is the simplification of a continuum (Johnson & Steiner, 2000), and like the syndromes theory, is a useful and simplified grouping framework for organising interactions.

### **Hawkmoth traits and the hawkmoth pollination syndrome**

In pollination-specialised flowers, the floral traits and pollinator traits often match physically or mechanically. Flowers with very long, narrow corolla tubes, for example, are suited for pollination by long-tongued functional groups such as hawkmoths (Nilsson, 1998; Anderson & Johnson, 2008; Muchhala & Thomson, 2009). A textbook example of floral specialisation to long-tongued pollinators is seen in the Malagasy star orchid (*Angraecum sesquipedale*) with its incredibly long spurs and one of its main pollinators, the Giant hawkmoth (*Xanthopan morgani praedicta*) which has a proboscis length that matches the length of the floral spurs (Darwin, 1862; Nilsson, 1987). In southern Africa, within the hawkmoth pollination syndrome, a distinct pollination guild of very long-tongued hawkmoths has been identified (Martins & Johnson, 2007; Johnson, 2010; Johnson & Raguso, 2016), and is represented by a single species, *Agrius convolvuli*, which has a proboscis length range of 90 mm to 110 mm. *A. convolvuli* hawkmoths are the exclusive pollinators of several plant species with flower corolla tubes or spurs longer than 10 cm, as these flowers mechanically exclude all shorter-tongued hawkmoths (Johnson & Raguso, 2016).

In pollination-specialised flowers, the floral traits and pollinator traits can also match temporally. Hawkmoths are typically most active at dusk (Luyt & Johnson, 2001; Micheneau, Johnson & Fay, 2009) and at dawn (Braun, *et al.*, 2012; Martins & Johnson, 2013), but recent studies have found that some hawkmoth species like *A. convolvuli* are

active throughout the night (Johnson & Raguso, 2016). Evening floral anthesis and scent emission in hawkmoth-pollinated flowers is common, and the timing often coincides with peak hawkmoth foraging activity (Loughrin, *et al.*, 1991; Raguso, *et al.*, 2003; Dötterl, Wolf & Jürgens, 2005; Balao, *et al.*, 2011).

Floral traits may also be energetically matched to their specialist pollinators. A common characteristic of hawkmoth-pollinated flowers is abundant nectar (Baker, 1961; Van der Pijl, 1961; Faegri & Van der Pijl, 1979; Grant & Grant, 1983; Haber & Frankie, 1989; Cota, 1993; Martins & Johnson, 2013). Hawkmoths feed in flight by hovering over flowers, which is energetically expensive (Micheneau, Johnson & Fay, 2009), so hawkmoths would be most attracted to flowers that secrete large volumes of sucrose-rich nectar to meet their energetic requirements.

### **Evolutionary mechanisms that lead to specialisation**

Within the hawkmoth pollination syndrome are some impressive examples of flower and pollinator trait-matches, well exemplified in the matching lengths of the Malagasy star orchid's spur and the proboscis of its pollinator, the Giant hawkmoth (Darwin, 1862). Although it is widely accepted that matches of this nature are due to pollinator-mediated floral specialisation, there is some debate about the underlying mechanisms that led to this high degree of specialisation (Arditti, *et al.*, 2012). Darwin (1862) postulated that the close match between the length of floral tubes and hawkmoth mouthparts could come about through a coevolutionary race: flowers with corolla tubes longer than their hawkmoth pollinators' tongues would force the hawkmoths to probe the tubes more deeply to reach nectar, maximising pollen transfer. Thus, selection would favour longer corolla tubes. Simultaneously, hawkmoths with tongues longer than the flower corolla tubes would have better access to nectar, so selection would favour moths with longer tongues. Darwin (1862) proposed that the increase in the length of pollinators mouthparts was facilitated by general size increases (natural selection in relation to their general conditions of life). Moth size increases could also have occurred in response to increased nectar foraging efficiency (Nilsson, 1998).

The coevolutionary model described by Darwin (1862) is one of several mechanisms by

which floral specialisation takes place (Anderson & Johnson, 2008; Johnson, 2010). Darwin's coevolutionary race (1862) describes a symmetrical relationship, but evolutionary relationships that result in specialisation in flowers can also be asymmetrical. This may occur when plants colonise new environments and then the floral traits adapt to the pre-existing traits of the available pollinator fauna, without the pollinators necessarily adapting (Whittall & Hodges, 2007). Alfred Wallace, cited by Muchhala and Thomson (2009), suggested that evolution can be one-sided at first and can then become coevolutionary, *i.e.*, a plant may adapt to a new pollinator, and when it reaches a point where the flower tube length exceeds the length of the longest-tongued pollinator in the community, the coevolutionary race begins. In the adaptive shift model described by Wasserthal (1993; 1997) the long tongues of hawkmoths are predicted to have evolved as an adaptation for predator avoidance. Jermy (1999) supported this hypothesis. Flowers with long tubes would be pollinated by existing, long-tongued hawkmoths, which would, through selective pressure, drive an increase in flower tube length to the point where the tube length exceeds the reach of that pollinator. At this point, the flower could shift to an existing, but longer-tongued hawkmoth pollinator. The extreme length of flower tubes would thus have evolved through a series of pollinator shifts (Wasserthal, 1993).

### **Prevalence of the hawkmoth pollination syndrome**

The hawkmoth pollination syndrome has been extensively studied, particularly in tropical and, more recently, subtropical systems. Grant and Grant (1979) observed hawkmoth pollination in cacti in tropical and subtropical central and south America, within the genera *Echinopsis*, *Hylocereus* and *Selenicereus*, and Grant (1985) recorded 106 native sphingophilous (hawkmoth-pollinated) plant species in the temperate North American flora. Haber and Frankie (1989) confirmed that hawkmoths are a major pollinator group in Costa Rican lowland dry forests, pollinating 10 % of tree species and various shrubs, herbs, lianas and epiphytes. In central and southern Africa, recent studies have recorded widespread hawkmoth pollination. Martins and Johnson (2013) recorded hawkmoth pollination in 25 plant species in Kenya, across numerous plant families. In South Africa hawkmoth pollination has been observed in numerous plant

species which include *Mystacidium venosum* in Kwa-Zulu Natal (Luyt & Johnson, 2001), *Bonatea speciosa* in the coastal forests of the southern Cape (Johnson & Liltved, 1997) and in *Habenaria epipactidea* in the Eastern Cape (Peter, *et al.*, 2009). In the eastern region of South Africa, long-tongued hawkmoths have been recorded pollinating plant species across multiple families, including Amaryllidaceae, Iridaceae, Capparaceae and Rubiaceae (Alexandersson & Johnson, 2002; Johnson, 2010). In southern Africa the guild of long-tongued hawkmoth pollinators is comprised of a single species, *Agrius convolvuli* (Martins & Johnson, 2007; Johnson, 2010; Johnson & Raguso, 2016).

### **Benefits and vulnerabilities associated with pollination specialisation**

For plants, a high degree of specialisation has its advantages, for example, hawkmoths are long-lived and are strong fliers, so are very effective pollen vectors in widely dispersed plant populations (Martins & Johnson, 2007; 2009; 2013). Hawkmoths also have the capacity to retain information while feeding (Kulbaba & Worley, 2012; Martins & Johnson, 2013), so they can learn to select rewarding flowers and remember rewarding species (this ensures pollinator fidelity) and can avoid recently visited flowers (Kulbaba & Worley, 2012), which is advantageous for self-incompatible plant species.

Being highly specialised for pollination by any one functional group or species may carry some risk too though (particularly if the plant species is pollinator-dependent and self-incompatible), because if the pollinator is lost from the system, the plant population can experience pollen limitation and reproductive failure (Bond, 1994; Kearns, Inouye & Waser, 1998; Waser & Price, 1998). In the Bahamas in 1996, hurricane Lili caused a severe decline of two key bird pollinators (the Bananaquits (*Coereba flaveola*) and Bahama Woodstars (*Calliphlox evelynae*)) and thereafter, the endemic shrub, *Pavonia bahamensis* (Malvaceae), suffered an 89 % loss in fruit set due to pollination limitation (Rathcke, 2000). On the North Island of New Zealand, the anthropogenic introduction of predators resulted in the extirpation of two major bird pollinators (the Bellbird (*Anthornis melanura*) and Stitchbird (*Notiomystis cincta*)), and due to the ensuing pollination failure, the endemic forest-understorey shrub, *Rhabdothamnus solandri*

(Gesneriaceae), experienced declines in seed production and population density (Anderson *et al.*, 2011). In the Cape Floristic Region of South Africa, Pauw (2007) found that in the absence of an oil-collecting bee (*Rediviva peringueyi*), six bee-specialised plant species failed to set seed. Two hawkmoth-pollinated *Brighamia* species in Hawaii had extremely low seed set and limited recruitment in fragmented island populations where their pollinators are suspected to have gone extinct (Gemmil *et al.*, 1998). In a small population of *Oxyanthus pyriformis* subsp. *pyriformis* in South Africa, when hawkmoths were absent, extremely low seed set and limited recruitment was also observed (Johnson *et al.*, 2004).

### **Vulnerability and risk compensation**

Evidence confirms that pollination specialisation can render plants vulnerable to reproductive failure in the absence of their pollinators (Larson & Barrett, 2000). Yet, in spite of the clear link between pollination failure and reproductive failure, few studies globally have been able to confirm that the absence of pollinators leads to plant extinction (Dunn, *et al.*, 2009; Pauw & Bond, 2011). This is because most extant angiosperms have breeding system compensation strategies or demographic compensation strategies that allow them to persist through periods of reproductive failure (Bond, 1994). Self-compatibility in plants is one such compensation trait. Self-compatible plants have mechanisms that ensure fertilisation with own pollen, thus allowing plants to escape the dependence on pollen vectors for seed set. Self-compatibility provides reproductive assurance in situations where pollinator availability is not guaranteed (Bond, 1994). Self-compatibility is seen across many taxonomic groups, often associated with isolated, peripheral environments (Busch, 2005).

Asexual reproduction also protects plant populations from reproductive failure (Bond, 1994) and can be viewed as a compensatory strategy, though it did not evolve exclusively for the purpose of compensating for pollination failure. Asexual reproduction (vegetative reproduction) is a widespread trait across angiosperms and is often associated with plant survival in disturbance-driven ecosystems.

## Predicting vulnerability

Bond (1994) developed a method for determining the vulnerability of a plant species to extinction in the face of a pollination mutualism collapse, using a variety of plant traits. Breeding system traits, demographic traits and dispersal traits are ranked from zero to one, with one representing the greatest vulnerability, and zero, the lowest (Bond, 1994). The high-risk traits (approaching one) include pollination specialisation, self-incompatibility, seed dependence (obligate seeding) and seed dispersal specialisation and the low-risk traits (approaching zero) include pollination generalisation, self-compatibility, an escape from seed dependence and no dispersal specialisation (Bond, 1994). The traits can be plotted against one another on a set of axes (Figure 1-1) and the plant species which exhibit both high-risk breeding system traits and high-risk demographic traits approach an overall vulnerability index value of one are most vulnerable to extinction in the face of pollination mutualism collapse (Figure 1-1, Box A) (Bond, 1994).

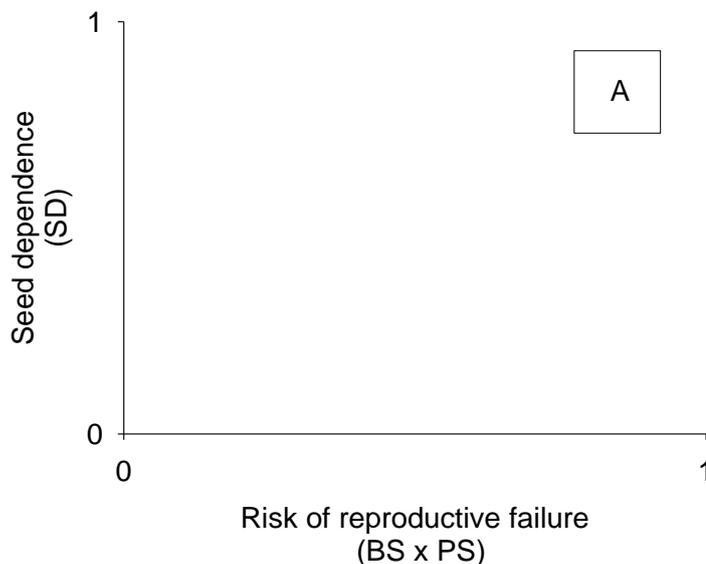


Figure 1-1: Extinction vulnerability plot adapted from Bond (1994), with demographic vulnerability (Seed dependence) plotted against breeding system vulnerability (Risk of reproductive failure). SD = seed dependence, BS = breeding system, PS = pollinator specificity. Plants with high risk breeding system traits and high-risk demographic traits are vulnerable to extinction in the face of a pollination mutualisms failure and fall into zone A.

Bond's (1994) vulnerability index is useful for determining the risk of a plant species to extinction, if its pollination and dispersal mutualisms collapse, but even a plant species with low-risk traits may still be at a high risk of extirpation within a particular environment. Habitat fragmentation, for example, has been found to threaten the survival of populations of generalised and specialised plant species similarly (Aizen, Ashworth & Galetto, 2002).

In addition to plant traits, environmental variables can strongly affect a plant population and determine its vulnerability to extirpation. Due to natural environmental fluctuations between seasons and across populations, the vulnerability score can change within a single population over time, and will also differ between spatially distinct populations. Although one can determine at any given time if a single plant population is experiencing reproductive failure and recruitment failure, it is much more challenging to confirm that the population is at risk of extirpation as a consequence, due to temporal variation in environmental conditions. A research project that tracks recruitment in one population over several seasons provides snapshot views of the population which can vary dramatically, because the pollinator population may naturally go through fluctuations, and so levels of reproductive success will differ seasonally. When assessing risk of extirpation, the spatial scale is also relevant, because across two distinct populations of a plant species, the pollinator diversity and pollinator abundance can vary, so the reproductive success and recruitment rates will differ.

The above example only takes into account the effect of pollinators on recruitment, but other environmental factors also determine vulnerability to extirpation: top-down factors such as antagonists (*e.g.* herbivores) and disturbance regimes (*e.g.* fire) as well as bottom up factors (*e.g.* water availability) affect recruitment rate, and also mortality rate in a population. All the environmental factors shape the demographic profile of the population need to be factored into a population-level risk assessment.

## **Predicting vulnerability with population-level demographic profiles**

As species extinction risk is very challenging to determine, it is more practical to determine the vulnerability of plant populations, and use an approach which can account for spatial and temporal fluctuations. In a population risk assessment, one should begin by exploring the breeding system traits and demographic traits of the plant species in question, to identify potential high-risk traits and determine if there are compensatory low-risk traits (Bond, 1994). Thereafter, the demographic profile of the population should be assessed, and the environmental processes that negatively alter the demographic profile of that population must be explored. The demography of a plant population refers to the fluctuations in population density and structure across space and over time (Jakob, *et al.*, 2010). The demographic profile of a population is shaped by processes that facilitate or limit recruitment (the production of genets), size-class transitions (increases or decreases in biomass) and mortality (Midgley, Lawes & Chamailí-Jammes, 2010).

Demographic bottlenecks refer to processes and events that kill or prevent reproduction in a large proportion of individuals in a population. Bottlenecks include processes that limit flower production and survival (*e.g.* florivory), processes that negatively impact pollinator populations and limit pollination (*e.g.* habitat fragmentation), processes that limit seed development, dispersal and recruitment (*e.g.* granivory), and processes that limit plant size-class transitions, from non-reproductive stages into reproductive stages (*e.g.* severe drought, fire and herbivory).

When demographic bottlenecks change the demographic profile of a population, the risk of extirpation can increase. When a disturbance event causes the mortality of a large number of established plants in a population of slow-growing trees, for example, an entire cohort will be lost from the demographic profile. Not only is the population more vulnerable to extirpation because it is smaller, but also because the reproductive potential that was stored in those lost trees (Higgins, Pickett & Bond, 2000) could take decades to replace, and prior to their replacement recruitment capacity is diminished.

It is important to note that even if bottlenecks do *not* alter the demographic profile of a given population, they may still increase its vulnerability to extirpation, by rendering the existing plants functionally obsolete (no longer productive members of their populations, *e.g.* the trees in a fragmented patch of forest that are isolated from pollinators and unable to set seed) (Janzen, 2001). The collapse of biotic interactions such as those between plants and their pollinators may occur long before one sees a change in the demographic profile of the plant populations.

Another important point to consider is that the demographic profiles of plant populations with different life history strategies will differ greatly under the same disturbance regimes. In a savanna ecosystem, for example, the typical demographic profile of a population of woody perennial plants that resprout after a fire, will look very different to the typical demographic profile of a population of non-sprouting (obligate seeding) annual plants which store fire-resistant seeds in seedbanks. In order to determine if environmental processes have negatively altered the demographic profile of a population, therefore, one must have some benchmark for what constitutes a “typical” demographic profile, and this will be determined by the life history strategy of the species (*e.g.* if it is resprouting or reseeded, perennial or annual), and the ecosystem in which it occurs (*e.g.* fynbos, savanna, grassland, *etc.*).

### **Plant life history strategies and demographic profiles**

The life history strategy of a plant species can shape the demographic profile of a population. Goldberg and Turner (1986) found that the population profiles of slow-growing, long-lived species typically showed erratic recruitment, low population density and age structures skewed towards larger, older plants. The population profiles of fast-growing plants with short life-spans, on the other hand, typically showed regular recruitment, high population density and had age/size structures skewed towards the youngest cohorts. The environment can also shape the demographic profile of a population. In many forest ecosystems, and in places where disturbances that compromise recruitment are limited, self-replacement rate in the plant populations is typically high, so the largest number of plants are often in the smallest size classes (McCarthy & Weetman, 2006; Wang, *et al.*, 2009; Giroldo & Scariot, 2015). The size-

class distribution will have a “classic reverse-J shape” (Lykke, 1998). In savanna ecosystems, where there are fluctuations in the frequency and intensity of ecosystem controlling variables such as fire, herbivory and rainfall, there are often also fluctuations in recruitment and mortality rates in plant populations, resulting in populations without the classic, reverse-J-shaped distributions, (Hall & Bawa, 1993; Lykke, 1998).

By assessing the demographic profile of a population at a given moment in time, and by assessing the change in population demography over time, one can gain insight into the effects of past perturbations (Shackleton, 1993), *i.e.*, see the effects of demographic bottlenecks and explore recruitment trends, determine if and where the population stores reproductive potential and get a clearer sense of whether the population is at risk of extirpation in its current environmental conditions. The same information can be used to make predictions about how the population will respond to changing environmental conditions, so thresholds of potential concern can be decided.

### **Demographic bottlenecks in savanna systems**

Many savanna plant species have life history traits that compensate for reproductive failure, because frequent and intense disturbance processes in these systems impose demographic bottlenecks that severely limit plant recruitment and survival (Midgley & Bond, 2001). Midgley and Bond (2001) identified several potential processes that cause bottlenecks in populations of African *Acacias* (woody perennials) in savanna ecosystems, and these include: 1) seed set limitation due to pollinator failure in outcrossing individuals, 2) seed limitation due to seed predation post-dispersal, 3) seed dispersal limitation in zoochorous species, 4) germination limitation due to lack of appropriate germination cues and 5) establishment limitation due to disturbance or climatic variables. These demographic bottlenecks may be present in other woody savanna species too, making this list a useful point of reference for demographic studies of woody savanna plant species.

The following section deals with establishment limitation due to disturbance or climatic variables, and germination limitation due to lack of appropriate germination cues.

### ***Fire***

Fires in savanna systems typically occur frequently and are of relatively low intensity, due to the long annual dry seasons combined with the rapid accumulation of highly flammable grassy biomass (Archibald, *et al.*, 2009). Frequent fires can keep resprouting savanna trees in reduced stages and thereby prevent their recruitment into larger, reproductive size-classes (Hoffmann, Orthen & Nascimento, 2003; Hoffman, *et al.*, 2009). Frequent, low-intensity fires more often limit recruitment than cause the mortality of established plants (Gignoux, Clobert & Menaut, 1997). Seedlings, however, can be easily killed by low-intensity fires, because they lack thick bark and typically cannot resprout (Midgley, Lawes & Chamailí-Jammes, 2010).

### ***Herbivory***

Herbivores can strongly limit plant growth, reproduction and survival (Maron & Crone, 2006) and thus dramatically impact woody plant demography (Barnes 1983; Sankaran, Augustine & Ratnam, 2013). In woody savanna species, browsing, like low intensity fires, most significantly affects size-class transitions, and less commonly causes mortality (Sankaran, Augustine & Ratnam, 2013). Seedlings, however, are also very vulnerable to mortality in the face of herbivory by small browser species (which consume more seedlings than larger browsing species (Augustine & McNaughton 2004; Moe, *et al.*, 2009; Midgley, Lawes & Chamailí-Jammes, 2010) and trampling by larger herbivores (Cumming & Cumming, 2003; Thompson Hobbs & Searle, 2005). Large herbivores such as the Common eland (*Taurotragus oryx*), Greater kudu (*Tragelaphus strepsiceros*) and African elephant (*Loxodonta africana*) prevent recruitment of small trees into larger size classes, by breaking off leaves and branches and stripping bark from the stems (Jacobs & Biggs, 2002). Elephants also push trees over, sometimes causing their mortality (Jacobs & Biggs, 2002; Scholes, Bond & Eckhardt, 2003).

## ***Florivory***

Florivory is a type of herbivory that is associated specifically with damage done to plant reproductive structures, and can affect plant fitness and population demography directly (where flower damage results in the loss of viable gametes), and indirectly (where flower damage renders the plant unattractive to pollinators) (Schemske & Horvitz, 1984; Krupnick, Weis & Campbell, 1999; McCall & Irwin, 2006; Cardel & Koptur, 2010). In Nebraska, an inflorescence-eating insect limited seed production, seedling establishment, plant density and maternal fitness in a population of the thistle, *Cirsium canescens* (Louda & Potvin, 1995).

Florivory can have serious negative impacts on populations of annual, reseeding plants, where the continued survival of populations depend on successful flower production seed survival each growing season (McCall & Irwin, 2006). If the seed set is diminished in one season, the population density will decrease, and the longevity of that population will be threatened. In small, sparse populations of annual or perennial plants, where flower density is low, florivory can also seriously limit plant reproductive success by lowering the number of flowers available for pollination and thus lowering seed set. Fewer available flowers in small populations can also lead to increased rates of self-pollination. Large plant populations may also be negatively impacted by florivory, because the high availability of food resources can support larger florivore populations, and the larger the florivore populations are, the more robust they are to stochastic extinction (Piessens, *et al.*, 2009). In addition, florivore population densities can increase exponentially with increased food resources, intensifying their impact in large plant populations (Colling & Matthies, 2004).

## ***Water***

During periods of increased water availability (high seasonal rainfall), germination rates increase (Gutiérrez & Meserve, 2003; Shannon, *et al.*, 2009) and so does net primary productivity. The abundance of plants in the population thus increases and their transitions into larger size classes is accelerated (Holmgren, *et al.*, 2006; Shannon, *et al.*, 2009). Droughts can therefore strongly limit germination and seedling survival

(Gerhardt, 1993; Bengtsson-Sjörs, 2006) as well as size-class transitions.

### ***Drought and herbivory***

Although periods of abundant water availability are good for plant productivity, they can have a negative impact on plant demography by altering herbivore spatial distributions and increasing browsing intensity across the landscape. Around water points where herbivore densities are high, increased trampling and extensive foraging on surrounding vegetation limits plant recruitment and growth rates (Owen-Smith, 1996, Shannon, *et al.*, 2009). Periods of drought can also alter herbivore utilisation and impact plant populations: during periods of water stress, herbivores increase their consumption of moist plant material to avoid dehydration (McCluney & Sabo, 2009; Shannon, *et al.*, 2009). As water stress intensifies, browsing and grazing intensifies (limiting plant recruitment and even causing mortality in established plants) until the herbivores migrate or die off.

### **Savanna plant life history strategies associated with disturbance**

The life history strategies of many perennial, woody savanna plant species are convergent, and reflect the necessary adaptations that have evolved to allow these plants deal with disturbance-induced damage and avoid mortality (Carpenter & Recher, 1979).

#### **Adaptations for fire and herbivory**

The traits common to many perennial, savanna plant species include low seed output, low seedling establishment rates and slow growth rates (in comparison to annual plants which typically have large seed outputs and fast growth rates) (Bond & Midgley, 2003). Turnover in perennial savanna species with these “low and slow” traits can take many years, as the populations have long generation times, resulting in damped demographic trends (no reverse-J demographic curves).

Low seed output and slow growth are inherently high-risk traits (Bond, 1994) and if savanna plant species with these traits were also dependent on seeds for propagation (non-resprouting), they would be at a high risk of extinction. However, most woody

savanna plants can compensate can resprout basally or epicormically (Midgley & Bond, 2001), and this inherently low-risk demographic trait or “compensatory trait” allows them an escape from dependence on seeds for propagation (Gignoux, Clobert & Menaut, 1997; Nefabas & Gambiza, 2007), and lowers extinction risk (Bond, 1994). Additional low-risk life history traits that ensure the survival of many woody savanna perennials in the face of fire and herbivory are thick bark (Gignoux, Clobert & Menaut, 1997; Nefabas & Gambiza, 2007) and the capacity to regrow bark.

#### Adaptations for florivory

Some life history strategies allow plants to avoid florivory, others allow plants to deter florivores, and others to tolerate them. Some plant species escape florivory through temporal variation in scent release, where floral anthesis and odour-signalling activities coincide with pollinator activity, but not antagonist activity (Balao, *et al.*, 2011). Other plant species have deceptive pollination systems, where they mimic mating partners or oviposition sites without offering any rewards (Kessler, *et al.*, 2015). These plant species can trick pollinators visually and thus avoid attracting antagonists with olfactory cues.

Some plants may escape florivory by being short-lived. The relationship between floral longevity and the impact of florivory has not been well studied, but Teixido, Méndez and Valladares (2011) recorded increased florivory by the larva of the moth *Cleonymia yvanii* (Noctuidae), on longer-lived flowers of the Mediterranean shrub, *Cistus ladanifer* (Cistaceae). McCall and Irwin (2006) postulate that increased flower longevity is linked to an increased likelihood of discovery by antagonists. However, longer-lived flowers have been found to experience more pollen removal, higher volumes and quality of pollen deposition and sometimes increased reproductive success (Ashman & Schoen, 1994; Teixido, Méndez & Valladares, 2011), so there are fitness benefits to both long-lived and short-lived flowers.

Coley, Bryant and Chapin’s growth rate theory (1985), if applied to flowers, predicts that flowers with long development times will have higher investments into defence than flowers which develop quickly, and that plants with many ephemeral flowers will

have a higher flower turnover and lower resource commitment to flower defence than plants which produce fewer, longer-lived flowers (McCall & Irwin, 2006). There are therefore two alternative strategies that could protect plants from the negative impacts of florivory: produce flowers that develop slowly, are resource expensive and long-lived, but well defended, or produce many flowers quickly that are resource cheap, short-lived and poorly defended.

To deter florivores, some plant species emit herbivore-detering compounds such as aliphatic ketones (Balao, *et al.*, 2011). In other plant species, mutualisms can offer protection from antagonists. Extrafloral nectaries (EFNs) on plants attract ants, and in some cases the ants defend those plants from foliar herbivory (Bentley, 1977; Horvitz & Schemske, 1984; Del-Claro, Berto & Réu, 1996; Nascimento & Del-Claro, 2010; Byk & Del-Claro, 2011). The ant-EFN mutualism could decrease herbivory of foliage and flowers, ensuring greater fruit set (Nascimento & Del-Claro, 2010).

To tolerate florivory, some plant species produce more flowers after they have experienced damage, by aborting damaged flowers and shunting resources into making new flowers (Olesen, 1992; McCall & Irwin, 2006). Other plants produce “reserve flowers” which develop selectively if other flowers are damaged (Ashman & Morgan, 2004; McCall & Irwin, 2006).

### *The study family, genera and species*

The Pedaliaceae family contains 79 species across 14 genera (Gormley, Bedigian & Olmstead, 2015). The fruit morphology and associated dispersal systems of the family are extremely variable, but the family is known for its spectacular and specialized fruit (Ihlenfeldt, 2004). The five genera in the family native to Southern Africa include *Ceratotheca*, *Dicerocaryum*, *Harpagophytum*, *Holubia*, *Pterodiscus*, *Sesamothamnus*, *Rogeria* and *Sesamum*. The five species in the genus *Sesamothamnus* include *Sesamothamnus benguellensis*, *Sesamothamnus busseanus*, *Sesamothamnus guerichii*, *Sesamothamnus lugardii* and *Sesamothamnus rivae* (The Plant List, 2013) and they all occur in Africa.

The study species, *Sesamothamnus lugardii* N. E. Br. Ex Stapf. (Transvaal Sesame-bush, Pedaliaceae) is a quintessential arid-savanna succulent shrub which can be found throughout Zimbabwe, as well as in southern Botswana and in South Africa, in the Limpopo River Valley (Coates Palgrave, 1977). *S. lugardii* shrubs typically grow in hot, dry climates on calcareous soils. The plants are stout and long-lived, and have disproportionately fat trunks, coppery flaking bark, spines and striking white flowers (Coates Palgrave, 1977) (Figure 1-2). *S. lugardii* shrubs flower between November and February (Coates Palgrave, 1977) but have been observed flowering in early October, after the first seasonal rains (A. Bijl, pers. obs.). *S. lugardii* flowers bloom in the evening, remain open for a single night and wilt the following morning (A. Bijl, pers. obs.). *S. lugardii* flowers occur in one to three short, raceme-like inflorescences and are large and sweetly scented. *S. lugardii* flowers are white to cream-coloured and sometimes suffused with purple (Ihlenfeldt, 2004). The calyxes are 5-partite, and the corolla tubes are long (8-10 cm), narrow (4-6 mm) and slightly curved, with a spur at the base (Ihlenfeldt, 2004). The spurs are typically 8 mm long (Coates Palgrave, 1977). Each flower has four stamens inserted at the throat of the corolla tube, and they dehisce in the evening (A. Bijl, pers. obs.). The stigma, which is bilobed, reaches the corolla mouth or can be exerted by up to 10 mm (Ihlenfeldt, 2004). *S. lugardii* pollen grains are large enough to see with the naked eye or a hand lens (x10) and occur in tetrads (A. Bijl, pers. obs.).

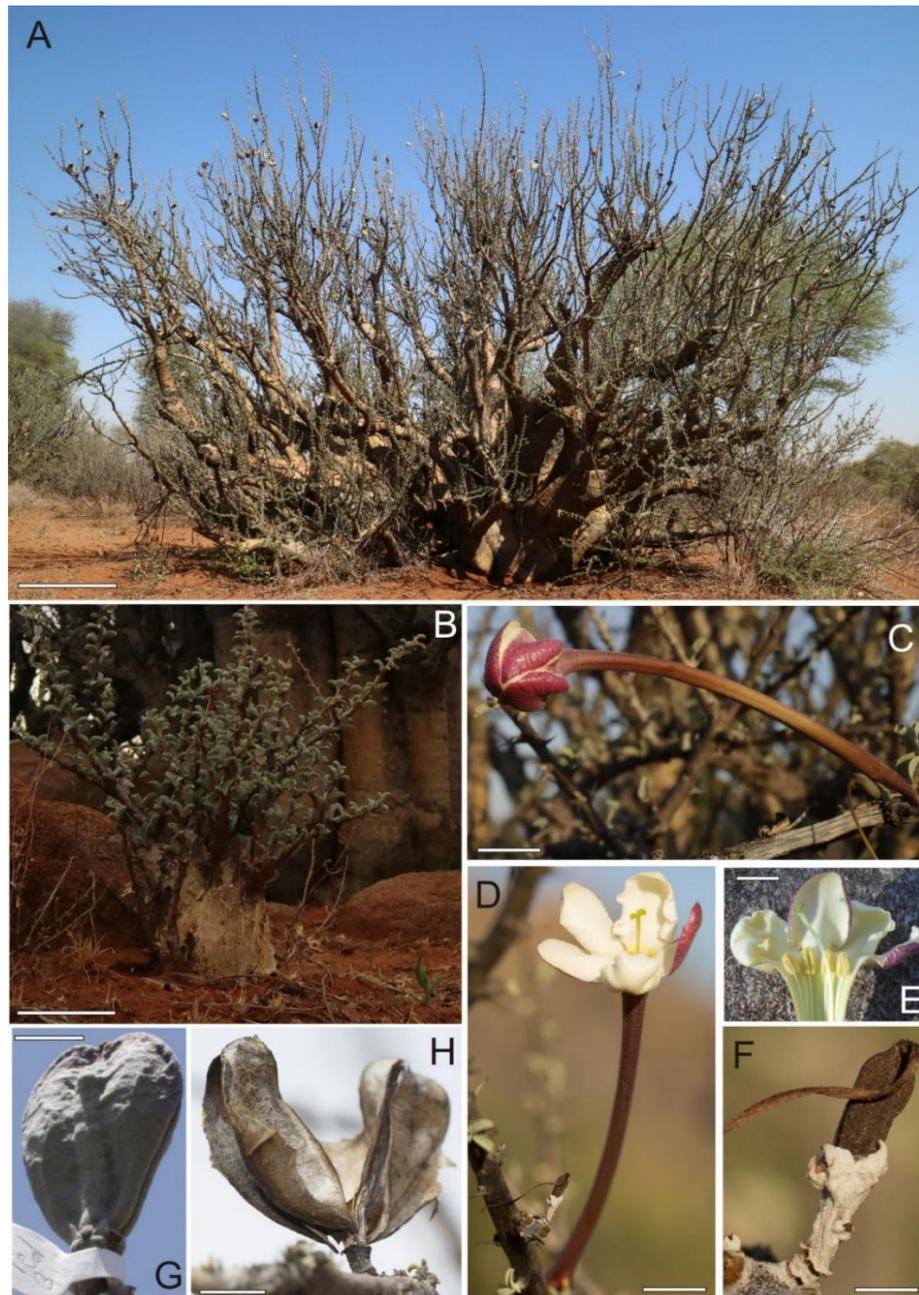


Figure 1-2: The vegetative morphology of a mature *S. lugardii* shrub (A). Scale bar = 1 m. A small *S. lugardii* sapling (B). Scale bar = 10 cm. The white flowers with long, slender corolla tubes, prior to evening anthesis (C), and after evening anthesis (D). Scale bar = 1 cm. A cross-section of the flower, displaying the bilobed stigma and four stamens and anthers attached to the corolla tube (E). Scale bar = 5 mm. A seed pod in the early stage of development (F), late stage of development (G) and after desiccation and dehiscence (H). Scale bars = 1 cm. Photographs by Alison Bijl (A, B, E, G) and Laurence Kruger (C, D, F, H).

## THESIS AIM

The aim of this thesis was to assess the breeding system, pollination syndrome and demography of the Transvaal Sesame-bush, *Sesamothamnus lugardii* N. E. Br. Ex Stapf. (Pedaliaceae) in two protected areas in South Africa, in order to: 1) understand the basic reproductive biology of *S. lugardii* and the ecosystem dynamics that affect this species at a population level, 2) identify the potential reproductive and demographic bottlenecks that affect the study populations of *S. lugardii*, 3) identify the life history strategies that provide resilience and buffer *S. lugardii* populations from extirpation and 4) make predictions about the thresholds beyond which populations of *S. lugardii* potentially become vulnerable to local extirpation.

## CHAPTER OUTLINES

In Chapter 1, I review the literature relevant to this study.

In Chapter 2, I assess the breeding system of *S. lugardii* to determine whether the species is dependent on biotic pollination for reproduction, and explore the degree of specificity in the plant-pollinator relationship. Additionally, I test for pollen-limitation and explore the effect of florivory on reproductive success.

In Chapter 3, I assess the demography of *S. lugardii* to determine if there are bottlenecks that put populations at risk of extirpation, and I explore whether the plants have compensation strategies that ensure resilience.

In Chapter 4, I synthesise the main findings in Chapters 2 and 3, and discuss the relevance of these findings for developing a conservation strategy. I also discuss the limitations and shortcomings of this study.

In Appendix A, I assess the breeding system and the demography of *Sesamothamnus guericchii*, using the theoretical frameworks and methodology developed in Chapters 2 and 3.

In Appendix B, I include a paper to which I have contributed, which is currently in review.

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## CHAPTER 2

### **Hawkmoth pollination and florivory in *Sesamothamnus lugardii* (Pedaliaceae): are flowers hiding in the dark?**

#### **ABSTRACT**

Pollinator-mediated selection is a key evolutionary driver of floral trait convergence and of plant-pollinator trait correlations, exemplified by the global hawkmoth floral syndrome. In South African savannas, long-tongued hawkmoths form a highly specialised pollinator guild for flowers with corolla tubes 8-10 cm in length. The succulent shrub, *Sesamothamnus lugardii*, has typical “hawkmoth traits”, and was found to be pollinated exclusively by long-tongued *Agrius convolvuli* hawkmoths. Evidence was obtained from direct observations and capture of hawkmoths, footage from motion-activated cameras and hawkmoth scales on flower stigmas. *S. lugardii* flowers open at dusk and remain open all night, which overlaps with the all-night foraging activity of *A. convolvuli* hawkmoths. *S. lugardii* nectar volume and composition are also consistent with the hawkmoth pollination syndrome. *A. convolvuli* hawkmoths carry pollen primarily on their proboscides and not on their faces or bodies, so long and short flowers receive equal volumes of pollen. There was thus no significant correlation between pollen deposition on stigmas and corolla tube lengths. Hand-pollination experiments confirmed that *S. lugardii* is an obligate outcrosser. Melolonthine scarab beetles feed destructively on *S. lugardii* flowers during anthesis, and hand-pollinated flowers protected from beetles produce more fruit than unprotected flowers. The beetles ceased to be active after nightfall, but greater fruit set in late-opening flowers (which avoid beetle predation) was not observed. Beetle florivory may be a selective force that delays the timing of floral anthesis, but remains unconfirmed.

#### **KEYWORDS**

*Sesamothamnus lugardii*, *Agrius convolvuli*, hawkmoth pollination syndrome, specialisation, pollen limitation, florivory

## INTRODUCTION

The relationships between plants and their animal pollinators illustrate selection in action (Herrera, 1996). Evidence of floral trait convergence driven by pollinators is seen across multiple plant species (often from disparate lineages): different plant species pollinated by the same pollinator functional types have similar suites of floral traits (Darwin, 1862; Van der Pijl, 1961; Grant & Grant, 1965; Faegri & Van der Pijl, 1979; Fenster, *et al.*, 2004; Ollerton, *et al.*, 2009).

A theoretical framework which groups pollinators according to their functional types, and groups suites of convergent floral traits according to each pollinator functional type, was conceptualised over a century ago by Federico Delpino (Waser, 2006), and much adapted by Baker (1961), Van der Pijl (1961), and Faegri and Van der Pijl (1979). This framework is known as the pollination syndromes theory and it is still used presently, because it is a useful framework for organising the incredible diversity in floral form and function. Pollination syndromes are defined using many floral traits: the timing of floral anthesis, the presence and qualities of floral scent and nectar, as well as flower colour, size and shape. Rodent-pollinated flowers, for example, grow low to the ground, are robust, secrete large volumes of sucrose-rich nectar and emit a “yeasty” odour in the evenings, to overlap with peak rodent activity (Johnson, Pauw & Midgley, 2001). Bat-pollinated flowers open at dusk and emit a musky odour (Muchhala & Thomson, 2009). Hawkmoth-pollinated flowers also have evening floral anthesis, nectar secretion and scent emission (Loughrin, *et al.*, 1991; Knudsen & Tollsten, 1993; Fleming, *et al.*, 2001; Martins & Johnson, 2013; Campbell, Jürgens & Johnson, 2016), but unlike rodent flowers, are delicate and have long and narrow corolla tubes (Baker, 1961; Faegri & Van der Pijl, 1979; Johnson, *et al.*, 2017), and unlike bat-pollinated flowers, hawkmoth flowers typically have a much sweeter aroma dominated by compounds such as linalool and nerolidol (acyclic terpene alcohols), as well as methyl benzoate and benzyl acetate (esters) (Knudsen & Tollsten, 1993; Balao, *et al.*, 2011).

Hawkmoths (Sphingidae) are key pollinators in tropical and subtropical systems around the world (Grant, 1985; Haber & Frankie, 1989; Martins & Johnson, 2013), and flowers pollinated by hawkmoths are often very highly specialised, dependent on a single

hawkmoth functional type or even a single hawkmoth species (Darwin, 1862; Alexandersson & Johnson, 2002; Martins & Johnson, 2007; Peter, *et al.*, 2009; Johnson, 2010; Martins & Johnson, 2013; Johnson & Raguso, 2016). Darwin (1862) postulated that the relationship between a plant and a pollinator could become highly specialised through pollinator-mediated selection, resulting in a close match between the floral traits and pollinator traits. Within the hawkmoth pollination syndrome are many impressive examples of flower-pollinator trait-matches, but the textbook example thereof is the correlation between the spur length of the Malagasy star orchid (*Angraecum sesquipedale*) and the proboscis length of its pollinator, the giant hawkmoth (*Xanthopan morgani praedicta*) (Darwin, 1862). Another example of such correlation is seen in the African iris, *Gladiolus longicollis*, where the length of the long-tubed morph is closely matched by the tongue length of its pollinator, the *Agrius convolvuli* hawkmoth (Anderson, Alexandersson & Johnson, 2010).

Darwin (1862) described the mechanism underlying the evolution of extreme flower tube length (and the trait correlation between long-tubed flowers and their specialist, long-tongued pollinators) as a “coevolutionary race”. He proposed that hawkmoths with tongues longer than the tubes of the flowers they were probing would have better access to nectar, and therefore, natural selection would favour moths with longer tongues. Flowers with tubes longer than moths’ tongues, however, would force the moths to probe the tubes more deeply to reach nectar and, in so doing, maximise pollen receipt and transfer. Selection would thus favour flowers with longer corolla tubes (Darwin, 1862). The increase in the length of the moth tongues was thought to be facilitated by a general size increase through natural selection, in relation to their general conditions of life and in response to increased nectar foraging efficiency (Nilsson, 1998).

Support for Darwin’s coevolutionary model has been found by studies showing that there is a fitness benefit associated with corolla tubes that exceed pollinator mouthparts in length (Nilsson, 1998; Alexandersson & Johnson, 2002; Anderson & Johnson, 2008; Muchhala & Thomson, 2009; Anderson, Alexandersson & Johnson, 2010). Other studies have challenged this coevolutionary model and suggested that, more commonly, plant-pollinator trait correlations are the result of one-sided (unilateral) adaptation

(Stebbins, 1970; Whittall & Hodges, 2007). Others have argued that the evolution of extreme flower-pollinator trait correlations are best explained by the alternative “adaptive shift” model (Wasserthal, 1993). Wasserthal (1993; 1997) argued that long tongues in hawkmoths evolved independently of long-tubed flowers, as an adaptation for predator avoidance. Flowers with long tubes could recruit and be pollinated by preadapted, long-tongued hawkmoths, which would (through selective pressure) drive an increase in flower tube length to the point where the flower tube length exceeds the reach of that particular hawkmoth species (Wasserthal, 1993; 1997). Upon reaching this point, the flower would recruit and shift to a new, longer-tongued hawkmoth pollinator if one was available, and thus the extreme length of flower tubes would have evolved through a series of pollinator shifts (Wasserthal, 1993; 1997). Although there is still much debate about the evolutionary processes that have led to the convergence seen in floral traits, and the correlations observed between plant and pollinator traits, it is widely accepted that pollinator-mediated selection is a key evolutionary driver of these processes.

Since Darwin’s (1862) seminal work on orchids, hawkmoth pollination has been extensively studied (see Baker, 1961; Van der Pijl, 1961; Grant & Grant, 1965; Faegri & Van der Pijl, 1979; Grant, 1985; Haber & Frankie, 1989; Luyt & Johnson, 2001; Peter, *et al.*, 2009; Martins & Johnson, 2013; Johnson & Raguso, 2016; Johnson, *et al.*, 2017), so the floral traits associated with the hawkmoth syndrome are well defined. Typical hawkmoth-pollinated flowers have white or cream corollas and long, narrow corolla tubes (Nilsson, 1998; Alexandersson & Johnson, 2002; Anderson & Johnson, 2008; Johnson, *et al.*, 2017), as well as abundant nectar, evening flower anthesis, evening scent production and short lifespans (Baker, 1961; Van der Pijl, 1961; Faegri & Van der Pijl, 1979; Grant & Grant, 1983; Haber & Frankie, 1989; Cota, 1993; Martins & Johnson, 2013).

Within the classic hawkmoth pollination syndrome is a distinct pollination guild, comprised of very long-tongued hawkmoths (Martins & Johnson, 2007; Johnson, 2010; Johnson & Raguso, 2016; Johnson, *et al.*, 2017). In South Africa, the long-tongued hawkmoth pollination guild, or pollination niche, outlined by Johnson (2010) is

represented by a single species, *Agrius convolvuli*, which has a proboscis length that ranges between 9-11 cm. *A. convolvuli* moths occur in open grasslands and savanna systems (Martins & Johnson, 2007; Johnson & Raguso, 2016) and are often the exclusive pollinators of plants in savanna systems with floral tubes or spurs longer than 10 cm, as such flowers mechanically exclude all shorter-tongued hawkmoth species (Martins & Johnson, 2013; Johnson & Raguso, 2016; Johnson, *et al.*, 2017). Flowers pollinated by this long-tongued hawkmoth pollination guild typically exhibit floral traits consistent with the classic hawkmoth syndrome (Martins & Johnson, 2013; Johnson & Raguso, 2016; Johnson, *et al.*, 2017).

From the perspective of plants, a high degree of pollination specialisation has its advantages. The advantage of specialisation for hawkmoth pollination is that hawkmoths are long-lived and are strong fliers, which makes them very effective as pollen vectors, even in widely dispersed plant populations (Martins & Johnson, 2007; 2009; 2013). Hawkmoths hover over flowers while feeding, which is energetically costly (Micheneau, Johnson & Fay, 2009), so they are selective in their foraging to make sure they meet their high energy requirements. As hawkmoths can make foraging decisions based on rewards (Kulbaba & Worley, 2012), their fidelity can be gained by a plant species. Hawkmoths can also avoid recently visited flowers (Kulbaba & Worley, 2012; Martins & Johnson, 2013), which makes them effective pollinators of self-incompatible plant species. Most hawkmoths forage nocturnally, so depend heavily on olfactory cues to navigate the nocturnal landscape (Knudsen & Tollsten, 1993; Balao, *et al.*, 2011), which explains why most hawkmoth-pollinated flowers produce large volumes of sucrose-rich nectar (Martins & Johnson, 2013; Johnson & Raguso, 2016; Johnson, *et al.*, 2017) and are strongly and sweetly scented (Knudsen & Tollsten, 1993; Balao, *et al.*, 2011). Nocturnal foraging by hawkmoths also drives selection for evening flower anthesis and evening scent production. These advantageous traits that come with hawkmoth specialisation ensure that flowers are most attractive during peak pollinator activity and are potentially obscure during antagonist activity. Flowers that can open and make rewards available at different times of the night or day, can avoid antagonists such as florivores and nectar robbers, without limiting pollinator attraction (Herrera, 1990; Nunes, *et al.*, 2016).

A high degree of pollination specialisation can also be disadvantageous to plants under certain circumstances, for example, when their specific pollinator is lost from the ecosystem. In the absence of their pollinators, plant species that are highly specialised and completely pollinator-dependent can experience severe pollen limitation and reproductive failure (Bond, 1994; Kearns, Inouye & Waser, 1998; Waser & Price, 1998; Pauw, 2007). In the Bahamas in 1996, hurricane Lili caused a decline of two key bird pollinators, the Bananaquits and Bahama Woodstars. Thereafter, the endemic shrub, *Pavonia bahamensis*, Malvaceae, suffered an 89 % loss in fruit set due to pollination limitation (Rathcke, 2000). Very low seed set and limited recruitment were observed when hawkmoths were absent in a fragmented population of *Oxyanthus pyriformis* subsp. *pyriformis* in South Africa (Johnson, *et al.*, 2004).

The risk of reproductive failure is especially high for pollinator-dependent plants in small, fragmented populations that become isolated (Johnson, *et al.*, 2004; Aguilar, *et al.*, 2006; Potts, *et al.*, 2010). Pollination specialisation can also be disadvantageous if the specialised floral traits that attract pollinators also attract antagonists which negatively impact plant fitness (Baldwin, *et al.*, 1997; Andrews, Theis & Adler, 2007; Nunes, *et al.*, 2016). Antagonists such as florivores (flower predators) can reduce the attractiveness of flowers to potential pollinators, if the floral components that attract pollinators (*e.g.* petals and nectar) are consumed (McCall & Irwin, 2006; Cardel & Koptur, 2010). Florivory can also limit the number of viable gametes in a population if the floral reproductive components are damaged (*e.g.* anthers and pistils) (McCall & Irwin, 2006; Cardel & Koptur, 2010). In a population of butterfly peas (*Centrosema virginianum*), for example, florivory by blister beetles (Coleoptera: Meloidae) was found to inhibit visitation by the bee pollinators, which resulted in diminished fruit set (Cardel & Koptur, 2010).

Although plant reproductive failure has been linked to pollination failure (Johnson, *et al.*, 2004; Pauw, 2007), there are very few cases where pollination failure has been linked to plant extinction (Dunn, *et al.*, 2009; Pauw & Bond, 2011), because many plant species have “compensation strategies” which allow them to persist through periods of reproductive failure (Bond, 1994), whether due to the absence of pollinators for long

periods of time (Kearns, Inouye & Waser, 1998), or due to antagonists damaging flowers (Olesen, 1992; Ashman & Morgan, 2004; McCall & Irwin, 2006; Teixido, Méndez & Valladares, 2011).

Bond (1994) described the vegetative/asexual reproduction as a “low-risk demographic trait” or compensation strategy, as this trait allows perennial plants to persist for long periods of time without needing to set seed. This capacity for persistence through vegetative reproduction ensures that populations do not collapse after a season of failed recruitment. Many pollination-specialised plants also have life history strategies to overcome the challenge of being simultaneously attractive to pollinators and antagonists (McCall & Irwin, 2006).

A common strategy seen across several pollination syndromes is the temporal shift in the timing of floral anthesis, scent emission and nectar secretion to match pollinator activity and limit discovery by antagonists (Herrera, 1990; Nunes, *et al.*, 2016). Additional flower traits that can protect plants from florivore damage are a short lifespan and small size: McCall and Irwin (2006) postulated that increased flower size and longevity are linked to an increased likelihood of discovery by antagonists. Teixido, Méndez and Valladares (2011) proposed that florivory could drive selection for smaller, more shorter-lived flowers, as these flowers would be more likely to avoid florivore detection.

Some plant species which cannot escape florivory through temporal shifts or obscurity, have strategies that allow them to tolerate it to a degree. There are plant species which can produce more flowers after they have experienced damage, by aborting damaged flowers and shunting resources into making new flowers (Olesen, 1992; McCall & Irwin, 2006). Other plants produce “reserve flowers” which develop selectively if the first wave of flowers is damaged (Ashman & Morgan, 2004; McCall & Irwin, 2006).

In some cases, the antagonistic florivores may simultaneously act as pollinators, which offsets the negative impact: Gottsberger (1977) found that beetle florivores may benefit plants if they also act as pollinators. In many plant species beetles are the primary pollinators (Bernhardt, 2000; Gibernau & Barabé, 2002) and the convergent floral traits

associated with beetle pollination include large nutritious petals, elevated numbers of stamens and carpels, and petals that close over the flattened centres of flower, creating a floral chamber which can be warmer than external ambient temperatures (Gibernau & Barabé, 2002). However, unless flowers are adapted for pollination by beetles and display at least some of the convergent traits associated with beetle pollination, it is unlikely that the beetle florivores are beneficial for pollination.

### **The study species**

The Transvaal Sesame Bush, *Sesamothamnus lugardii* N. E. Br. Ex Stapf. (Pedaliaceae) has extremely striking flowers: they are white, sweetly scented, and have impressively long, narrow corolla tubes of 8-10 cm (Figure 1-3B). The flowers open in the evening, emit a strong, sweet scent dominated by the monoterpene compound linalool (SD Johnson, unpublished data), and last a single night until corollas are sloughed off the following morning (A. Bijl, pers. obs.). A variety of settling moth species and one hawkmoth species have been observed flying around the *S. lugardii* shrubs in the evenings, making it likely that *S. lugardii* is moth-pollinated. The extreme length of the flower corolla tubes makes it likely that *S. lugardii* is specialised for pollination by a long-tongued hawkmoth, and these long corolla tubes could have evolved in a Darwinian coevolutionary race with a long-tongued hawkmoth pollinator. In a study population of *S. lugardii* at Mapungubwe National Park, swarms of melolonthine scarab beetles (Melolonthinae) have been observed visiting flowers during floral anthesis and appear to feed destructively on the petals and styles (Figure 1-3F). The beetles leave shortly after sunset, once darkness falls. Some flowers open after beetle activity has ceased, making it possible that evening floral anthesis is not only a temporal adaptation driven by moth-selection, but also a florivore-avoidance adaptation.

## Hypotheses

I hypothesised that:

- 1) Hawkmoths are the most likely pollinators of *S. lugardii*, because *S. lugardii* floral traits are consistent with those that have been characterised for the hawkmoth pollination syndrome.
- 2) *S. lugardii* flowers are highly specialised for pollination by long-tongued *A. convolvuli* hawkmoths, based on the extreme lengths of *S. lugardii* flower corolla tubes (*ca.* 10 cm).
- 3) *A. convolvuli* hawkmoths impose directional selection on flower tube lengths, in that flowers with longer tubes receive more pollen than short-tubed flowers.
- 4) *S. lugardii* flowers are pollinator-dependent (obligate outcrossers), as many highly specialised flowers are often dependent on pollinators for reproduction.
- 5) The melolonthine beetles observed feeding on *S. lugardii* flowers during floral anthesis reduce the number of flowers available for pollination, by damaging flowers.
- 6) The flowers damaged by melolonthine beetles would not be successfully pollinated and would thus not develop into fruit.
- 7) The flowers which open late in the evening, after peak beetle activity, have the benefit of a temporal escape from florivory, because late-opening flowers should receive fewer, if any, visits from melolonthine beetles and therefore experience less damage than early-opening flowers.

## Questions addressed in this study

1. Based on visitor behaviour, visitation time and visitor frequency, which flower visitors are the most likely pollinators of *S. lugardii*?
2. Do flower visitors that interact with the reproductive structures act as pollen vectors of *S. lugardii*?
3. Do moth scales on stigmas provide evidence of pollination?
4. Is there a relationship between corolla tube length and pollen count on the stigma?
5. Is there a general fit between flower tube length and hawkmoth mouthparts?
6. Is the timing of floral anthesis and the activity time of hawkmoth visitors synchronous?
7. Is nectar volume, nectar composition and floral scent emission in *S. lugardii* flowers consistent with traits identified for the convolvulus hawkmoth pollination guild?
8. What breeding system does *S. lugardii* have: is the species an obligate outcrosser?
9. Is fruit production in *S. lugardii* populations compromised when pollinators are excluded?
10. Are the study populations pollen-limited?

11. Is florivory by melolonthine beetles reducing the number of flowers available for pollination, and thereby compromising plant fecundity?

- i. Are melolonthine beetles present at both study locations?
- ii. Is the timing of floral anthesis and the activity of melolonthine beetles synchronous?
- iii. Do late-opening flowers receive fewer beetle visits than early-opening flowers?
- iv. Does beetle visitation affect pod formation negatively?
- v. Are melolonthine beetles damaging flowers in a way that lowers plant fecundity (stigma/style damage)?
- vi. Does stigma damage lower the chances of pod development?

## METHODS

### The study species and study sites

*Sesamothamnus lugardii* N. E. Br. Ex Stapf. (Transvaal Sesame-bush, Pedaliaceae) shrubs are striking in their vegetative and floral morphology, with disproportionately fat trunks, coppery flaking bark and sharp spines (Figure 1-2A & B). *S. lugardii* shrubs produce flowers between November and February (Coates Palgrave, 1977). *S. lugardii* flowers are large and sweetly scented, have very long, narrow corolla tubes of 8-10 cm and spurs at the base of the tubes that are typically 8 mm long (Coates Palgrave, 1977) (Figure 1-2C & D). The flowers open in the evening, last only a single night, and corollas are sloughed off in the mornings (A. Bijl, pers. obs.).

In this study, two populations of *S. lugardii* were assessed in South Africa: in the Limpopo province at Mapungubwe National Park and at Morongwa Private Safari Lodge (Figure 2-2). Fieldwork at Mapungubwe National Park (S22.216525, E29.387180) was done on a large population of shrubs at the base of Mapungubwe hill. Data were collected over four years during the flowering season. In 2014, data were collected from November 11<sup>th</sup> to November 15<sup>th</sup> 2014. In 2015, data were collected from October 22<sup>nd</sup> to October 31<sup>st</sup>, and from November 6<sup>th</sup> to November 12<sup>th</sup>. In 2016, fieldwork was done from October 10<sup>th</sup> to October 14<sup>th</sup>. Fieldwork at Morongwa Private Safari Lodge (S23.984940, E27.088443) was done on a small population of shrubs growing on rocky dolomite outcrops. Data were collected only in 2015, from November 13<sup>th</sup> to November 17<sup>th</sup>. Henceforth, “Mapungubwe” refers to the plant population at Mapungubwe National Park and “Morongwa” refers to the plant population at Morongwa Private Safari Lodge. Data collected at Mapungubwe in 2014 and 2015 is denoted Mapungubwe (2014/15), and data collected at Mapungubwe in 2016 is denoted Mapungubwe (2016). Data collected at Morongwa in 2015, is denoted Morongwa (2015).

## Flower visitor observations

Direct moth observations were done opportunistically during fieldwork sessions. At Mapungubwe in 2014, moth observations were done from the 11<sup>th</sup> – 15<sup>th</sup> November 2014, and in 2015, from the 22<sup>nd</sup> – 31<sup>st</sup> October, and the 6<sup>th</sup> – 12<sup>th</sup> November. At Morongwa in 2015, moth observations were done from the 13<sup>th</sup> – 17<sup>th</sup> November.

Bushnell Natureview motion-activated cameras (model 119740, Bushnell Corporation, Overland Park, Kansas) were used to identify flower visitors at Mapungubwe (2015) from the 22<sup>nd</sup> – 31<sup>st</sup> October and the 6<sup>th</sup> – 12<sup>th</sup> November. These cameras use passive-infrared detection (PIR), and the lenses supplied allow close focusing either 460 mm or 250 mm from the subject. The cameras were set at maximum sensitivity, with infrared illumination at the lowest setting and shutter speed at the highest setting, and the video duration was set at 30s. Forty flowers in total were recorded. As the flowers are short-lived and wilt the morning after anthesis, each flower was recorded for a single night only. Each afternoon at 16:00, prior to flower anthesis, a camera trap was directed towards a selected flower, configured to record a 30-second video when triggered, left on throughout the night, and manually switched off the following morning at 10:00. At Morongwa (2015), no cameras were available, so only visual observations of visitors were made. The video footage was assessed to identify visitors, record the time and frequency of visits and determine how visitors were utilising flowers. The impact that each flower visitor had on the opening night and the morning after opening was determined in relation to flower reproductive success: behaviour associated with likely movement of pollen was recorded as “positive”, whereas actions that damaged the stigma or anthers, or reduced the attractiveness of flowers on the opening night, were recorded as “negative.”

Flower visitors were opportunistically captured by hand or with a hand-held net at Mapungubwe (2014/2015) and Morongwa (2015), to determine whether they were picking up *S. lugardii* pollen on their bodies. If visitors were carrying pollen, the number of pollen tetrads were counted (using a hand lens) and the location of the pollen tetrads was noted. *S. lugardii* pollen tetrads are large and distinct enough to identify to species.

### **Moth scales as evidence for pollination**

At Mapungubwe (2015) and Morongwa (2015) flowers were assessed for evidence of moth scales and pollen. Flowers due to open each sample night were marked with unique metal identification tags (twisted around the stems) in the afternoons. The following mornings ( $\pm 12$  hours after anthesis) flower stigmas were inspected with a hand lens for moth scales and pollen tetrads. If present, scales and pollen tetrads were counted.

To assess whether moth scales on flower stigmas provide evidence of pollination across the two study locations, the relationship between moth scale presence and pollen presence was assessed using an events by trials GLM with a binomial distribution and logit link function. Scale presence (present or absent), location (Mapungubwe or Morongwa) and the interaction term of scale presence and location (S x L) were used as the predictor variables, and pollen presence events as the outcome variable.

The relationship between scale presence and pollen tetrad count was tested using a GLM with a negative binomial distribution and log link function. Scale presence (present or absent), location (Mapungubwe or Morongwa) and the interaction term of scale presence and location (S x L) were used as the predictor variables, and pollen tetrad count as the dependent variable. It was assumed that pollen present on the flower stigma prior to the corolla being sloughed off was cross-pollen, because the stigma and anthers are spatially separated, so accidental self-pollination would be unlikely. Self-pollination due to pollinator activity was, however, not accounted for and could have been possible.

### **Pollinator-mediated selection on flower tube length**

At Mapungubwe (2015) and Morongwa (2015), flower corolla tubes were measured the mornings ( $\pm 12$  hours after anthesis) and flower stigmas were inspected with a hand lens for pollen tetrads. If present, pollen tetrads were counted.

To assess the relationship between flower corolla tube length and pollen tetrad number, a GLM with a negative binomial distribution and log link function was used. Corolla

tube length (cm), location (Mapungubwe or Morongwa) and the interaction term of corolla tube length and location (T x L) were used as the predictor variables, and pollen tetrad count on stigmas was used as the dependent variable.

To assess whether flowers with corolla tubes longer than the moths' proboscides received more pollen than flowers with corolla tubes shorter than the moths' proboscides, the mean moth proboscis length for each location was calculated, and flowers were assigned to the "long" group, and if their tube lengths exceeded the moth proboscis length, the "short" group. The difference in pollen tetrad count between the long and short flowers across the two locations was assessed using a GLM with a negative binomial distribution and log link function. Corolla tube length (short or long), location (Mapungubwe or Morongwa) and the interaction term of corolla tube length and location (T x L) were used as the predictor variables, and pollen tetrad count as the dependent variable.

To determine whether tube length was under stabilising or directional selection at each location, a GLM with a negative binomial distribution and log link function was used. For each location (Mapungubwe 2015 and Morongwa 2015), corolla tube length (cm) and corolla tube length squared were used as predictor variables, and pollen tetrad count on stigmas was used as the outcome variable. For each location the regression coefficient for the squared term was recorded to report directional ( $\beta$ ) and non-directional ( $\gamma$ ) selection differentials. Non-linear selection differentials were doubled, based on methods outlined in Stinchcombe *et al.* (2008).

### **Plant-pollinator functional fit**

A sample of flower corolla tube lengths and style lengths were measured in Mapungubwe (2014/15) and Morongwa (2015). Captured hawkmoth proboscis lengths from Mapungubwe (2014/15) and Morongwa (2015) were also measured. The flower corolla tube lengths and style lengths were compared with hawkmoth proboscis lengths at each location and between locations to assess whether the flower (corolla tube and style) length distributions and moth proboscis length distributions matched.

## **Plant-pollinator temporal fit: flower anthesis and pollinator activity**

### ***Floral anthesis***

To record flower opening time in the *S. lugardii* study populations, a sample of flowers due to open each night were marked with a unique identification tag prior to opening, and were then observed at roughly ten-minute intervals between 16:00 and 21:00. During anthesis, petals unfurled sequentially, and the time at which the first petal was fully open was recorded as “open time” for each flower, because at this point the reproductive structures were exposed and flowers were accessible to visitors.

Each flower was assigned to a 30-minute “open time” bin, and the cumulative number of flowers open per time bin was plotted for each sample population and each year separately (Mapungubwe 2014, Mapungubwe 2015 and Morongwa 2015). For each population and year, an opening time distribution was created using a GLM with a binomial distribution and logit link function. The number of flowers per time bin represented the trials, and the cumulative number of flowers open per time bin, the events. Numeric time bins were used as the predictor variables in each model. The coefficients calculated in the logistic regressions for each location and year were substituted into Equation 1 (the curve of a binomial logistic regression) to create a model curve for each population, and to determine the peak rate of flower opening time for each population (where  $P = 0.5$ ).

### **Equation 1:**

$$X = \text{Ln} \left( \frac{P}{1 - P} \right) = \beta_0 + \beta_1$$

$\beta_0$  and  $\beta_1$  = the regression coefficients

$P$  = probability of the flower being open

$X$  = time

For peak rate,  $P = 0.5$

### ***Pollinator activity***

The peak time of floral anthesis in each population was recorded and compared to the activity time of the identified pollinators, as determined from the data from the motion-activated cameras.

### **Nectar volume and concentration**

Nectar was sampled in Mapungubwe in 2016. Nectar was accessed by cutting flower tubes open right down to the spur and drawing it out using capillary action.

Microcapillary tubes (50  $\mu$ l) were used and nectar volume was measured directly from capillary tubes. Using an Eclipse hand refractometer (0–50%; Bellingham and Stanley, Tunbridge Wells, UK) nectar sugar concentration was determined.

### **Breeding system and pollinator exclusion**

A sample of flowers due to open each night were identified and marked with unique metal identification tags and covered with fine-mesh drawstring bags. Once the flowers had opened, the flowers were either left untreated (testing for autogamy), hand-pollinated with pollen from a randomly selected conspecific plants flowers (cross-pollinated positive control), or pollinated with their own pollen (testing for self-incompatibility). The bags remained on for several weeks until fruit had begun to form. In all cases of hand-pollination, the pollen was applied over the entire stigmatic surface with the tip of a toothpick, and a clean tip was used for the treatment of each flower.

To assess the difference in fruit set between treatments across the two study locations, an events by trials GLM with a binomial distribution and logit link function was run. The number of flowers sampled represented the trials, and the number of flowers that developed into pods, the events. The predictor variables were treatment (self, cross or autogamy), location, year nested within location (to deal with data from Mapungubwe 2014/15 and Morongwa 2015) and the interaction term of treatment and location (T x L).

To assess the effect of pollinator exclusion, a sample of flowers were tagged, but left untreated and uncovered (representing natural pollination rates) and fruit set was compared to the untreated and bagged flowers (autogamy flowers).

To assess the difference in fruit set between treatments across the two study locations, an events by trials GLM with a binomial distribution and logit link function was run. The number of flowers sampled represented the trials, and the number of flowers that developed into pods, the events. The predictor variables were treatment (bagged untreated and open natural), location, year nested within location (Mapungubwe 2014/15 and Morongwa 2015) and the interaction term of treatment and location (T x L).

### **Natural pollination rate and pollen limitation**

Each afternoon flowers due to open the same night were marked with tags. Tagged flowers were either left open and untreated (natural pollination rates) or left open and cross-pollinated by hand with pollen from a randomly selected conspecific plant's flower (open crossed). I returned three weeks later when fruit had begun developing, to count fruit set. To assess the difference in fruit set between treatments across the two study locations, an events by trials GLM with a binomial distribution and logit link function was run. The number of flowers sampled represented the trials, and the number of flowers that developed into pods, the events. The predictor variables were treatment (open natural pollination and open cross-pollination), location, year nested within location (Mapungubwe 2014/15 and Morongwa 2015) and the interaction term of treatment and location (T x L).

### **The impact of florivory on plant fecundity**

The effect of excluding florivores (while controlling for breeding system) was assessed by comparing the pod production in open, cross-pollinated flowers with pod production in bagged, cross-pollinated flowers. To assess the difference in fruit set between treatments across the two study locations, a trial by events GLM with a binomial distribution and logit link function was run. The number of flowers sampled represented the trials, and the number of flowers that developed into pods, the events. The predictor

variables were treatment (bagged crossed and open crossed) location, year nested within location (Mapungubwe 2014/15 and Morongwa 2015), and the interaction term of treatment and location (T x L).

### ***Beetle presence at the study locations***

The same flowers that were assessed for flower opening time were used for this component of the study. The selected flowers were observed at roughly ten to fifteen-minute intervals over the course of the evening, between 16:00 and 21:00. For each flower, the opening time was recorded, and whether it was visited by beetles (yes or no) over the course of the observation period was recorded.

To compare beetle presence between the locations, a GLM with a binomial distribution and logit link function was run, with location as the predictor variable, and beetle visit (yes or no) as the outcome variable.

### ***Beetle activity over time***

At Mapungubwe (2015), beetle activity over time was assessed using a GLM with a binomial distribution and logit link function, with time (numeric) and time squared (numeric) as the predictor variables, and beetle presence (yes or no) as the outcome variable.

### ***Flower opening time and beetle visits***

For each flower, the time at which it opened was recorded. If a beetle was observed on the flower at any point during the evening observations, beetle presence was recorded for that flower. For Mapungubwe, a GLM with a binomial distribution and logit link function was run with flower opening time as the predictor variable, and beetle presence (yes or no) as the outcome variable.

Flowers that begin opening after 19:00 were considered “late flowers” because they opened after peak beetle activity (between 18:30 and 19:00). Flowers at Mapungubwe

(2015) were assigned an “early” or “late” opening time status, and the proportions of beetle visits were compared between the groups using a GLM with a binomial distribution and logit link function, with flower opening status (early or late) as the predictor variable, and beetle presence (yes or no) as the outcome variable.

### **Beetle damage and plant fecundity**

The effect of beetle florivory on flower stigmas and styles was assessed, because if beetle florivory damaged these structures, then plant fecundity would be compromised. The same flowers that were assessed for flower opening time at Mapungubwe (2015) were used for this component of the study. The selected flowers were observed at roughly ten to fifteen-minute intervals over the course of the evening, between 16:00 and 21:00.

To determine whether beetle presence resulted in stigma or style damage, for each flower, beetle presence or absence was recorded. Additionally, the condition of the flowers’ stigmas and visible portions of the styles was recorded when the final flower observations were done at 21:00 (predated or not). A GLM with a binomial distribution and logit link function was run with beetle presence (yes or no) as the predictor variable and stigma/style damage (yes or no) as the outcome variable.

To determine whether beetle presence had a negative effect on pod formation at Mapungubwe (2015), a GLM with a binomial distribution and logit link function was run with beetle presence (yes or no) as the predictor variables and pod formation (yes or no) as the outcome variable.

To assess the difference in pod development between flowers with and without stigma damage across the two study locations, an events by trials GLM with a binomial distribution and logit link function was run. The number of flowers sampled with and without stigma/style damage at each location represented the trials, and the number of flowers that developed into pods, the events. The predictor variables were stigma/style damage (damaged or undamaged), location (Mapungubwe and Morongwa) and the interaction term of stigma/style damage and location (L x Sd).

### **Notes on statistical analyses**

All statistical analyses were done using IBM's SPSS® Statistics, version 23 software, and for all generalised linear models (GLMs), the chi-square likelihood ratio ( $\chi^2$ ) was used as the test statistic to assess the significance (p) of the model effects. Where necessary, for post-hoc tests, the Dunn-Šidák correction (Šidák, 1967) was used to adjust the alpha level (significance) when multiple pairwise comparisons were done.

## RESULTS

### Visitor identification, activity time and visit frequency

Over five nights at Mapungubwe in 2015, between 18:30 and 20:10, seven *Agrius convolvuli* hawkmoths were observed foraging for nectar from *S. lugardii* flowers, and 18 *A. convolvuli* hawkmoths were observed flying around near *S. lugardii* shrubs. At Morongwa in 2015, two *A. convolvuli* hawkmoths were observed foraging for nectar between 18:30 and 19:40, and one was observed flying around near *S. lugardii* shrubs at 19:22. The hawkmoths were very light sensitive, and would fly away if a torch was directed towards them. With red light it was easier to observe them at a close range, but this made them difficult to spot and observe at a distance.

A total of 212 flower visits were recorded with Bushnell Natureview motion-activated cameras, on *S. lugardii* flowers (n = 40) at Mapungubwe. Flowers were visited mostly at night, by a variety of insects and at dusk by White-bellied sunbirds. I recorded 160 visits on flowers during the night and 52 visits during the subsequent mornings (Table 2-1). The most common night-time pollinators were *A. convolvuli* hawkmoths, which approached flowers at night on 55 occasions, and on 23 occasions, also probed the *S. lugardii* flowers (Table 2-1) (Supplementary video footage S 2-1). Among animals that could contribute to pollination, the most common flowers visitors in the morning were solitary bees (n = 7) and African honey bees (*Apis mellifera scutellata*) (n = 5) (Table 2-1).

Visits by White-bellied Sunbirds (*Cinnyris talatala*), melolonthine scarab beetles, Christmas beetles (*Anoplognathus* spp.) and Carpenter bees (*Xylocopa* spp.) resulted in flower damage, mainly because of nectar robbing when they pierce the corolla. Visits by settling moths (Noctuidae) were considered neutral, as they did not result in pollination. All recorded visits by these small moths showed them moving about on the petals of the flowers, but not making contact with the anthers or stigmas of flowers. Visits by ants, cockroaches, ichneumon wasps and cicadas were also neutral as these visitors did not interact with flower reproductive structures (Table 2-1).

*A. convolvuli* hawkmoths were active throughout the nights, from 18:00 to 05:30

(Figure 2-3). Melolonthine scarab beetle activity and White-bellied sunbird activity was bimodal, with visits in the evenings from 16:30 to 19:00 and in the mornings from 05:00 to 09:00 (Figure 2-3). In the mornings, solitary bees were active between 06:30 and 08:30 and African honey bees, from 05:30 to 07:00 (Figure 2-3). No melolonthine scarab beetles were observed on flower opening nights at Morongwa, but were seen frequently at Mapungubwe (Supplementary video footage S 2-2).

At Morongwa, no camera footage was collected, but *A. convolvuli* hawkmoths ( $n = 4$ ) were observed visually, foraging for nectar between 19:00 and 20:10 at night. At Mapungubwe (2014), the flower visitors captured included a solitary bee ( $n = 1$ ), small settling moths ( $n = 5$ ), melolonthine scarab beetles ( $n = 10$ ) and *A. convolvuli* hawkmoths ( $n = 2$ ). Only *A. convolvuli* hawkmoths were found to have pollen on their bodies, located primarily on their proboscides. At Morongwa (2015), three *A. convolvuli* hawkmoths were captured and all had pollen on their bodies. All the captured *A. convolvuli* hawkmoths were carrying large amounts of pollen on their proboscides (Table 2-2).

### **Moth scales as evidence for pollination**

At Mapungubwe there was no significant relationship between moth scale presence and pollen presence on flower stigmas, but at Morongwa, flowers were more likely to have pollen on the stigmas when moth scales were present (GLM  $\chi^2(3) = 17.637$ , Scale presence  $p = 0.012$ ) (Figure 2-4). There was, however, no significant relationship between moth scale presence and the *number* of pollen tetrads deposited on stigmas (Figure 2-5).

Location, however, was a statistically significant predictor of pollen tetrad count on flower stigmas (GLM  $\chi^2(3) = 9.596$ , Location  $p = 0.045$ ) (Figure 2-5), because flowers at Mapungubwe had far fewer pollen tetrads on their stigmas than those at Morongwa.

### **Pollinator-mediated selection on flower tube length**

Corolla tube length was not a significant predictor of pollen tetrad count on flower stigmas at either Mapungubwe or Morongwa ( $\chi^2(2) = 9.800$ , Tube length  $p = 0.568$ , Location  $p = 0.737$ ) (Figure 2-7).

At Morongwa, long flowers (flowers with corolla tubes longer than the mean hawkmoth proboscis length (calculated from the captured sample)) did not receive significantly more pollen than short flowers (flowers with corolla tubes shorter than the mean hawkmoth proboscis length) (Figure 2-8). At Mapungubwe this could not be tested as no flower corolla tubes exceeded the length of the mean moth proboscis length (Figure 2-6; Figure 2-8). Short flowers at Mapungubwe received significantly less pollen than short flowers at Morongwa (GLM  $\chi^2(2) = 9.759$ , Location  $p = 0.002$ ), but the difference in pollen receipt between long flowers could not be tested across the locations, because Mapungubwe did not have any long flowers.

### **Testing for directional and stabilising selection**

The GLMs assessing the effects of tube length on pollen tetrad deposition on stigmas were not significant. (GLM  $\chi^2(2) = 0.936$ ,  $p = 0.626$ ). For corolla tube length at Mapungubwe (2015), the directional selection differential ( $\beta \pm SE$ ) was  $2.243 \pm 4.986$  ( $p = 0.660$ ), and non-directional selection differential ( $\gamma \pm SE$ ) was  $-0.228 \pm 0.457$  ( $p = 0.625$ ). For corolla tube length at Morongwa (2015), the directional selection differential ( $\beta \pm SE$ ) was  $1.740 \pm 1.543$  ( $p = 0.303$ ), and stabilising selection differential ( $\gamma \pm SE$ ) was  $-0.116 \pm 0.144$  ( $p = 0.292$ ).

## Plant-pollinator functional fit

### *Flower length measurements and hawkmoth proboscis length measurements*

*S. lugardii* flowers have long, narrow corolla tubes ranging from 7.9 - 13.5 cm at Mapungubwe, and 5 - 13.3 cm at Morongwa. The style lengths at Mapungubwe range from 9.4 - 14.1 cm, and 7.5 - 13.5 cm at Morongwa. The mean corolla tube length at Mapungubwe was  $10.77 \pm 1.17$  cm ( $n = 63$ ), and the mean style length was  $11.90 \pm 1.18$  cm ( $n = 39$ ). At Morongwa, the mean corolla tube length was  $10.28 \pm 1.33$  cm ( $n = 99$ ), and the mean style length was  $11.22 \pm 1.13$  cm ( $n = 98$ ). Corolla tube lengths differ significantly between Mapungubwe and Morongwa ( $t = 2.522$ ,  $p = 0.012$ ) and so do style lengths ( $t = 2.959$ ,  $p = 0.003$ ).

*A. convolvuli* hawkmoth proboscides were extremely long, ranging from 12.2 - 13.3 cm at Mapungubwe, and 9.4 - 10.9 cm at Morongwa. The mean *A. convolvuli* hawkmoth proboscis length at Mapungubwe was  $12.75 \pm 0.78$  cm ( $n = 2$ ) and mean hawkmoth proboscis length at Morongwa was  $10.43 \pm 0.90$  cm ( $n = 3$ ). Proboscis length differences between Mapungubwe and Morongwa were marginally non-significant ( $t = 2.956$ ,  $p = 0.060$ ), but the small sample size meant that this comparison had little statistical power.

At Mapungubwe, both the mean flower corolla tube length and the mean style length are shorter than the mean *A. convolvuli* hawkmoth proboscis length (Table 2-3), but not significantly (tube length  $t = 2.269$ ,  $p = 0.024$ ; style length  $t = 0.967$ ,  $p = 0.334$ ). At Morongwa the mean corolla tube length is only slightly shorter than the mean moth proboscis length, but not significantly ( $t = 0.215$ ,  $p = 0.830$ ) and the mean style length exceeds the mean moth proboscis length, but not significantly ( $t = 1.107$ ,  $p = 0.269$ ) (Table 2-3).

## **Plant-pollinator temporal fit**

### *Timing of floral anthesis and hawkmoth activity*

*S. lugardii* flowers at Mapungubwe (2014) began opening between 16:00 and 16:30, and at Mapungubwe (2015) began a little later, between 16:30 and 17:00. At Morongwa (2015), flower opening began much later, between 19:00 and 19:30. The modelled peak flower opening time at Mapungubwe (2014), was at 18:18 (GLM  $\chi^2(1) = 106.217$ ,  $p < 0.005$ ), and at Mapungubwe (2015), was earlier, at 18:06 (GLM  $\chi^2(1) = 478.008$ ,  $p < 0.005$ ) (Figure 2-9). At Morongwa (2015), the peak flower opening time was much later in the evening than the Mapungubwe populations, at 19:15 ( $\chi^2(1) = 250.285$ ,  $p < 0.005$ ) (Figure 2-9).

At Mapungubwe (2015), *S. lugardii* flowers began opening between 16:30 and 17:00 in the evening, and the peak rate of flower opening occurred at 18:06 (Figure 2-9). The activity of *A. convolvuli* hawkmoths recorded with Bushnell Natureview motion-activated cameras in Mapungubwe (2015) began at 18:00, and continued throughout the night to 05:30 the following morning (Figure 2-3). *A. convolvuli* hawkmoths were also visually observed foraging for nectar from *S. lugardii* flowers between 18:30 and 21:00 on multiple evenings. *A. convolvuli* hawkmoth activity was high when flower opening rate was at its peak, and *A. convolvuli* hawkmoths were active throughout the nights while flowers were open (Figure 2-3 & Figure 2-4).

### **Nectar volume and concentration**

At Mapungubwe (2016), the measured nectar volume per *S. lugardii* flower ranged from 2.2  $\mu$ L to 19  $\mu$ L, with a mean  $\pm$  SE volume of  $6.06 \pm 3.62$   $\mu$ L (Table 2-4). The nectar concentration ranged from 12.5 % to 33 %, with a mean concentration of  $21.87 \pm 4.98$  % (Table 2-4).

### **Breeding system**

The proportion of cross-pollinated flowers that developed into pods was significantly higher than any other treatment (self-pollinated or autogamy) at both Mapungubwe and

Morongwa. In the hand-pollination breeding system experiments, 57.1 % of all cross-pollinated flowers, 4% of all self-pollinated flowers and 0 % of all untreated/autogamy flowers developed into pods (across locations and years collectively). Only treatment (cross-pollinated, self-pollinated and autogamy/untreated) was a significant predictor of pod development (GLM  $\chi^2(6) = 30.118$ , Treatment  $p = 0.000028$ ) (Figure 2-10).

### **Pollinator exclusion**

At Mapungubwe, 0 % of the pollinator-excluded (bagged and untreated) flowers developed into fruit and only 6.4 % of the pollinator-exposed (open and untreated flowers) developed into fruit. At Morongwa 0 % of the pollinator-excluded flowers developed into pods, but 24.6 % of pollinator-exposed flowers developed into pods. Surprisingly, treatment was not significant ( $\chi^2(4) = 18.386$ , Treatment  $p = 0.177$ ), but location (with year nested within) was marginally non-significant ( $p = 0.053$ ) (Figure 2-11). Excluding pollinators does not significantly influence pod development, but some location-related process does.

### **Natural pollination rate and pollen limitation**

At Mapungubwe, seven of 109 open (un-bagged) and untreated flowers developed into pods and zero of five open, pollen-supplemented flowers developed into pods. At Morongwa, 16 of 65 open and untreated flowers developed into pods and eight of 18 open, pollen-supplemented flowers developed into pods (Figure 2-12). Treatment was not significant, but location (year nested within) was marginally non-significant (GLM  $\chi^2(4) = 25.968$ , Location  $p = 0.053$ ) (Figure 2-12). This suggests that the effect of supplementing pollen does not significantly influence fruit set, but again, some location-related process does.

### **Effect of florivory on hand pollination experiment outcomes**

To explore potential predation effects, I compared open cross-pollinated flowers with bagged and cross-pollinated flowers, and at both locations more bagged flowers developed into fruit than open/un-bagged flowers (Figure 2-13). Treatment (bagged or open) was a significant predictor of fruit set (GLM  $\chi^2(4) = 7.802$ , Treatment  $p = 0.012$ ) (Figure 2-13).

### ***Beetle presence across study locations***

At Mapungubwe (2015), 29 of the 62 flowers assessed were visited by beetles, but at Morongwa, no beetles were observed. Location was thus a significant predictor of beetle presence (GLM  $\chi^2(1) = 25.061$ , Location  $p < 0.005$ ) (Figure 2-14).

### ***Beetle activity over time***

Beetle presence varied significantly over time (GLM  $\chi^2(2) = 57.707$ , Time  $p = 2.945E^{-13}$ ) and beetle activity was highest between 18:30 and 19:00 (Figure 2-15).

There was no significant relationship between the time that a flower opens, and its chances of being visited by beetles (GLM  $\chi^2(33) = 43.096$ ,  $p = 0.112$ ), and nor did the opening time of flowers (early or late) determine the number of beetle visits a flower received (GLM  $\chi^2(1) = 0.052$ ,  $p = 0.820$ ). Beetle visits were slightly lower on late-opening flowers than on early-opening flowers, but the difference was not significant ( $t = 0.222$ ,  $p = 0.825$ ) (Figure 2-16).

## **Beetle damage and plant fecundity**

### ***Beetle visitation and style damage***

At Mapungubwe (2015), flowers visited by beetles did not have significantly more stigma/style damage than unvisited flowers (GLM  $\chi^2(1) = 1.963$ ,  $p = 0.161$ ) (Figure 2-17).

### ***Beetle visitation and pod formation***

Pod development was slightly higher in flowers not visited by beetles, but the difference was not significant ( $t = 1.353$ ,  $p = 0.183$ ), (GLM  $\chi^2(1) = 2.603$ ,  $p = 0.107$ ) (Figure 2-18).

### ***Stigma/style damage and pod outcomes***

At Mapungubwe (2015), 7.5 % of the observed flowers had signs of stigma/style damage (four out of 53) and only one of the four damaged flowers developed into a pod. 92.5 % (49 out of 53) of the flowers were undamaged, but also only one of the 49 undamaged flowers developed into a pod. At Morongwa (2015), 10 % of the observed flowers (2 out of 20) had stigma/style damage and neither of the damaged flowers developed into pods. Eighteen of 20 flowers were undamaged, and half of the undamaged flowers (9) developed into pods. Pod development was significantly higher at Morongwa than at Mapungubwe for undamaged flowers, but for damaged flowers, was similarly low. Neither stigma damage nor location were significant predictors of pod development, but the interaction term of stigma damage and location was significant (GLM  $\chi^2(3) = 22.674$ ,  $L \times Sd p = 0.021$ ) (Figure 2-19).

## DISCUSSION

At Mapungubwe, direct observations and video footage from motion-activated cameras showed that *S. lugardii* flowers are visited by a wide variety of insects throughout the night, when flowers are open (Table 2-1; Figure 2-3). Small settling moths and *A. convolvuli* hawkmoths were the most frequent visitors, but only *A. convolvuli* hawkmoths interacted with the flowers' reproductive structures during visits, by hovering over flowers and inserting their proboscides into the extremely narrow corolla tubes, probing for nectar (Figure 2-1C & D; Table 2-1). Their proboscides would brush against the anthers at the neck of the corolla tubes, and against the protruding stigmas (Supplementary video S 2-3). Settling moths moved about on the flower petals, but did not, in any recorded observations, make contact with the anthers or stigmas of flowers.

Only *A. convolvuli* hawkmoths were found to be *S. lugardii* pollen vectors. Pollen was visible on the proboscides of hawkmoths photographed while feeding on flowers (Fig 2-1C) and each moth captured was carrying over 160 pollen tetrads, primarily on its proboscis (Table 2-2). These findings confirm that *A. convolvuli* hawkmoths are the only effective pollinators of *S. lugardii*. At Mapungubwe, the mean corolla tube length was 10.8 cm and at Morongwa, was 10.3 cm, which confirms that *S. lugardii* flowers fit into the convolvulus hawkmoth pollination guild, as flowers within this pollination guild typically have corolla tubes longer than 10 cm (Martins & Johnson, 2013; Johnson & Raguso, 2016; Johnson, *et al.*, 2017).

Moth scales provided additional evidence for hawkmoth pollination. On most flower stigmas, moth scales and pollen tetrads co-occurred, and it was found that flowers were more likely to have pollen present on the stigmas when moth scales were present as well (Figure 2-4). There was, however, no significant relationship between scale presence and the *number* of pollen tetrads counted on the stigmas (Figure 2-5). This finding is not unexpected: as *A. convolvuli* hawkmoths transport pollen on their proboscides, contact between their bodies and flower stigmas is not necessary for pollen transfer, so they can deposit pollen without depositing scales.

At Morongwa, the co-occurrence of moth scales and pollen was greater, because there

were many flowers in the population with tube lengths greater than the mean hawkmoth proboscis length, so the likelihood of the flower stigmas and the hawkmoths' bodies coming into contact was higher than at Mapungubwe, where all flower tubes were shorter than the mean hawkmoth proboscis length (Figure 2-6).

If deeper probes by hawkmoths ensure more efficient pollen placement or larger volumes of pollen deposited on flower stigmas, then flowers with corolla tubes longer than the hawkmoths' mouthparts should have a fitness advantage over shorter flowers, and the population should experience directional selection for longer corolla tubes (Darwin, 1862). In the case of *S. lugardii*, however, there was no selective advantage to having corolla tubes longer than *A. convolvuli* hawkmoth proboscides, because the "long" flowers received no more pollen than the "short" flowers. *A. convolvuli* hawkmoths effectively transport large volumes of pollen on their proboscides, so long and short flowers receive similar volumes of pollen, and there is no greater benefit enjoyed by longer-tubed flowers that force hawkmoths to probe more deeply to reach nectar. I found no clear evidence for either directional or stabilising selection in this study.

There was a morphological fit between *S. lugardii* corolla tube lengths and *A. convolvuli* hawkmoth mouthparts, as these traits were closely correlated in each population (Table 2-3). On average, *A. convolvuli* hawkmoth proboscides were slightly longer than flower corolla tubes (Table 2-3). At Mapungubwe, both the hawkmoth proboscides and corolla tube lengths were significantly longer than at Morongwa, but within locations, the hawkmoth proboscis lengths and flower lengths matched. These site-level correlations in flower and pollinator traits reflect what Anderson and Johnson (2008) called a "classical Darwinian coevolutionary scenario," where reciprocal co-adaptations occur as a result of coevolution at a population level. Anderson and Johnson (2008) found a similar trend in the proboscis lengths of the long-tongued fly (*Prosoeca ganglbaueri*) and the corolla tube lengths of *Zaluzianskya microsiphon* (the flies' primary food source): plant-pollinator traits were strongly correlated with one another in study sites where they co-occurred, but varied significantly across their geographical ranges.

Evening floral anthesis and scent emission are common traits in moth-pollinated flowers (Loughrin, *et al.*, 1991; Knudsen & Tollsten, 1993; Fleming, *et al.*, 2001), as these traits ensure that flowers are most attractive when their pollinators are most active. A “temporal fit” between *S. lugardii* flowers and *A. convolvuli* hawkmoths was confirmed in this study. At Mapungubwe (2015), flower anthesis began between 16:30 and 17:00, and the peak rate of flower opening occurred at 18:06 (Figure 2-9). Flowers would remain open all night, and in the mornings, corollas would be sloughed off. *A. convolvuli* hawkmoths became active at 18:00, corresponding with peak flower opening time, and remained active throughout the nights when flowers were open, with the last hawkmoth visits occurring at 05:30 (Figure 2-3).

It was interesting to note that between Mapungubwe (2014) and Mapungubwe (2015), peak flower-opening time differed by 12 minutes, where between Mapungubwe (2015) and Morongwa (2015), the peak rate of flower-opening differed by 69 minutes (Figure 2-9). Within-site variation is much smaller than between-site variation. This site-level variation in peak opening time may be driven by pollinator selection, but is particularly difficult to assess in a long-lived plant species such as *S. lugardii*, because the rate of turnover in the population can be extremely slow. To test whether there has been a shift in opening time between two generations could take decades. In this study I did not attempt to test whether pollinator selection causes a shift in the timing of anthesis between flowering seasons or years within individual *S. lugardii* shrubs.

Flowers in the convolvulus hawkmoth pollination guild typically secrete large volumes of sucrose-dominated nectar (Martins & Johnson, 2013; Johnson & Raguso, 2016; Johnson, *et al.*, 2017), and the nectar and scent traits of *S. lugardii* flowers were consistent with this pollination guild. The mean nectar volume measured in *S. lugardii* flowers in Mapungubwe (2016) was  $6.06 \pm 3.62 \mu\text{L}$  (Table 2-4), which fell within the range recorded for hawkmoth-pollinated orchids (Johnson, 1995; Johnson & Liltved, 1997; Luyt & Johnson, 2001). The mean nectar volume measured in *S. lugardii* was even higher than that recorded for terrestrial savanna orchids which are known to attract *A. convolvuli* hawkmoths in South Africa (Peter, *et al.*, 2009; Balducci, 2018).

The scent profiles of *S. lugardii* flowers are dominated by the terpene alcohol, linalool (3,7-dimethyl-1,6-octadien-3-ol) (SD Johnson, unpublished data), a floral volatile found in several other sphingophilous flowers (Raguso & Pichersky, 1995; Miyake, Yamaoka & Yahara, 1998; Raguso, 2016).

In the breeding system experiments, pod development was significantly higher in cross-pollinated flowers than in self-pollinated flowers (Figure 2-10), confirming that *S. lugardii* is an obligate outcrosser. *S. lugardii* flowers also have no mechanism for ensuring self-pollination, as flowers tested for autogamy failed to develop into pods (Figure 2-9). *S. lugardii* flowers are dependent on pollinators for fertilisation as no flowers produced pods when pollinators were excluded from flowers with mesh bags (Figure 2-11).

In the pollinator-exclusion control group (flowers left open and available to pollinators) pod development was very low at Mapungubwe, and significantly higher at Morongwa, which was unexpected (Figure 2-11). A location-level process appeared to be impacting pod outcomes, and the difference was attributed to the presence of florivores at Mapungubwe. Predation of *S. lugardii* flowers by melolonthine scarab beetles was observed during flower anthesis at Mapungubwe, but these beetles were absent at Morongwa, which may explain why pod development in un-bagged flowers was extremely low at Mapungubwe and higher at Morongwa (Figure 2-11).

In the pollen-limitation experiments, very few pods developed at Mapungubwe, regardless of whether they were supplemented with pollen or not (Figure 2-12) and this was attributed to florivore damage of open flowers, as pod development was significantly higher at Morongwa (where melolonthine scarab beetles were absent) than at Mapungubwe, where beetles were present (Figure 2-12). At Morongwa, flowers are not pollen limited, as there was no significant difference in pod development between open flowers un-supplemented with pollen, and open flowers supplemented with conspecific (cross) pollen (Figure 2-12). At Mapungubwe, it was difficult to draw conclusions about pollen limitation, because pod development was extremely low in all flowers. I thus decided to explore the effects of florivory by melolonthine scarab beetles.

Florivory can affect plant fitness by lowering the attractiveness of flowers to potential pollinators, and by limiting the number of viable gametes if pistils are damaged (McCall & Irwin, 2006; Cardel & Koptur, 2010). During their visits, the melolonthine scarab beetles appeared to be feeding destructively on the petals, stigmas and styles of *S. lugardii* flowers, thus making flowers less attractive to the *A. convolvuli* hawkmoths, and damaging the reproductive structures, preventing fertilisation and pod development.

Melolonthine scarab beetles were only present at Mapungubwe (Figure 2-14) and their activity was synchronised with floral anthesis. At Mapungubwe (2015), melolonthine scarab beetles were observed interacting with *S. lugardii* flowers in the late afternoons and evenings, between 16:30 and 19:00, and their activity was highest between 18:00 and 19:00 (Figure 2-15) when the rate of flower opening peaked in Mapungubwe (2015) (Figure 2-9).

As beetle activity dropped off rapidly between 19:00 and 19:30, flowers opening after 19:00 were considered late-opening flowers. I expected that the *S. lugardii* flowers which opened later in the evening, after peak beetle activity, would have the benefit of a temporal “escape” from florivory, *i.e.*, the late-opening flowers were expected to receive fewer, if any, visits from beetles. Contrary to expectation, there was no significant relationship between the time at which a flower opened in the evening, and its chance of being visited by beetles (Figure 2-16), so late anthesis does not offer an escape from beetle florivory.

I found that stigma and style damage did not differ significantly between early and late flowers at Mapungubwe (2015) (Figure 2-16), so questioned whether beetle visits were damaging floral reproductive structures. At Mapungubwe (2015), the relationship between beetle presence and stigma damage yielded surprising results: flowers visited by beetles did not have significantly more stigma/style damage than unvisited flowers (Figure 2-17), and although pod development was slightly higher in flowers not visited by beetles (Figure 2-18), the difference was not significant.

I explored the link between stigma/style damage, and pod development. At Morongwa (2015) flowers with stigma damage produced fewer pods than flowers without stigma

damage, but the difference was not significant, and at Mapungubwe (2015) there was also no significant difference in pod development for flowers with damaged and undamaged stigmas. The results of this study showed neither a clear link between beetle visitation and stigma damage, nor a clear link between stigma damage and pod development, but the sample sizes were small, so should be replicated in future studies. I also did not explore the role of any other flower and pod predators, which may have affected my study flowers.

## CONCLUSION

*S. lugardii* flowers are white, sweetly scented and have long, narrow corolla tubes. Floral anthesis occurs in the evening and once open, the flowers emit a strong, sweet scent. *S. lugardii* flowers only last only a single night before the corollas are sloughed off, so they have short lifespans (A. Bijl, pers. obs.). These floral traits are convergent with the those that define the hawkmoth pollination syndrome, and the extremely long corolla tubes of the *S. lugardii* flowers (*ca.* 10 cm) make it a likely candidate for pollination by long-tongued hawkmoths in the convolvulus hawkmoth pollination guild. In this study, I confirmed that *A. convolvuli* hawkmoths were visiting *S. lugardii* flowers, using visual and camera observations. Observations of *S. lugardii* pollen on *A. convolvuli* hawkmoth proboscides also confirmed that *A. convolvuli* hawkmoths are effective pollinators of *S. lugardii*. *A. convolvuli* hawkmoth scales were found on flower stigmas in association with *S. lugardii* pollen tetrads, which provided further support for hawkmoth pollination. I also found the nectar and scent traits of *S. lugardii* flowers consistent with other flower species pollinated by *A. convolvuli* hawkmoths.

There was a mechanical fit between the length of *S. lugardii* flower corolla tubes and *A. convolvuli* hawkmoth proboscis lengths, and there was a temporal fit between flower anthesis and hawkmoth activity. These trait matches confirmed that *S. lugardii* flowers are adapted (and specialised) for pollination by *A. convolvuli* hawkmoths exclusively. In this study, I did not find sufficient evidence to confirm directional selection on floral tube length, so cannot be sure that a Darwinian coevolutionary race is currently driving the lengthening of *S. lugardii* corolla tubes (Darwin, 1862).

I found that *S. lugardii* flowers are self-incompatible and dependent on *A. convolvuli* moths for cross-pollination.

At Mapungubwe it was impossible to determine whether or not the *S. lugardii* study population was experiencing pollen limitation. Pod development was extremely low in both samples of treatment flowers used to test for pollen limitation (those supplemented with cross-pollen and those untreated), because the samples were left open and unprotected from antagonists for several weeks. Florivorous melolonthine beetles were

suspected of damaging almost all the sample flowers at Mapungubwe. At Morongwa, melolonthine beetles were absent, and far fewer of the sample flowers left open were damaged, so pod development was not as badly compromised and pollen limitation could be tested. The Morongwa population was not pollen-limited.

I expected that florivory by melolonthine beetles was damaging flower stigmas and styles at Mapungubwe, reducing the number of flowers that could develop into pods. Stigma damage, however, was similar at Mapungubwe and Morongwa, where beetles were present and absent, respectively, so I could not confirm that melolonthine beetles were the only culprits causing stigma damage. Small caterpillars were occasionally observed inside the corolla tubes of *S. lugardii* flowers at both populations, and were seen to feed on the anthers and styles of the flowers, often in flowers that were still developing. In the hand-pollination flower samples, the flowers which were un-bagged could have been damaged by caterpillars during the period between treatment and pod assessment (several un-observed weeks). During this period, pod predators would also have had access to unprotected pods, and a ground squirrel was seen gnawing a developing *S. lugardii* pod in the field once (A. Bijl, pers. obs.). Herbivores like eland and kudu feed on *S. lugardii* foliage (A. Bijl, pers. obs.), and could also damage the pods, but were not observed doing so in this study. I did not explore the effects of flower predators other than melolonthine beetles, or the effects of pod predators such as squirrels, kudu and eland, all of which could be contributing to the reproductive bottleneck observed, and would be worth considering in future assessments.

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## TABLES AND FIGURES

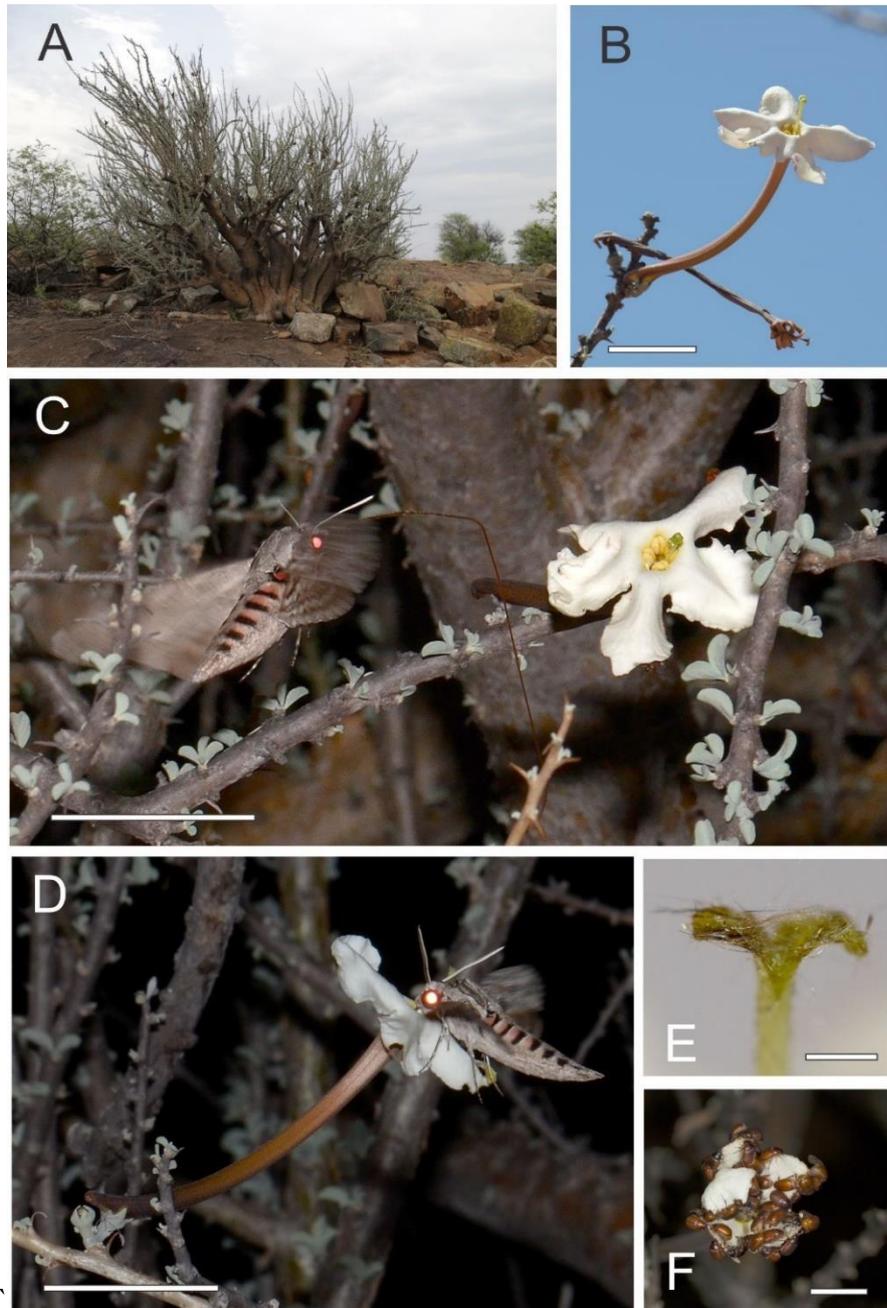


Figure 2-1: The vegetative morphology of a mature *S. lugardii* shrub (A). A *S. lugardii* flower shortly after late afternoon anthesis (B). Scale bar = 3 cm. An *A. convolvuli* hawkmoth with its proboscis extended, approaching a *S. lugardii* flower (C). Scale bar = 5 cm. An *A. convolvuli* hawkmoth foraging for nectar in a *S. lugardii* flower (D). Scale bar = 4 cm. Moth scales on the stigmatic surface of a *S. lugardii* flower (E). Scale bar = 1 mm. Melolonthine scarab beetles feeding on a *S. lugardii* flower during floral anthesis (F). Scale bar = 8 mm.

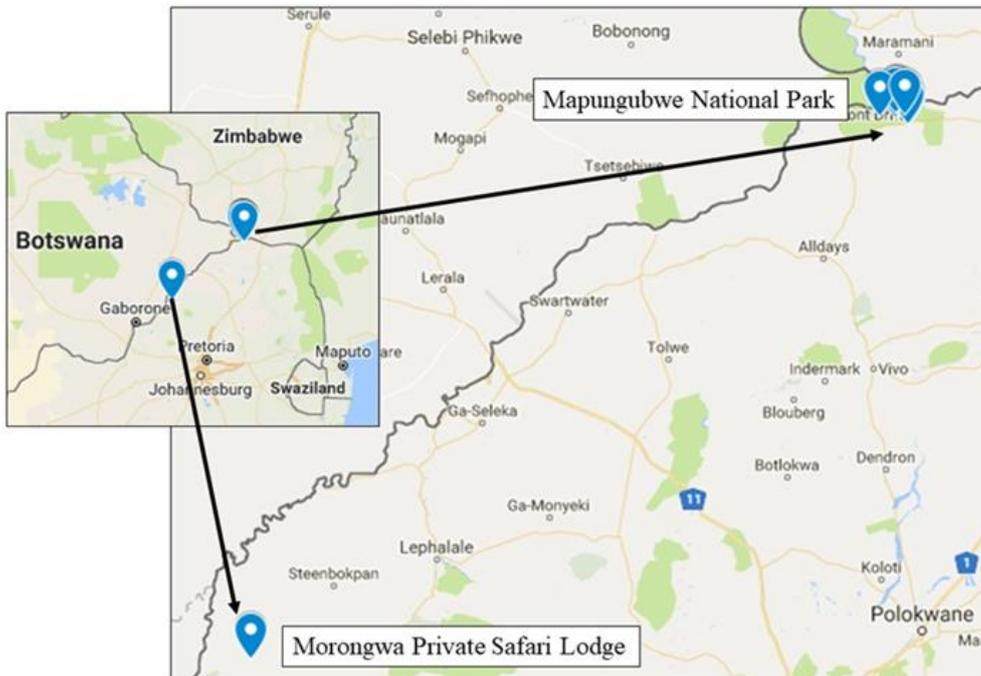


Figure 2-2: Map of the sites in South Africa (Limpopo Province) where *S. lugardii* populations were studied, at Mapungubwe National Park and Morongwa Private Safari Lodge.

## Flower visitor observations

Table 2-1: Table indicating the total number of flower visitors and the number of visits in each impact category (positive or negative) on the opening night of flowers and during the mornings after the opening night, recorded for 40 flowers at Mapungubwe National Park (2015).

Visitor	Flower opening night interactions			Morning after flower opening interactions			Most common behaviour
	Total	Positive	Negative	Total	Positive	Negative	
Hawkmoth ( <i>Agrius convolvuli</i> )	55	23		0			Foraging for nectar
African honey bee ( <i>Apis mellifera scutellata</i> )	1	1		5	5		Foraging for pollen
Cockroach ( <i>Blatella</i> spp)	11			0			Foraging for pollen
Solitary bee (multiple spp)	0			7	7		Foraging for pollen
White-bellied Sunbird ( <i>Cinnyris talatala</i> )	6		5	14			Robbing nectar from spur
Carpenter bee ( <i>Xylocopa</i> spp)	0			1		1	Robbing nectar from spur
Scarab beetle (subfamily Melolonthinae)	5		5	0			Flower predation
Christmas beetle ( <i>Anoplognathus</i> spp)	1		1	0			Flower predation
Small settling moth (multiple spp)	55			0			Walking around on petals
Grasshopper ( <i>Chortophaga</i> spp)	7			2			Walking around on petals
Sugar ant ( <i>Camponotus</i> spp)	6			14			Walking around on petals
Cocktail ant ( <i>Crematogaster</i> spp)	6			7			Walking around on petals
Bal-byter ant ( <i>Camponotus</i> spp)	3			1			Walking around on petals
Ichneumon wasp (Family Ichneumonoidea)	3			1			Walking around on petals
Tiny cicada ( <i>Platypleura</i> spp)	1			0			Walking around on petals
<b>Total</b>	<b>160</b>	<b>24</b>	<b>11</b>	<b>52</b>	<b>12</b>	<b>1</b>	

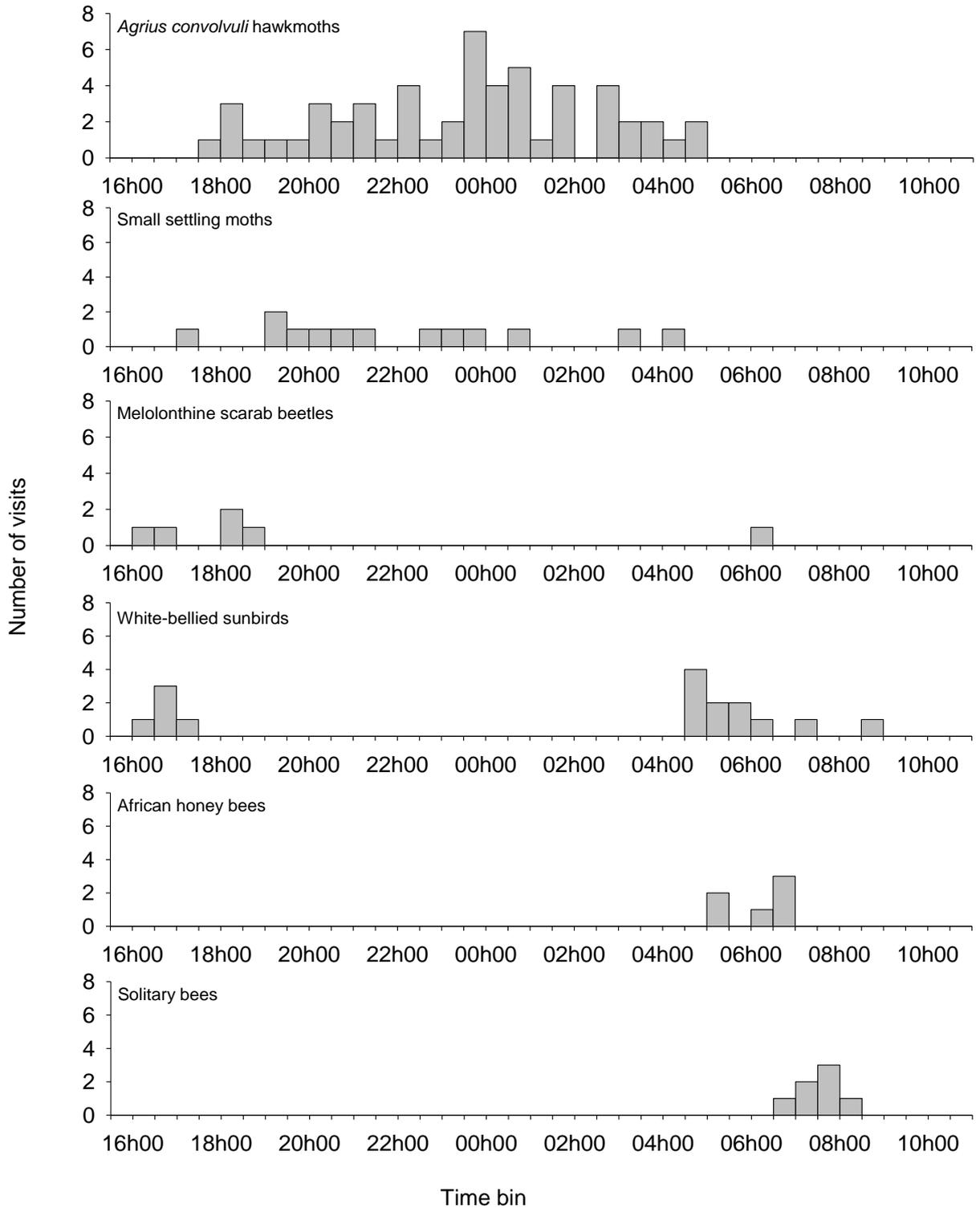


Figure 2-3: The frequency distributions of most common positive and negative visitors of *S. lugardii* flowers, recorded with Bushnell Natureview motion-activated cameras at Mapungubwe (2015).

## Pollen vector confirmation

Table 2-2: Captured *A. convolvuli* hawkmoth proboscis lengths and pollen tetrad counts.

Location	Proboscis length	Pollen grains
Mapungubwe	13.3	172
	12.2	199
Morongwa	10.9	165
	11	189
	9.4	206

## Moth scales as evidence of pollination

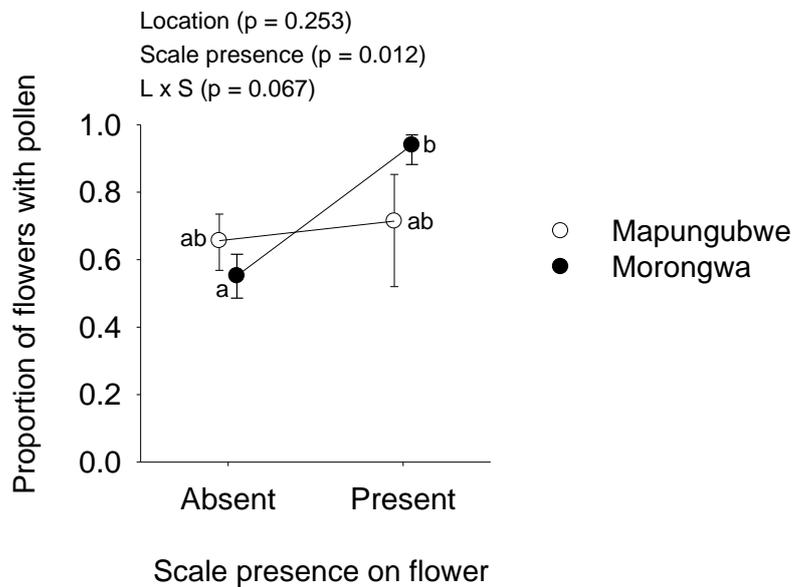


Figure 2-4: Scatter plot of the mean ( $\pm$  SE) proportion of flowers with pollen present on styles in the absence of moth scales (Absent) and in the presence of moth scales (Present) across the two study locations (Mapungubwe and Morongwa). Mapungubwe is indicated by white circles and Morongwa is indicated by black circles. Means that share letters are not significantly different.

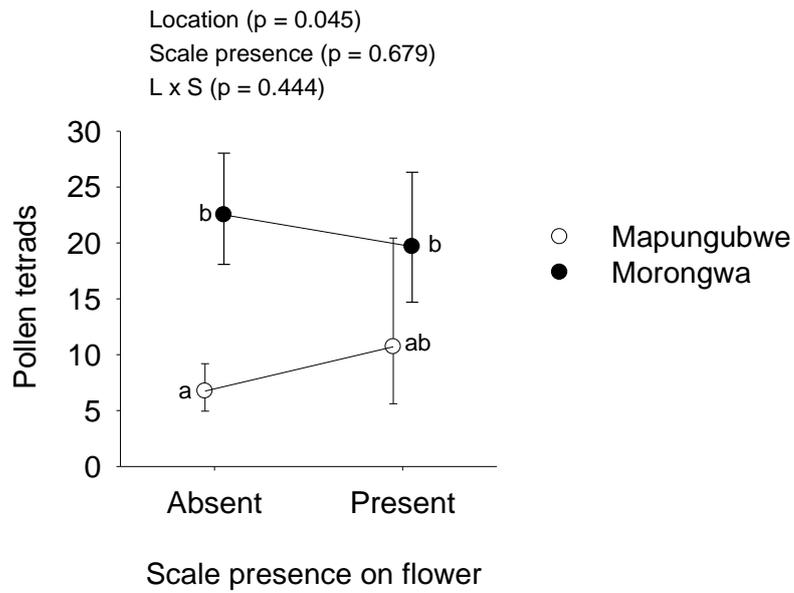


Figure 2-5: Scatter plot of the mean ( $\pm$  SE) number of pollen tetrads present on styles in the absence of moth scales (Absent) and in the presence of moth scales (Present) across the two study locations (Mapungubwe and Morongwa). Mapungubwe is indicated by white circles and Morongwa is indicated by black circles. Means that share letters are not significantly different.

### Pollinator mediated selection on flower tube length

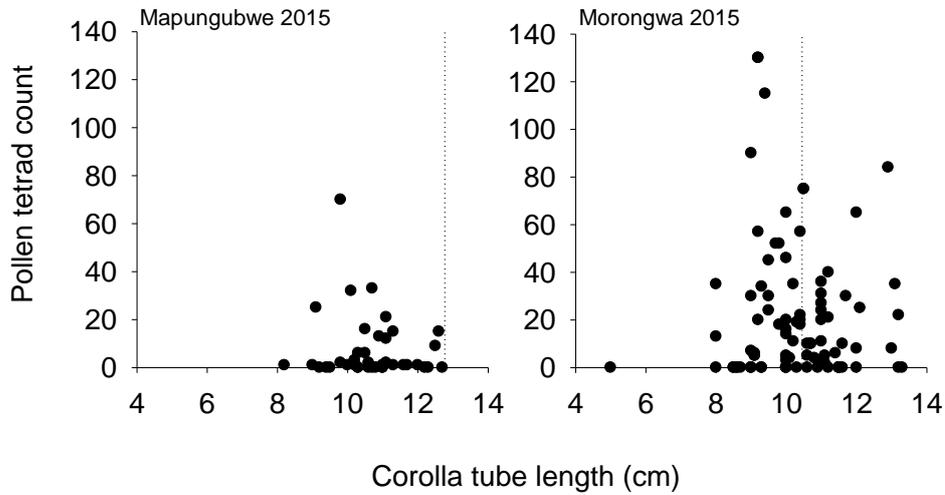


Figure 2-6: Scatter plot of the number of pollen tetrads present on flower stigmas across the two study locations (Mapungubwe and Morongwa) for each corolla tube length measured (cm). The mean proboscis lengths of captured *A. convolvuli* hawkmoths in each location are indicated on the graphs by black, dotted lines.

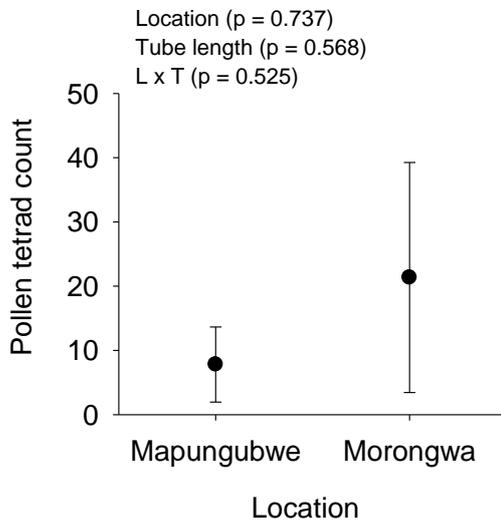


Figure 2-7: Scatter plot of the mean ( $\pm$  SE) number of pollen tetrads present on flower stigmas across the two study locations (Mapungubwe and Morongwa).

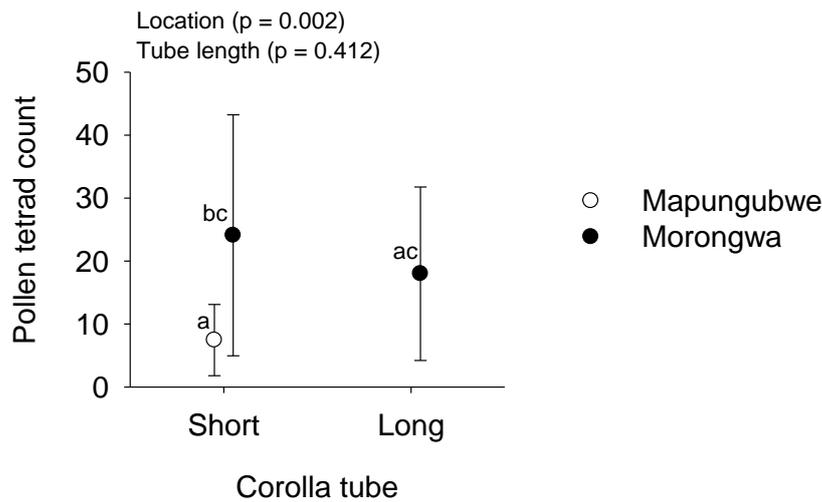


Figure 2-8: Scatter plot of the mean number of pollen tetrads present on flower stigmas of flowers shorter or longer than the mean *A. convolvuli* hawkmoth proboscis length (Short or Long). Mapungubwe is indicated by white circles and Morongwa is indicated by black circles. Means that share letters are not significantly different.

### Plant-pollinator functional fit

Table 2-3: The mean flower corolla tube length (cm) and mean moth tongue length (cm) ratios, as well as the mean flower style length (cm) and mean moth tongue length (cm) ratios, calculated for *S. lugardii* flowers in Mapungubwe (2015) and Morongwa (2015). Measurements that share letters are not significantly different.

Location	Corolla tube/moth tongue	Style/moth tongue
Mapungubwe	0.85	0.93
Morongwa	0.99	1.08

## Plant-pollinator temporal fit: Flower anthesis and pollinator activity

### Floral anthesis

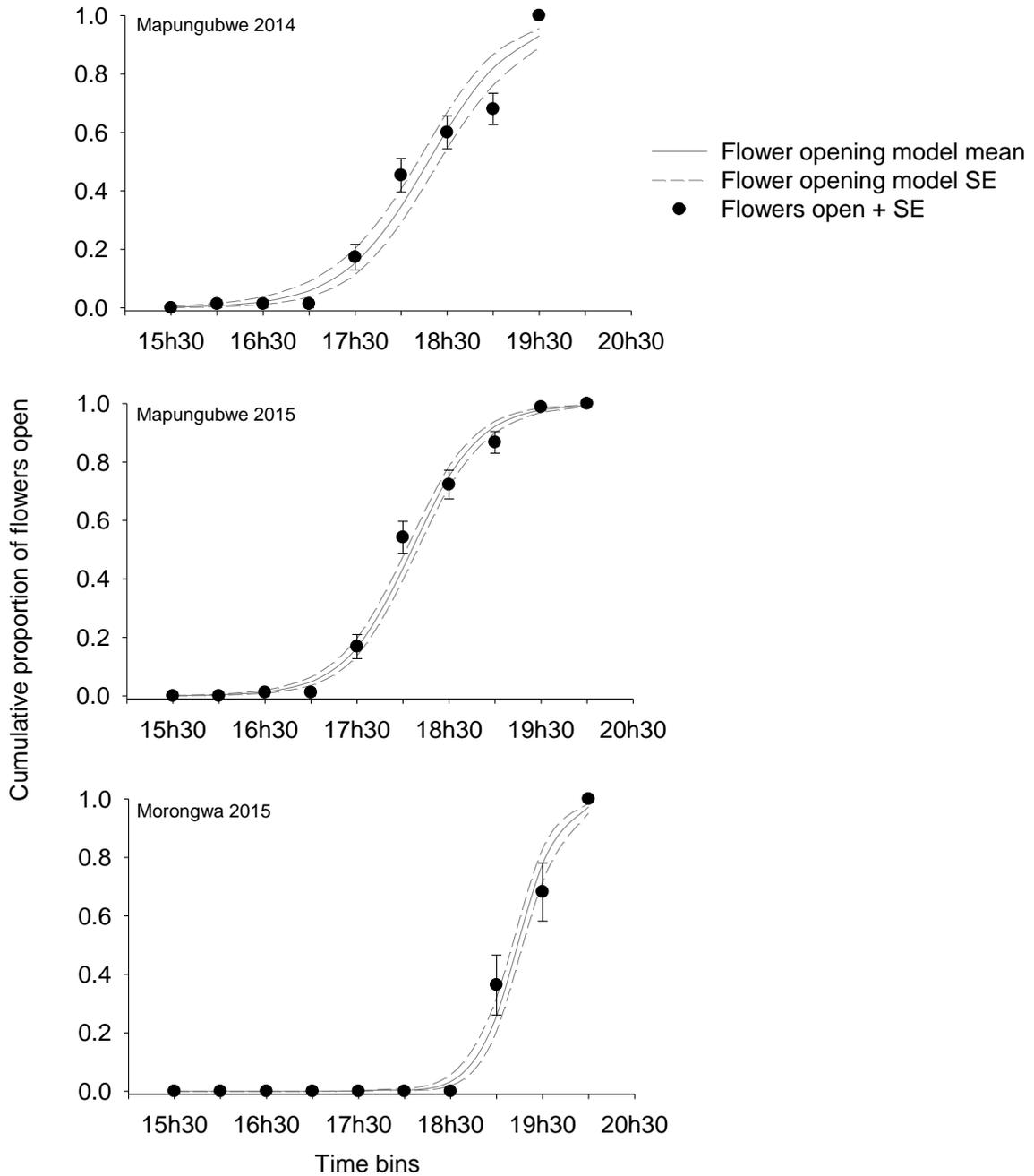


Figure 2-9: The cumulative number of *S. lugardii* flowers open per 30-minute time bin, at Mapungubwe (2014 and 2015) and at Morongwa (2015). The observed number of flowers open indicated by black circles, and the GLM binomial logistic regression model means and predicted standard errors indicated with dotted and solid grey lines respectively.

## Nectar volume and concentration

Table 2-4: A summary of nectar volume ( $\mu\text{L}$ ) and concentration (%) recorded in a sample of flowers at Mapungubwe (2016).

Variable	Range	Mean $\pm$ SD (n)
Nectar volume ( $\mu\text{L}$ )	2.2 - 19	$6.06 \pm 3.62$ (27)
Nectar concentration (%)	12.5 - 33	$21.87 \pm 4.98$ (23)

## Breeding system

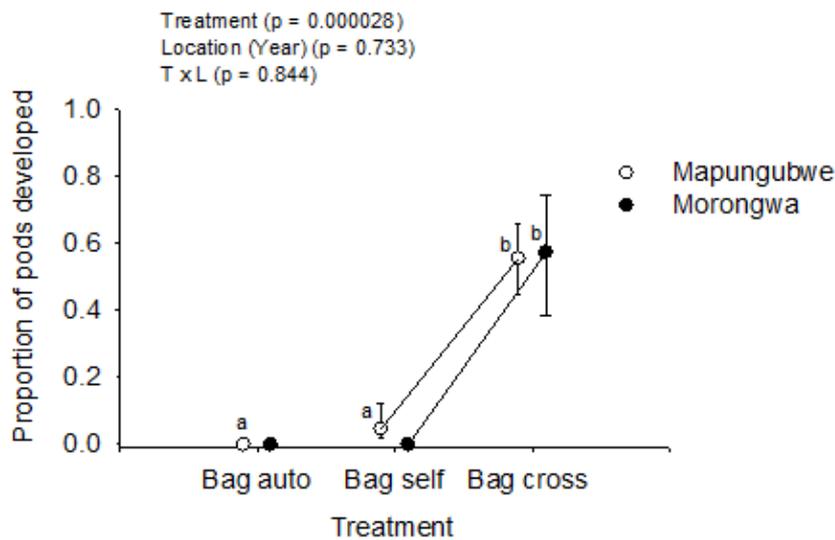


Figure 2-10: The mean ( $\pm$  SE) proportion of pods that developed in hand-pollination experiments, where flowers were left untreated and bagged (Bag auto), pollinated with own pollen and bagged (Bag self) or pollinated with a conspecific plant's pollen and bagged (Bag cross). Mapungubwe is indicated by white circles and Morongwa is indicated by black circles. Means that share letters are not significantly different.

## Pollinator exclusion

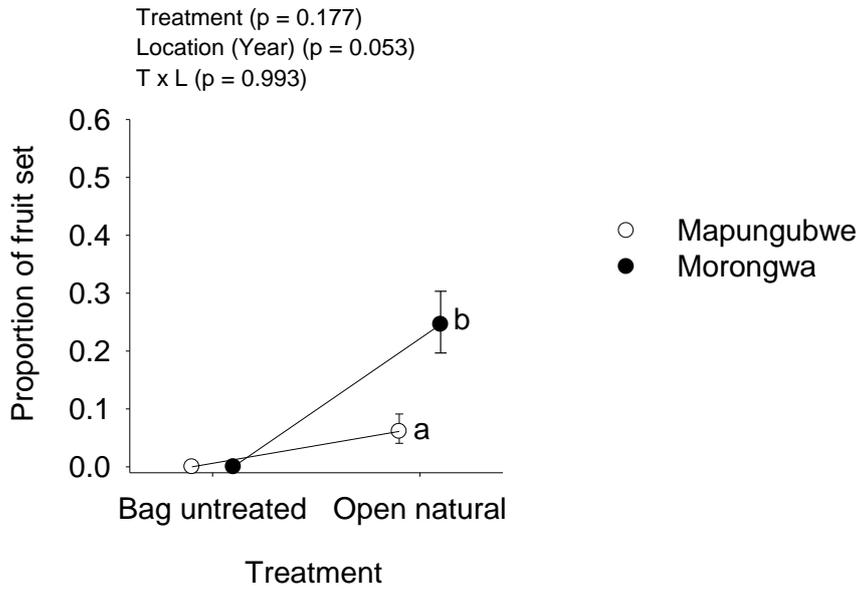


Figure 2-11: The mean ( $\pm$ SE) proportion of pods that developed in hand-pollination experiments, where flowers were left untreated and bagged to exclude pollinators (Bag untreated), or left open and accessible to pollinators (Bag cross). Mapungubwe is indicated by white circles and Morongwa is indicated by black circles. Means that share letters are not significantly different.

## Natural pollination rate and pollen limitation

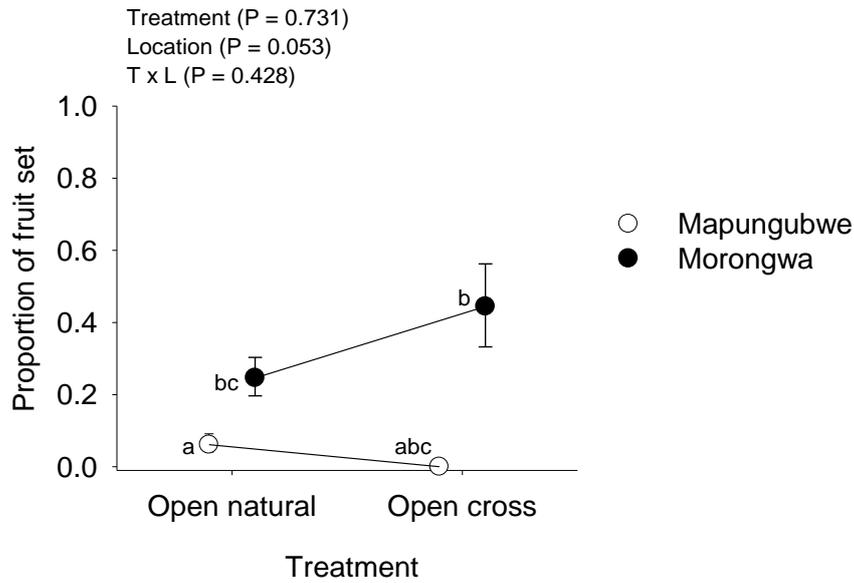


Figure 2-12: The mean ( $\pm$  SE) proportion of pods that developed in hand-pollination experiments, where flowers were left untreated and open to natural pollination (Open natural) or left open and supplemented with cross-pollen (Open cross). Mapungubwe is indicated by white circles and Morongwa is indicated by black circles. Means that share letters are not significantly different.

## The impact of florivory on plant fecundity

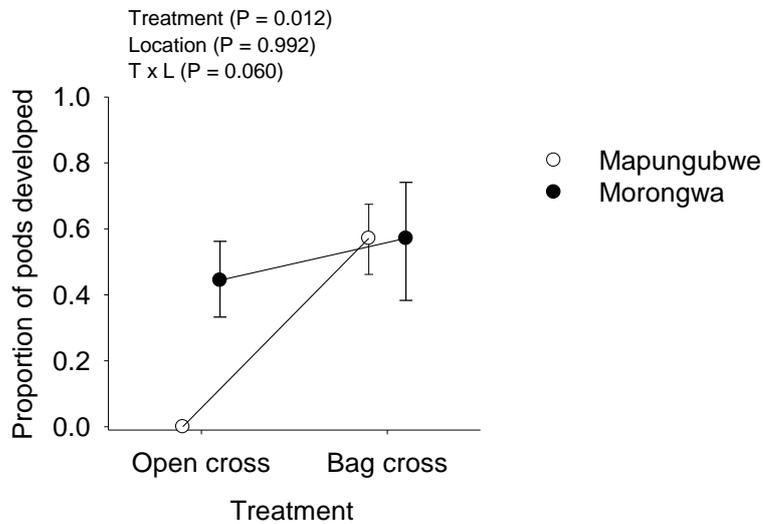


Figure 2-13: The mean ( $\pm$  SE) proportion of pods that developed in hand-pollination experiments, where flowers were cross-pollinated by hand and left open (Open cross) or cross-pollinated by hand and left open (Bag cross). Mapungubwe is indicated by white circles and Morongwa is indicated by black circles.

## Beetle presence at the study locations

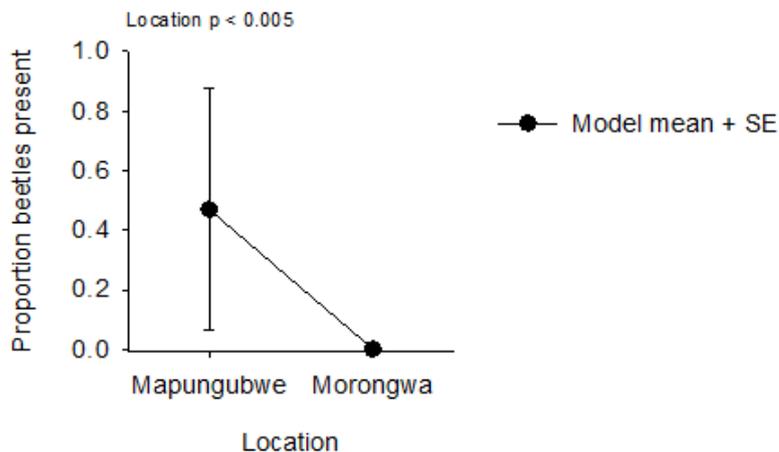


Figure 2-14: The mean ( $\pm$  SE) proportion of beetles present at Mapungubwe (2015) and Morongwa (2015).

***Beetle activity over time***

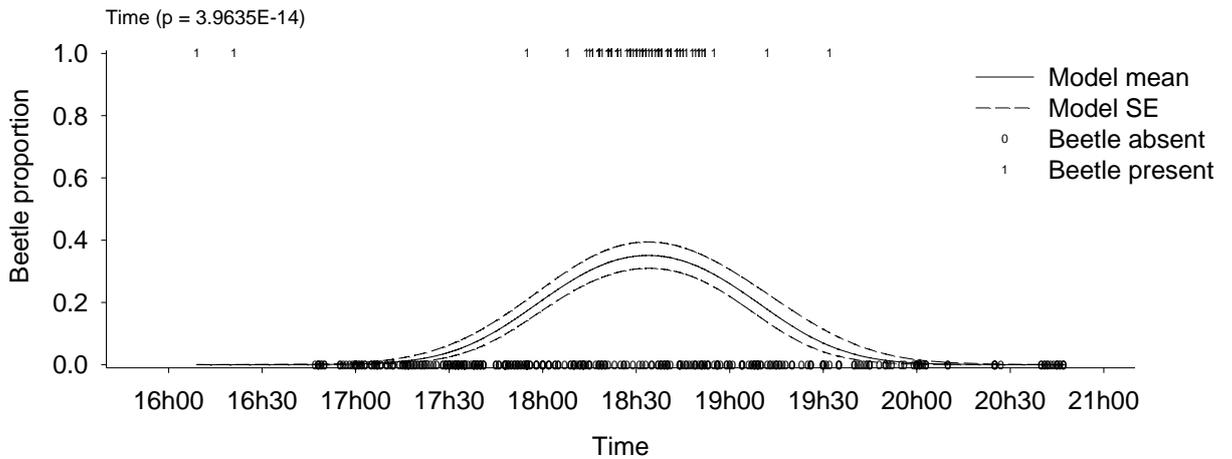


Figure 2-15: Beetle presence over time modelled with a binomial logistic GLM, for Mapungubwe (2015), with binary beetle observation results (absence = 0 and presence = 1) plotted over time. The significance value of the predictor variable, as determined by the GLM, is recorded above the graph.

***Flower opening time and beetle visits***

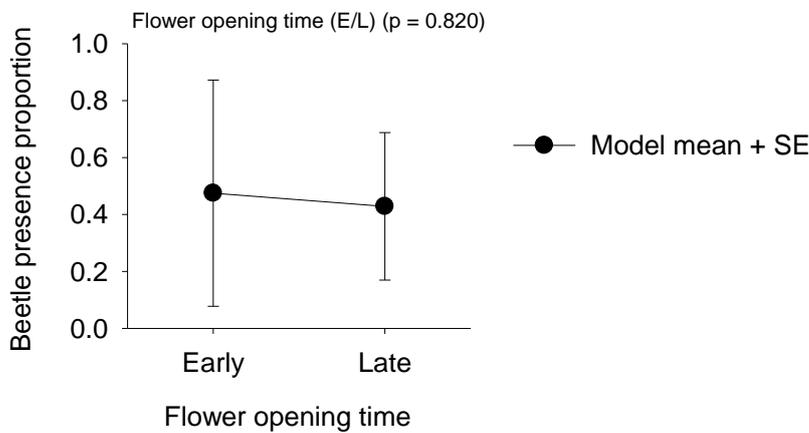


Figure 2-16: The mean proportion of beetles observed on early- (<19h00) and late- (>19h00) opening flowers at Mapungubwe (2015). The significance value of the predictor variable, as determined by the GLM, is recorded above the graph.

## Beetle visitation and style damage

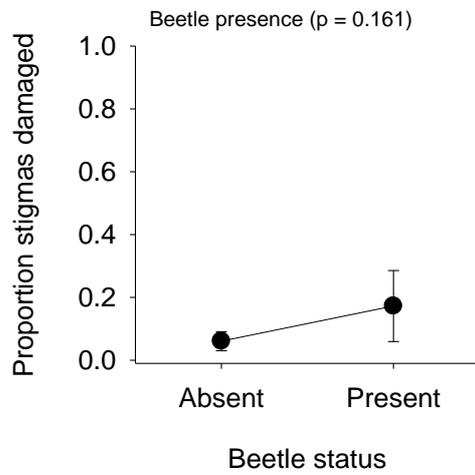


Figure 2-17: The mean proportion of flowers that have signs of stigma damage at Mapungubwe (2015) when visited by beetles (Present) or ignored by beetles (Absent).

### ***Beetle visitation and pod formation***

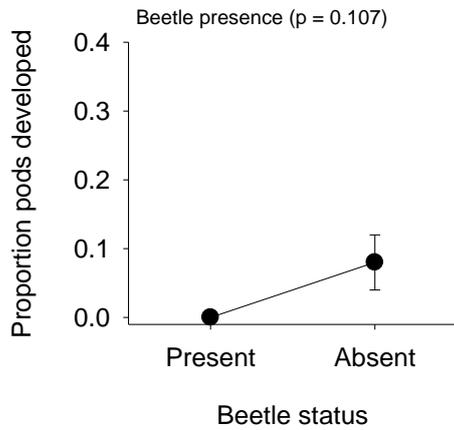


Figure 2-18: The mean proportion of pods that developed at Mapungubwe (2015) from flowers visited by beetles (Present) and flowers not visited by beetles (Absent).

### ***Stigma/style damage and pod outcomes***

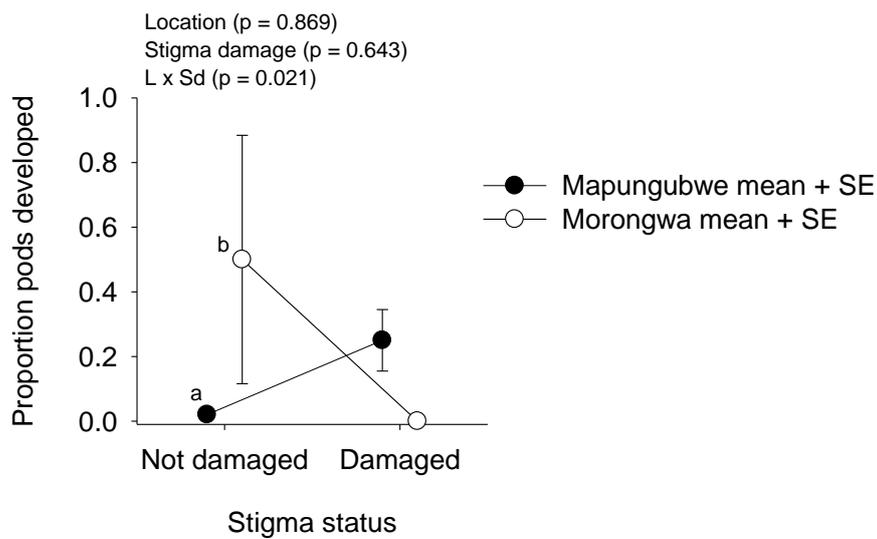


Figure 2-19: The mean ( $\pm$  SE) proportion of pods that developed at Mapungubwe (2015) when stigmas were damaged (Damaged) and when stigmas were undamaged (Not damaged). Means that share letters are not significantly different.

## CHAPTER 3:

### **The demography of *Sesamothamnus lugardii* (Pedaliaceae): the message in a bottleneck.**

#### **ABSTRACT**

The vulnerability of *Sesamothamnus lugardii* to extirpation in the face of a pollination mutualism collapse was assessed, because *S. lugardii* has high-risk breeding system traits (specialised for hawkmoth pollination and self-incompatible). The demographic traits of *S. lugardii* were assessed, to determine if they were also high-risk, rendering the populations vulnerable to extirpation. Two *S. lugardii* populations were assessed in arid savanna ecosystems in Limpopo, South Africa. In both systems seedlings were scarce and the populations were experiencing severe recruitment bottlenecks, due to predation of flowers and immature seed pods, limited germination, and poor survival of seedlings. The populations were dominated by large shrubs, most exhibiting some canopy loss and bark loss, attributed to herbivory by eland and kudu. Shrubs of all sizes showed signs of bark regrowth and, in larger shrubs particularly, vigorous resprouting. Once *S. lugardii* shrubs grow large, they are highly resilient to herbivory and drought, ensuring longevity. This lowers the demographic dependence of *S. lugardii* populations on seeds, because the resilient shrubs store reproductive potential and keep the risk of extirpation low. An increase in the rate of mortality of established shrubs would increase the risk of extirpation rapidly in the study populations, because all the reproductive potential in the populations will be lost, and there are almost no smaller cohorts in the populations that could grow to replace the lost adults.

#### **KEYWORDS**

*Sesamothamnus lugardii*, Mapungubwe National Park, demographic profiles, woody savanna perennials, size-class distributions, demographic bottlenecks, extinction risk

## INTRODUCTION

Pollination mutualisms are key interactions that influence the demography of plant populations, because they influence reproductive output. Over 80 % of angiosperm species are dependent on biotic pollinators (Mitchell, *et al.*, 2009; Ollerton, Winfree & Tarrant, 2011), and when pollination mutualisms collapse, the plant populations that are dependent on these interactions experience reproductive failure, and may even face local extirpation and ultimately extinction. In the Bahamas, hurricane Lili had caused a severe decline of two key bird pollinators, and as a result the endemic shrub, *Pavonia bahamensis* (Malvaceae) suffered pollen limitation and an 89 % loss in fruit set (Rathcke, 2000). In New Zealand, the anthropogenic introduction of predators caused the extirpation of two major bird pollinators, which resulted in pollination failure in populations of the endemic shrub, *Rhabdothamnus solandri* (Gesneriaceae), and led to declines in shrub population densities (Anderson, *et al.*, 2011). In the Cape Floristic Region of South Africa, the absence of an oil-collecting bee (*Rediviva peringueyi*) resulted in six bee-specialised plant species failing to set seed (Pauw, 2006). Two hawkmoth-pollinated *Brighamia* species in Hawaii had extremely low seed set and limited recruitment in populations where their hawkmoth pollinators were absent due to habitat fragmentation, and the same was observed for a small population of *Oxyanthus pyriformis* subsp. *pyriformis* in South Africa, when hawkmoths were absent (Johnson, *et al.*, 2004).

In a seminal paper, Bond (1994) developed a practical approach for determining the vulnerability of a plant species to extinction risk in the face of a mutualism collapse, based on breeding system traits, dispersal traits and demographic traits. These traits get ranked from zero to one, with one representing the greatest vulnerability to extinction, and zero, the lowest. The “high-risk” traits (approaching a value of one) include pollination specialisation, self-incompatibility, seed dependence (obligate seeding) and seed dispersal specialisation. The “low-risk” traits (approaching zero) include pollination generalisation, self-compatibility, an escape from seed dependence and no dispersal specialisation.

Plant species with high-risk breeding system traits, high-risk dispersal traits and high-risk demographic traits, approach an overall vulnerability value of one (Figure 3-1, Box A), and are most vulnerable to extinction. Such species, however, are uncommon. Many plants which have high-risk demographic traits also have compensatory low-risk breeding system traits, and *vice versa* (Bond, 1994).

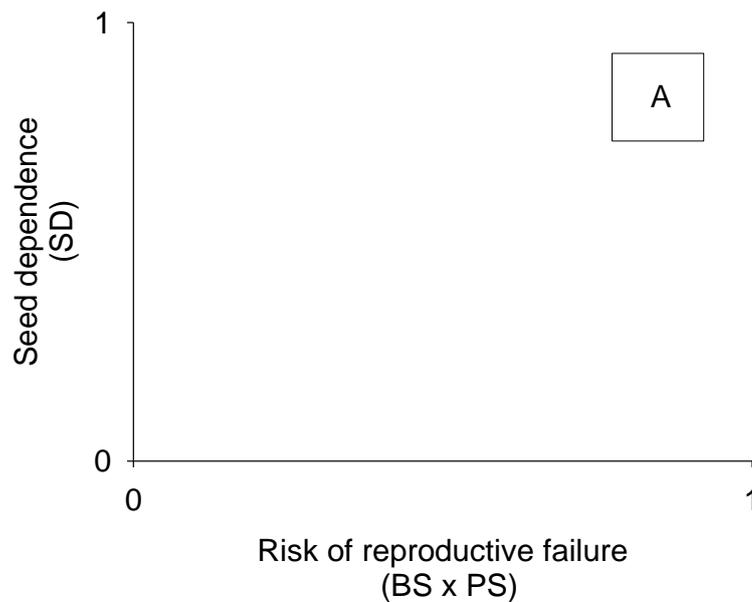


Figure 3-1: Extinction vulnerability plot adapted from, with demographic vulnerability (seed dependence) plotted against breeding system vulnerability (risk of reproductive failure). SD = seed dependence, BS = breeding system, PS = pollinator specificity. Plants which approach an overall vulnerability score of one fall in zone A, the high-risk zone.

In South African Fynbos ecosystems, many plants in the Proteaceae family produce few seeds and cannot resprout after fire (Kearns & Inouye, 1997), making them demographically dependent on seeds (a high risk trait). These species, however, are often also pollination-generalists or are self-compatible (low risk traits). These low-risk traits ensure continued plant survival by providing a higher degree of reproductive assurance in environments where pollinator availability or fidelity is uncertain ( Bond, 1994; Johnson & Steiner, 2000; Busch, 2005). These low-risk pollination strategies also allow plant populations to persist when specific pollinators are lost from the ecosystem. When plants have both high-risk traits and compensatory low-risk traits, the overall

“vulnerability score” is reduced. Motten (1986) assessed wildflowers in a deciduous temperate forest in North Carolina, and Bond (1994) noticed that most of the pollination-specialised plants clustered at the low-risk end of the seed-dependence axis (Figure 3-1), because they could reproduce vegetatively and were long-lived.

Vegetative reproduction by clonal growth or resprouting can buffer plants from reproductive failure for very long periods of time (Bond & Midgley, 2001), for example, some populations of the South African fynbos shrub, *Ixianthes retzioides* (specialised for pollination by oil-collecting bees), have been separated from their pollinators by habitat fragmentation, yet have persisted for decades in spite of reproductive failure, through vegetative reproduction (Kearns & Inouye, 1997). In angiosperms, vegetative reproduction is a very widespread demographic trait (or life history strategy) that has evolved across multiple ecosystems, not only to compensate for pollination failure, but also to ensure survival in the face of other disturbance processes that limit plant recruitment and cause mortality. In moist tropical forests, for example, where persistent shade and falling debris limit seedling and sapling survival, vegetative reproduction is a key persistence strategy (Poorter, *et al.*, 2010). In savanna systems, resprouting in response to both fire and herbivory is a very common survival strategy (Gignoux, Clobert & Menaut, 1997; Nefabas & Gambiza, 2007; Sankaran, Augustine & Ratnam, 2013).

Using Bond’s (1994) approach, one can assess the traits of a plant species and predict its inherent vulnerability to extinction in the face of a mutualism collapse. However, for predicting the risk of a plant population to extirpation, a detailed assessment of demography of that population is necessary. The demography of a plant population refers to the fluctuations in population density and structure (size-class distribution) over space and time (Jakob, *et al.*, 2010). The demography of a population, therefore, is influenced by both the life history strategy of the species in question (reproductive and demographic traits), and the environmental processes that affect the population. A population’s demographic profile can be shaped by the environmental processes that facilitate or limit recruitment (the production of genets), size-class transitions (increases or decreases in biomass) and mortality (Midgley, Lawes & Chamailí-Jammes, 2010).

Although a plant species may have low-risk breeding system and demographic traits, its populations may still experience demographic bottlenecks that put them at a high risk of extirpation. Bottlenecks refer to processes and events that kill or prevent reproduction in a large proportion of individuals in a population. Bottlenecks can be caused by changes in environmental conditions, such as the loss of pollinators from an ecosystem due to habitat fragmentation, the onset of a severe drought in a mesic system, a large increase or decrease in herbivore or florivore numbers in an ecosystem or, in fire-prone systems, the suppression of fire.

In savanna ecosystems, top-down disturbance processes such as fire and herbivory can severely limit plant recruitment and survival (Midgley & Bond, 2001) by creating demographic bottlenecks. Fires in mesic savannas are typically frequent (occurring every one to three years) and of fairly low intensity, but can damage flowers and seeds, kill seedlings and prevent the transitions of small plants into larger, reproductive size-classes (Gignoux, Clobert & Menaut, 1997; Higgins, Bond & Trollope, 2000; Hoffmann, Orthen & Nascimento, 2003; Sankaran, Ratnam & Hanan, 2004; Grange & Duncan, 2006; Hoffmann, *et al.*, 2009; Midgley, Lawes & Chamailí-Jammes, 2010). Herbivores also impose limitations on recruitment and survival: selective browsing by small herbivores kills seedlings (Augustine & McNaughton, 2004; Moe, *et al.*, 2009; Midgley, Lawes & Chamailí-Jammes, 2010) and so does trampling by large and small herbivores alike (Cumming & Cumming, 2003; Thompson Hobbs & Searle, 2005). Browsing by mega- and meso-herbivores can also prevent the transition of smaller plants into larger, reproductive size classes (Scholes & Walker, 1993; Jacobs & Biggs, 2002; Scholes, Bond & Eckhardt, 2003).

Due to the demographic bottlenecks created by these frequent and intense top-down disturbance processes, most woody, perennial savanna trees and shrubs are capable of some form of vegetative reproduction, which allows them to persist through periods of failed seed set and recruitment. Regeneration through sexual reproduction is actually considered a relatively rare occurrence in savanna ecosystems (Bengtsson-Sjörs, 2006).

In disturbance-driven savannas, the populations of plants that do well all have some form of stored reproductive or regenerative potential. In a population of resprouting

savanna plants, those plants that persist through disturbance events and reach reproductive maturity represent a store of reproductive potential in the population (Higgins, Pickett & Bond, 2000). In a population of non-resprouting, disturbance-sensitive plants, those plants that reach maturity and set seed between disturbance events ensure regenerative potential, because although those plants do not survive disturbance events, the regenerative capacity of the population is stored in the seeds they produced.

Resprouting species typically allocate more resources to below-ground structures than to above-ground growth, compared to non-sprouters (Bond & Midgley, 2003). Consequently, plant growth in resprouters is slower, saplings are slower to reach maturity and seed output is lower than in non-sprouters (Bond & Midgley, 2003). These co-occurring “low and slow” traits in resprouting species make plants in small size classes very vulnerable to disturbance, but once the plants grow large enough, and have accumulated sufficient belowground storage to resprout, they become highly disturbance-tolerant, so these low and slow traits result in a life history strategy of persistence. Obligate seeders, however, typically have a short life-cycle, rapid accumulation of above-ground biomass and a fast growth rate, which allows the plants to complete their life cycles and set seeds between disturbance events. Such species typically also have larger seed output than resprouters, and have resilient seeds and large seedbanks, in which they store their reproductive potential (Higgins, Pickett & Bond, 2000). These traits are rarely seen in woody savanna species (Nano & Clarke, 2011), because populations of obligate seeders have a high degree of demographic dependence on seeds, and this is not an adaptive strategy in frequently disturbed ecosystems.

The characteristics of a population’s demographic profile can provide insight into the life history strategy of the species, reveal whether the population stores reproductive potential, provide insight into the effects of past perturbations (one can see the effects of demographic bottlenecks) (Shackleton, 1993) and can reveal whether the population is at risk of extirpation in its current environmental conditions. This information can be

used to make predictions about how the population will respond to changing environmental conditions.

Goldberg and Turner (1986) found that slow-growing, long-lived species typically have erratic recruitment, low population density and age structures skewed towards larger, older plants. Short-lived, fast-growing plants, however, typically have regular recruitment, high population densities and age/size structures skewed towards the youngest cohorts (Goldberg & Turner, 1986). In ecosystems such as forests, where disturbances are limited or absent, the self-replacement rate in plant populations is typically high, and the largest number of plants are usually in the smallest size classes (McCarthy & Weetman, 2006; Wang, *et al.*, 2009; Giroldo & Scariot, 2015). The size-class distributions of these plant populations often have a “classic reverse-J” shape (Lykke, 1998). In arid savanna ecosystems, where there are fluctuations in the frequency and intensity of ecosystem controlling variables such as fire, herbivory and rainfall, there will be corresponding fluctuations in recruitment and mortality events in the plant populations, resulting in populations without the classic, reverse-J-shaped demographic profiles (Hall & Bawa, 1993; Lykke, 1998).

## **Study aim**

*Sesamothamnus lugardii* flowers are highly specialised for pollination by a single hawkmoth species (*Agrius convolvuli*) and are also self-incompatible (Chapter 2), so *S. lugardii* has high-risk breeding system traits. Little is known about the demography of the species, so in this chapter, I will explore the demographic traits of *S. lugardii*, to determine whether it has any low-risk demographic traits that offset its breeding system vulnerability. I will use Bond's vulnerability index (1994) to determine the inherent risk of *S. lugardii* to extinction in the face of a pollination mutualism collapse. In addition to describing the life history strategy of the species, I will explore the demographic profiles of two *S. lugardii* populations, identify the key environmental factors that shape the demography of these populations, and consider how variations in environmental conditions could alter the risk of extirpation in these populations.

## **Chapter objectives**

I will describe the physical characteristics of *S. lugardii* plants to better understand the life history strategy of the species.

I will use demographic profiles to determine potential demographic bottlenecks, explore the recruitment strategy of the species, identify where the populations store reproductive potential, and determine the degree of demographic dependence that the *S. lugardii* populations have on seeds.

I will assess how *S. lugardii* shrubs respond to disturbance, specifically herbivory (as fire has been suppressed at the study sites), and thereby determine if *S. lugardii* shrubs are prone to, or tolerant of disturbance.

I will determine if *S. lugardii* has low-risk demographic "compensation traits" that allow populations of this highly specialised, self-incompatible species to persist through periods of pollination and reproductive failure.

## METHODS

### The study species

*Sesamothamnus lugardii* N. E. Br. Ex Stapf., the Transvaal Sesame-bush (Pedaliaceae), is an arid-savanna succulent shrub which occurs throughout Zimbabwe, as well as in southern Botswana and in South Africa, in the Limpopo River Valley (Coates Palgrave, 1977). *S. lugardii* shrubs typically grow in hot, dry climates and are often found on rocky ridges and in calcareous soils. These perennial shrubs are stem succulents and are typically multi-stemmed, with tertiary branches that are highly ramified (Figure 1-1, A). The shrubs have sharp spines on the secondary and tertiary branches, and the bark is finely flaking and yellow-brown to coppery in colour (Coates Palgrave, 1977). *S. lugardii* flowers are white, sweetly scented, have impressively long, narrow corolla tubes and open in the evening (Figure 1-1, C and D). They are pollinated by only one hawkmoth species, *Agrius convolvuli*, (Figure 2-1, C and D) and are self-incompatible, which makes *S. lugardii* completely dependent on its hawkmoth pollinator for fertilisation and seed set. The seed pods are green and fleshy during development (Figure 1-1, G), but harden and desiccate with maturation and dehisce to release winged, wind dispersed seeds (Coates Palgrave, 1977) (Figure 1-1, H).

### Field sites

Two populations of *S. lugardii* were assessed in the Limpopo province of South Africa: one at Mapungubwe National Park (Mapungubwe), and one on a private game farm called Morongwa Private Safari Lodge (Morongwa) (Figure 3-2). In this chapter, “Mapungubwe” refers to the plant populations at Mapungubwe National Park and “Morongwa” refers to the plant population at Morongwa Private Safari Lodge. Both the Mapungubwe and Morongwa study sites are located in arid savanna ecosystems. The mean annual precipitation typically ranges between 300-400 mm at Mapungubwe, and the mean annual rainfall around Morongwa is about 500 mm (Mucina & Rutherford, 2006). In 2015 when the Morongwa site was surveyed, the area received between 50-100 mm of rainfall, so was experiencing a severe drought.

At both study locations trees and shrubs were sparsely interspersed, grass biomass was relatively low and *S. lugardii* shrubs were observed to be the locally dominant shrub species in small patches around the landscape. At Mapungubwe, *S. lugardii* shrubs were found near large, rocky, sandstone outcrops and on open, sandy plains comprised of both quartz-rich sandy soils and loamy sands derived from calcareous and sandstone rock. At Morongwa the shrubs were found on and around a series of rocky dolerite ridges and on the surrounding sandy plains comprised of loose, red sands.

### **Data collected**

At Mapungubwe, several small sub-populations of *S. lugardii* shrubs were surveyed on three occasions over a ten-year period (Figure 3-3), and one population was surveyed once at Morongwa. At Mapungubwe, study sites B1 to B5 were surveyed from November 18<sup>th</sup> - 19<sup>th</sup> in 2005, sites F1 to F4 were surveyed from November 8<sup>th</sup> - 10<sup>th</sup> in 2008 and site A1 was surveyed from November 11<sup>th</sup> - 15<sup>th</sup> in 2014. At Morongwa, the study site was surveyed from November 13<sup>th</sup> - 17<sup>th</sup> in 2015.

At Mapungubwe, the sub-populations assessed each year were analysed together as a single large population. At Morongwa, only one large patch of shrubs was found on the farm and was analysed as a single small population. In each patch, all the shrubs were sampled.

The physical characteristics of *S. lugardii* as a species were observed visually and described, so that inferences could be made about the life history strategy of the species.

For each population at Mapungubwe (2005, 2008 and 2014) and Morongwa (2015), the height (m) of each shrub was recorded. These data were used to create demographic profiles and height-class distributions, and to do so, shrubs were categorised according to biologically meaningful height-classes (Table 3-1). Height distributions were chosen over diameter distributions, because in savanna systems where herbivore densities are high and trees experience frequent disturbance, measures of basal stem diameter can over-estimate the “true size” of the tree (Holdo, 2007). In disturbed savanna systems, tree height provides a realistic size metric for population analysis.

At Mapungubwe (2005 and 2008), the browsing herbivores associated with *S. lugardii* shrubs were determined using dung presence near shrubs as a proxy for residence time. If dung was present within a 5 m radius of an *S. lugardii* shrub, it was recorded and identified (to herbivore species).

For each shrub in the study populations assessed at Mapungubwe (2008), herbivore impact was quantified by estimating the percentage of bark removed (% stem stripped) and the percentage of canopy loss (% branches broken). Recovery was recorded if a shrub showed signs of bark regrowth (yes or no) and signs of epicormic resprouting (yes or no).

At Mapungubwe (2014), and Morongwa (2015), the height (m), numbers of flowers and number of seed pods was recorded for each shrub, to determine the proportion of shrubs in each size class producing reproductive structures, and to assess the reproductive output (abundance of structures) across size-classes. Reproductive output was then compared between the Mapungubwe (2014) and Morongwa (2015) populations.

### **Data analysis**

Demographic profiles were created for populations at Mapungubwe in 2005, 2008 and 2014 and at Morongwa in 2015, by ranking all the shrubs according to height, and graphing the distributions using histograms. A moving window analysis was then done for each distribution, where, for each population, shrub heights were ranked and then the mean height and standard deviation of an initial fixed subset of heights was calculated. To compute the next mean height and standard deviation, the fixed subset was shifted forward (by excluding the first number of the series and including the next value in the subset). The fixed subset value was calculated by dividing the sample size by 10, and rounding up to the nearest whole number. The standard deviation values for each population were then plotted as a line graph overlaying the demography histogram. Large peaks in the standard deviation line act as indicators of large height-changes in the ranked population (potential demographic bottlenecks).

Size-class distributions for the populations were created using shrub heights. *S. lugardii* shrubs were assigned to biologically meaningful height-classes (Table 1-2) based on their measured heights. For each population (Mapungubwe 2005, 2008 and 2014, and Morongwa 2015), the frequency of shrubs in each height class was calculated and displayed with a bar graph.

To determine the herbivore species impacting *S. lugardii* shrubs at Mapungubwe (in 2005 and 2008) the proportions of shrubs in each population with dung in close proximity were calculated. The proportion of dung present per herbivore species was used as a proxy for residence time of herbivores.

The mean percentage of bark removed and canopy lost was calculated for each size class in each population, and displayed in a table and with stacked bar graphs. The proportion of shrubs showing signs of recovery from bark stripping and canopy loss in each size class and population was also calculated and displayed with scatter plots.

For the Mapungubwe (2014) and Morongwa (2015) populations, the proportions of shrubs in each size class producing flowers and pods were calculated using events by trials GLMs with binomial distributions and logit link functions. For each population, the number of shrubs in each height class represented the trials, the number of flowers or pods respectively, the events, and the predictor variable was the height class (seedling, small sapling, *etc.*).

For both the Mapungubwe and Morongwa populations, binomial logistic GLMs with logit link functions were run, with shrub height as the predictor variable, and with flowers or pods, respectively, present (yes or no) as the outcome variable. The shrub heights associated with the modelled peak rate of increase in the probability of having flowers and pods were then calculated using the regression coefficients of the GLMs, substituted into Equation 1.

**Equation 1:**

$$X = \text{Ln} \left( \frac{P}{1 - P} \right) = \beta_0 + \beta_1$$

Where  $\beta_0$  and  $\beta_1$  are the regression coefficients

$P$  = probability of a shrub having reproductive structures

$X$  = Shrub height

For peak rate of change  $P = 0.5$

The mean number of flower structures (developing and open flowers) and pods on shrubs in each height class were calculated for the Mapungubwe (2014) population and the Morongwa (2015) population, and displayed with scatter plots.

**Notes on statistical analyses**

All statistical analyses were done using IBM's SPSS® Statistics, version 23 software, and for all generalised linear models (GLMs), the chi-square likelihood ratio ( $\chi^2$ ) was used as the test statistic to assess the significance (p) of the model effects.

## RESULTS

### Structural characteristics of *S. lugardii* shrubs

*S. lugardii* shrubs are stem succulents, and often have disproportionately fat trunks relative to their height (Figure 3-4, A and C). Many *S. lugardii* shrubs are multi-stemmed, with highly ramified secondary and tertiary branches (Figure 3-4, A). *S. lugardii* shrubs also have sharp spines on the secondary and tertiary branches (Figure 3-4, B), so appear to be well defended against herbivory. In 2015, a few seedlings were germinated from seed in a nursery by P. Viljoen, and the stems of these new seedlings were soft and fleshy, with no protective bark (Figure 3-4, G). Within a year, these seedlings had developed the flaking bark characteristic of the species, and while some seedlings were single-stemmed, others were multi-stemmed, despite being grown under the same conditions and excluded from disturbance in the controlled nursery environment (A. Bijl pers. obs.).

Almost all the shrubs in the study populations at Mapungubwe (where herbivores were present) showed signs of damage in the form of broken secondary and tertiary branches and also showed signs of bark damage (Figure 3-4, D and E). Many of the larger shrubs at Mapungubwe had old strip scars (where bark had previously been removed) which were sealed or partially sealed, and many shrubs that had experienced canopy loss appeared to be resprouting (Figure 3-4, F). At Morongwa, where herbivores were almost entirely excluded, canopy loss in the form of broken branches, and bark damage (bark stripped) appeared minimal (A. Bijl, pers. obs.).

Almost no seedlings or saplings were observed in the surveyed populations at Mapungubwe or Morongwa.

### Demographic profiles, height-class distributions and bottlenecks

The demographic profiles showed that all surveyed populations of *S. lugardii* comprised mostly large shrubs, with almost no small seedlings (<15 cm) and saplings (15-100 cm) (Figure 3-5).

In all the assessments at Mapungubwe and Morongwa, very small proportions of the populations were comprised of shrubs under one metre tall (Table 3-2). At Mapungubwe (2005, 2008 and 2014), there appear to be recruitment bottlenecks, indicated by peaks in the standard deviations of the moving window analyses. These peaks show that very small plants are missing from the population (Figure 3-6). A recruitment bottleneck was not so evident at Morongwa (2015), as there were no big peaks in standard deviation (Figure 3-5).

The height-class distributions of all the surveyed populations approximated the shape of a Gaussian/normal curve, with very few plants in the smallest and largest height classes. Only one seedling, and few very large shrubs were observed at Mapungubwe (2008) (Figure 3-6). In all the populations at Mapungubwe and Morongwa, the majority of shrubs were “medium” shrubs (2.1 - 4 m tall) (Figure 3-6). At Morongwa, however, there were more “small” shrubs (1.1 - 2 m tall) than at Mapungubwe. (Figure 3-6).

#### **Herbivore species associated with *S. lugardii* shrubs at Mapungubwe**

The herbivore species most often associated with *S. lugardii* shrubs at Mapungubwe in 2005 were eland (*Taurotragus oryx*), impala (*Aepyceros melampus*) and kudu (*Tragelaphus strepsiceros*), with their dung present at over 45 % of shrubs (Figure 3-7). Fewer than 20 % of shrubs had steenbok (*Raphicerus campestris*), giraffe (*Giraffa Camelopardalis*) and elephant (*Loxodonta africana*) dung in close proximity (Figure 3-7). At Mapungubwe (2008), the herbivore species most often associated with shrubs were kudu and eland, with dung at over 40 % of shrubs (Figure 3-7). Fewer than 10 % of shrubs had elephant dung or zebra (*Equus burchelli*) dung in close proximity (Figure 3-7).

## **Herbivory and plant response**

At Mapungubwe (2008), shrubs in all the height classes except the seedlings experienced canopy loss (Figure 3-8) (Table 3-3), but few shrubs had over 30 % of their canopy damaged, so overall canopy loss was low (Figure 3-9). Shrubs in all height classes except the seedlings experienced extensive bark loss. The mean percentage of bark damage ranged between 30.5 % and 66.9 %, and was most extensive in the large shrubs (Figure 3-8; Table 3-3).

Less than half of the plants in the smaller height classes (large saplings and small shrubs) showed signs of epicormic resprouting, but in the larger height classes (medium, large, and very large shrubs) the proportion of shrubs in the population that were resprouting increased to 70 % (Figure 3-9). Most shrubs showed signs of bark regrowth (83 % of shrubs). In the very large shrub height class, bark regrowth was recorded in almost all the shrubs (90 %) (Figure 3-9).

## **Reproductive maturity of shrubs at Mapungubwe and Morongwa**

At both the Mapungubwe (2014) and Morongwa (2015) populations, the proportion of *S. lugardii* shrubs with reproductive structures (flowers and pods) increased as shrub height increased (Figure 3-10). At Mapungubwe and Morongwa over 80 % of the medium and large shrubs had flowers, and 100 % of the very large shrubs had flowers (Figure 3-10). At both the Mapungubwe (2014) and Morongwa (2015) populations, over 70 % of the medium shrubs produced pods and 100 % of the large and very large shrubs produced pods (Figure 3-10). The *S. lugardii* shrubs in small size classes were unlikely to have flowers or pods, where shrubs over four metres tall were almost always guaranteed to have reproductive structures (Figure 3-11).

At Mapungubwe (2014), the height at which a *S. lugardii* shrub's probability of having flowers increased beyond a 50 % chance was 1.3 m (GLM  $\chi^2(1) = 4.800$ ,  $p = 0.028$ ), and at Morongwa (2015), was 2 m (GLM  $\chi^2(1) = 49.155$ ,  $p < 0.005$ ) (Figure 3-11). At Mapungubwe (2014), the height at which a shrub's probability of having pods increased beyond a 50 % chance was 1.9 m (GLM  $\chi^2(1) = 9.665$ ,  $p = 0.002$ ), and at Morongwa (2015), was 2.1 m (GLM  $\chi^2(1) = 84.180$ ,  $p < 0.005$ ) (Figure 3-11).

At Mapungubwe, *S. lugardii* shrubs produced more flowers than at Morongwa, but at Morongwa, despite the lower flower production, shrubs produced more pods than at Mapungubwe. At Mapungubwe, the medium shrubs (2.1 - 4 m tall) produced almost five times as many flowers as medium shrubs at Morongwa, and at Mapungubwe, the large shrubs (4.1 - 6 m tall) produced over three times as many flowers as large shrubs at Morongwa (Figure 3-12). There were no shrubs in the very large height class (6.1 m - 8 m tall) at Mapungubwe, but at Morongwa, one was observed and it produced as many flowers as the average large shrub at Mapungubwe (40 flowers) (Figure 3-12). The medium shrubs at Morongwa produced twice as many pods as the medium shrubs in Mapungubwe, and the large shrubs at Morongwa produced just over twice as many pods as large shrubs at Mapungubwe (Figure 3-12). The very large shrub observed in Morongwa produced almost triple the number of pods that the average large shrub at Morongwa produced (Figure 3-12).

## DISCUSSION

*S. lugardii* flowers are highly specialised for pollination by a single hawkmoth species (*Agrius convolvuli*) and are also self-incompatible. These two high-risk breeding system traits could make *S. lugardii* vulnerable to extirpation in the face of a pollination mutualism collapse, if it does not have some compensatory low-risk demographic traits (Bond, 1994).

At a first glance *S. lugardii* appeared demographically vulnerable. This demographic vulnerability was associated with the lack of recruitment clearly evident in the Mapungubwe populations, and evident to a lesser degree at the Morongwa population (Figure 3-5). Small plants (under a metre tall) were missing from the populations surveyed at Mapungubwe, and only one seedling was found in all the surveys over the years (Figure 3-6). At Morongwa small shrubs were also mostly missing from the population, as very few plants under a metre tall were found (Figure 3-5). Very large shrubs (over six metres tall) were also scarce in the study populations, but this was not cause for concern, as six metres is the upper height limit described for *S. lugardii* shrubs (Coates Palgrave, 1977).

Due to there being few very small shrubs, and few very large shrubs, the height-class distributions of the Mapungubwe and Morongwa *S. lugardii* populations approximated Gaussian distributions (Figure 3-6), and not the “reverse-J” shape of “classic” undisturbed forest ecosystems with high rates of recruitment (Lykke, 1998). Distributions like these of *S. lugardii* are not unusual in populations of long-lived, perennial species (Shackleton, 1993): Goldberg and Turner (1986) found that populations with demographic profiles skewed towards larger, older plants, were typical of populations of slow-growing and long-lived species, with erratic recruitment. Hall and Bawa (1993) and Lykke (1998) found that plant species with low seed output and low seedling establishment rates also had distribution-curves skewed toward larger individuals.

Based on the demographic profiles of the *S. lugardii* populations surveyed, there are recruitment bottlenecks that cause limited establishment and seedling survival at Mapungubwe and to a lesser degree at Morongwa.

Midgley and Bond (2001) identified the major demographic bottlenecks that limit recruitment in woody savanna plants, and these include: 1) seed set limitation due to pollinator failure in outcrossing individuals, 2) seed limitation due to seed predation post-dispersal, 3) seed dispersal limitation in zoochorous species, 4) germination limitation due to lack of appropriate germination cues and 5) establishment limitation due to disturbance or climatic variables. Some of these may be relevant to *S. lugardii* as it is a woody savanna plant.

*S. lugardii* is an outcrossing species, so pollinator failure can affect its demography. At Morongwa I did not observe pollen limitation, but at Mapungubwe the results were unclear. Seed predation post-dispersal was not explored in this study, so the effect of that potential demographic bottleneck on *S. lugardii* populations remains unknown. Seed dispersal limitation is unlikely, however, as *S. lugardii* is wind dispersed and thus has no specialised dispersal mutualisms (a low-risk demographic trait (Bond, 1994)).

A demographic bottleneck that may well be affecting *S. lugardii* populations at Mapungubwe and Morongwa, is germination limitation due to a lack of appropriate germination cues. In arid systems where water is limited, rainfall is often a key germination cue (Gutiérrez & Meserve, 2003; Levine, McEachern & Cowan, 2008) and during periods of drought, germination and seedling survival is limited (Gerhardt, 1993; Bengtsson-Sjörs, 2006). I did not do any seed germination experiments in the study populations, so could not draw conclusions about germination cues for *S. lugardii*, but as Mapungubwe experienced many years of below-average rainfall between 2005 and 2014, and only one seedling was observed during that period, it may be that recruitment in *S. lugardii* is water-limited.

The establishment limitation bottleneck that is due to disturbance or climatic variables may also be affecting *S. lugardii* populations. I did not explicitly test establishment limitations due to disturbance processes such as fire and herbivory, but some inferences

could be made regarding these processes. In the study populations the fuel load was too low to support fires, and fires were suppressed by management. Meso- and mega-herbivores, however, were abundant at Mapungubwe (Selier & Page 2015). Selective browsing by smaller herbivores, and trampling by large and small herbivores alike could have killed many seedlings (Cumming & Cumming, 2003; Augustine & McNaughton 2004; Thompson Hobbs & Searle, 2005; Moe, *et al.*, 2009; Midgley, Lawes & Chamailí-Jammes, 2010).

Although not specified by Midgley and Bond (2001) as one of the major demographic bottlenecks that limit recruitment in woody savanna plants, I suspect that an additional bottleneck that is very relevant to *S. lugardii* populations, and perhaps other species too, is seed-set limitation due to predation of flowers and fruit *prior to* dispersal.

In Chapter 2, the predation of flowers and developing fruit was explored. Melolonthine scarab beetles were seen to damage flower stigmas at Mapungubwe (A. Bijl, pers. obs.), and this predation bottleneck was thought to limit pod development, although clear evidence confirming this bottleneck was not found due to limited sampling. Small caterpillars were also observed inside the corolla tubes of *S. lugardii* flowers at the Mapungubwe and Morongwa populations, and were seen to feed destructively on the anthers and styles of the flowers (Figure 3-13, D), but I did not test their contribution to reproductive failure in populations. Pod predators could also have been responsible for the pre-dispersal recruitment bottleneck in *S. lugardii* populations: the pods take several weeks to mature, during which time they are green and fleshy (Figure 1-1, G), providing a good source of nutrients and water to herbivores in these arid ecosystems. Although I once found a chewed pod (Figure 3-13, C) I did not explore further whether pod predators were responsible for a recruitment bottleneck. Melolonthine scarab beetles, caterpillars and pod predators probably all contribute to reproductive failure in *S. lugardii* populations, and together, create a severe recruitment bottleneck. Herbivory (which includes florivory, pod predation and seedling predation) is likely the most influential top-down disturbance process limiting recruitment in *S. lugardii* populations, and water-limitation, the most influential bottom-up factor. It would be worth exploring this pre-dispersal predation bottleneck in greater detail in future assessments.

Once established, *S. lugardii* shrubs are well adapted to survive herbivory. In savanna systems, spines evolved as a means of deterrence to herbivory by large browsers and medium-sized mixed feeders (Shipley, 2007; Hanley, *et al.*, 2007; Charles-Dominique, *et al.*, 2016). As sharp spines were observed on the secondary and tertiary branches of *S. lugardii* shrubs (Figure 3-4, B), *S. lugardii* shrubs appear well adapted for herbivory. Based on the branching structure, *S. lugardii* shrubs can also resprout epicormically, which is an adaptive trait that allows persistence in the face of herbivory. In plants capable of resprouting, browsing can have the same effect as hedge-clipping: it increases the canopy density by increasing the density of tertiary branches (Danell, Bergstrom & Edenius, 1994; Bond & Midgley, 2003; Martínez & López-Portillo, 2003; Moncrieff, 2010). Most *S. lugardii* shrubs observed were multi-stemmed and had highly ramified secondary and tertiary branches (Figure 3-4, A and B).

*S. lugardii* shrubs are further adapted for herbivory, because they are stem succulents (Figure 3-4, C). Stem succulent (pachycaul) plants store starch as their reserve substance rather than water (Eggli & Nyffeler, 2009), and this stored starch allows the shrubs to rapidly resprout and replace biomass lost to browsing. Pachycauly is also beneficial in arid conditions, because plants can use their stored starch to grow rapidly right at the start of the vegetative period (Eggli & Nyffeler, 2009). *S. lugardii* shrubs also appear resilient to herbivory because they are able to regrow bark that has been removed. At Mapungubwe, many shrubs had patches of exposed cambium where bark had been removed (Figure 3-4, D), but also had strip scars which were completely sealed (Figure 3-4, F), indicative of bark regrowth.

At Mapungubwe, I explored in more detail whether *S. lugardii* shrubs could recover from canopy loss and bark damage. Less than half of the small shrubs (1.1 - 2 m tall) were resprouting, but most of the larger plants were (Figure 3-9). The largest shrubs in the population were most resilient to browser-induced canopy loss. The majority of shrubs with bark damage showed signs of bark regrowth, regardless of shrub size, but as with canopy recovery, the likelihood of bark recovery was highest in the largest shrubs (6.1 - 8 m tall) (Figure 3-9). *S. lugardii* shrubs are therefore well adapted to survive disturbance in the form of herbivory once they have grown beyond the smaller size

classes. Their persistence traits (epicormic resprouting and bark regrowth) allow them to recover from disturbance and transition into larger size classes.

Resprouting species typically allocate more resources to below-ground structures than to above-ground growth, compared to non-sprouters, so plant growth is slower and saplings are slower to reach maturity (Bond & Midgley, 2003). These slow-growing plants spend a longer time, comparatively, in small size classes, and are thus vulnerable in systems where disturbances are frequent. Once the plants grow large enough and can resprout and regrow bark, however, they become highly disturbance-tolerant. Due to the recruitment bottlenecks imposed by herbivory and fire in savanna systems, slow-growing, long-lived species typically have erratic recruitment, low population density and age structures skewed towards larger, older plants (Goldberg & Turner, 1986). *S. lugardii* shrubs are resprouters, the seedlings are fleshy and defenceless, vulnerable to mortality, and the populations at Mapungubwe and Morongwa have size structures skewed towards larger, older plants. Therefore, *S. lugardii* is likely a slow-growing species.

I set out to determine the size at which *S. lugardii* shrubs reach reproductive maturity. At Mapungubwe and Morongwa, shrubs smaller than one-metre tall were unlikely to have flowers or pods (Figure 3-10), and those that did, had very few reproductive structures (Figure 3-12). The numbers of reproductive structures (flowers or pods) increased with shrub height, and the shrubs in the largest height-classes in each population produced the largest numbers of flower and pods (Figure 3-12). At Mapungubwe, the height at which a shrub is more likely than not to have reproductive structures, is 1.3 m, and at Morongwa, is 2 m (Figure 3-11). It appears that *S. lugardii* shrubs have a minimum flowering height, and are slow to reach reproductive maturity, but once they grow beyond 1.3 m tall, their reproductive potential is high.

Flower output was explored across the locations to determine if *S. lugardii* shrubs could compensate for flower loss due to florivory. Medium shrubs and large shrubs in Mapungubwe produced more flowers than shrubs of the same size in Morongwa and it is possible that the larger volumes of flowers produced per shrub at Mapungubwe are to compensate for the numbers lost to florivory. Some plant species have been found to

produce more flowers after they have experienced damage, by aborting damaged flowers and shunting resources into making new flowers (Olesen, 1992; McCall & Irwin, 2006). Other plants produce “reserve flowers” which develop selectively if the first wave of flowers are damaged (Ashman & Morgan, 2004; McCall & Irwin, 2006). This may be what is happening in populations of *S. lugardii* at Mapungubwe.

Despite the greater flower output of Mapungubwe shrubs, pod development and survival were still limited. Medium and large shrubs in Morongwa produced twice as many pods as those at Mapungubwe (Figure 3-12) in spite of the fact that shrubs of the same size across the locations were equally likely to produce pods. The difference in pod numbers was probably due to both flower and pod predation at Mapungubwe, where florivores and herbivores were prevalent. At Morongwa, most florivores were absent and most herbivores were excluded from the study site by fences. At Mapungubwe, impala, kudu and eland impose a “browse trap” on shrubs under 4 m tall, through feeding on and damaging shrub canopies. Once the shrubs grow beyond the browse trap, pod density is high. Populations of *S. lugardii* shrubs that have escaped the browse trap are buffered from recruitment failure due to flower- and seed-limitation.

The demographic dependence of *S. lugardii* populations on seeds is low as long as the large shrubs in a population persist and retain their reproductive potential. Populations comprised of large shrubs are buffered by the storage effect from the negative consequences of reproductive failure, and can persist for decades through periods of failed recruitment. The storage effect refers to the various mechanisms that allow plant populations to persist during unfavourable periods and increase under favourable conditions (Higgins, Pickett & Bond, 2000). If the rate of mortality in a population of plants is low, it can create a storage effect in that population, where the reproductive potential of the population is retained in the persistent, reproductively mature plants (Warner & Chesson, 1985). Reproductive potential in the *S. lugardii* populations at Mapungubwe and Morongwa is stored in the resilient established plants that are able to produce reproductive structures, or are at least large enough to tolerate disturbance and have the potential to grow into height-classes that can produce seeds.

Seedlings and saplings were absent in the populations of *S. lugardii* assessed at Mapungubwe and Morongwa, due to several environmental processes creating demographic bottlenecks. Although populations of *S. lugardii* are severely affected by recruitment bottlenecks, the established shrubs are highly resilient to herbivory and climatic stressors such as drought. The large, established *S. lugardii* shrubs are very long-lived due to this resilience, and it is within these large, long-lived shrubs that the reproductive potential of the populations is stored. Longevity is a key resilience strategy in *S. lugardii* populations. Due to the low-risk demographic traits observed in *S. lugardii* shrubs, the high-risk breeding system traits (pollination specialisation and self-incompatibility) are compensated for, and do not, at present, threaten the continued survival of the *S. lugardii* populations at Mapungubwe and Morongwa.

## CONCLUSION

Due to their stem succulence, capacity for epicormic resprouting, ability to regrow bark after damage, and spinescence, established *S. lugardii* shrubs are well adapted for survival in arid savanna ecosystems where herbivores are prevalent. The life history strategy of *S. lugardii* appears to be one of slow growth and maturation and great persistence. The persistence traits are evident in the larger shrubs, especially those taller than 4 m. Shrubs only seem to have a reasonable capacity for persistence once they are taller than 1 m, because extremely few shrubs smaller than this were found across all the populations surveyed over almost a decade.

The absence of seedlings and saplings in the populations was thought to be the result of severe recruitment bottlenecks imposed by florivores that reduce the flower output, and herbivores that eat seedlings, damage established shrub canopies and feed on pods. The likelihood of fire imposing a recruitment bottleneck in these systems was ruled out, as, in both study sites, fire was suppressed by management. Fire is also not typically a key disturbance factor in arid areas (Bond, 2005; Coestee & Wigley, 2016).

In future studies, it would be useful to explore the fate of fruit, seeds and seedlings in the field, to determine how florivory, herbivory and drought impose demographic bottlenecks on recruitment and seedling survival. In populations where droughts primarily limit recruitment, one can expect episodic recruitment events to occur, and these patterns should be evident in the demographic profiles. In the study populations of *S. lugardii*, episodic recruitments were not clear, and this is likely due to the steady attrition of seedlings due to herbivory. Exclusion experiments that isolate field sites from herbivores would shed more light on the factors that cause the most severe recruitment bottlenecks.

In this study, I aimed to determine the vulnerability of *S. lugardii* populations to extirpation in the face of a pollination mutualism collapse, by assessing whether *S. lugardii* has low-risk demographic “compensation strategies” that would allow populations of this highly specialised, self-incompatible species to persist through periods of pollination and reproductive failure. *S. lugardii* shrubs are slow-growing and

very resilient to disturbance once they grow beyond the sapling stage. The established shrubs are highly tolerant to herbivory, and can become very large and long-lived, creating a storage effect in the populations. The storage effect allows the Mapungubwe and Morongwa populations to persist through periods of reproductive failure and greatly reduces the demographic dependence of these populations on seeds. This is how *S. lugardii* populations compensate for limited recruitment and the demographic vulnerability associated therewith.

If the mortality rate in the established population remains low, as is the case for *S. lugardii* populations at Mapungubwe and Morongwa currently (only two shrubs were found toppled and killed in all the years of study (Figure 3-13 B), the populations will continue to be at a low risk of extirpation. Should some environmental change cause an increase in the currently very low rate of mortality of established *S. lugardii* shrubs at Mapungubwe and Morongwa, the risk of extirpation would rapidly and seriously increase, because all the reproductive potential in the population is stored in the larger shrubs, and there are no smaller cohorts in the populations that could grow to replace the lost adults.

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## TABLES AND FIGURES



Figure 3-2: Map of the study sites in South Africa where *S. lugardii* populations were found, in Limpopo (Mapungubwe National Park) and North West (Morongwa Private Safari Lodge).

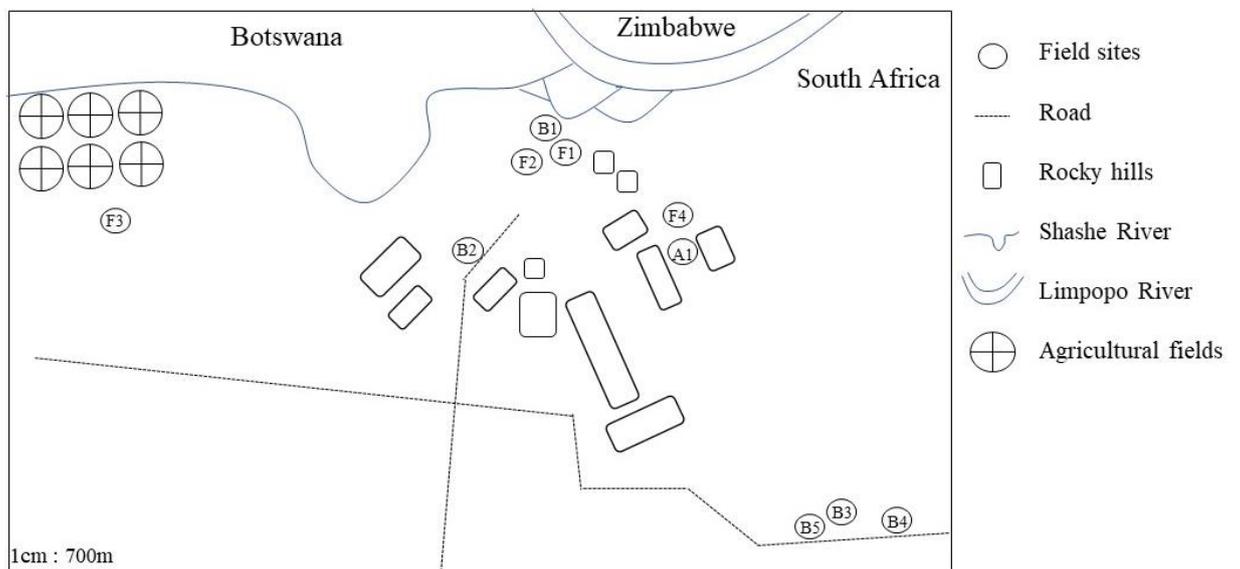


Figure 3-3: Sketch of the field sites surveyed at Mapungubwe National Park, near the confluence of the Shashe River and Limpopo River. Field sites B1- B5 were surveyed in 2005, field sites F1- F4 were surveyed in 2008 and field site A1 was surveyed in 2014.

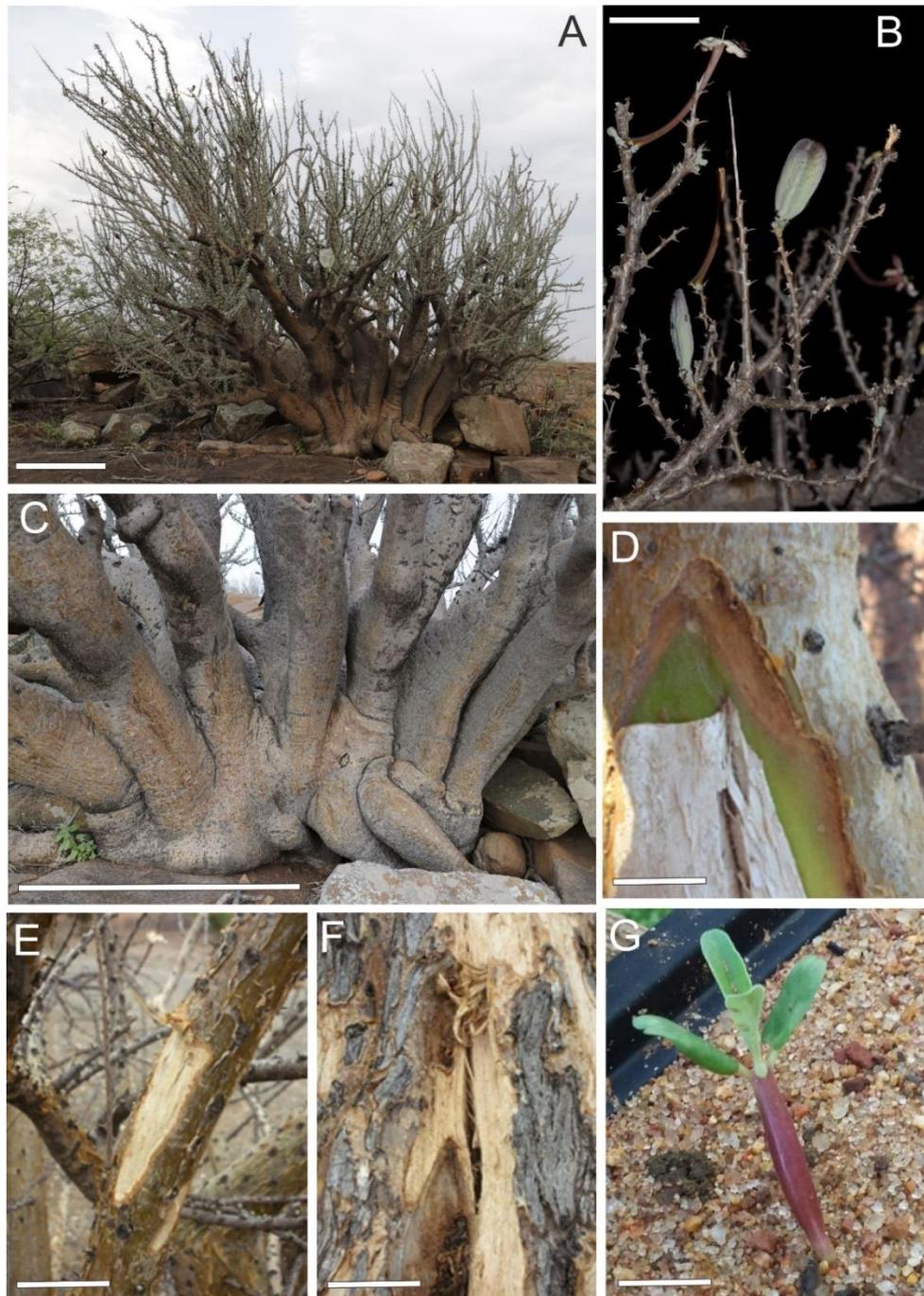


Figure 3-4: A large *S. lugardii* shrub with highly ramified secondary and tertiary branches (A). Scale bar = 1 m. A shrub with sharp spines on the secondary and tertiary branches (B). Scale bar = 10 cm. The thick, succulent stem of a large shrub (C). Scale bar = 1 m. The stem of a shrub with signs of bark stripping (D). Scale bar = 5 cm. A stem with superficial bark removal (E). Scale bar = 10 cm. A shrub showing signs of bark regrowth (F). Scale bar = 5 cm. A seedling, with a soft and fleshy stem (G). Scale bar = 1 cm. Photographs by Alison Bijl (A, C), Pieter Viljoen (G), Laurence Kruger (D, E, F) and Steven Johnson (B).

Table 3-1: Biologically meaningful height-class (cm) categories for *S. lugardii* shrubs.

Height classes	Height class description
0 to 15 cm	Seedling
15.1 to 30 cm	Small sapling
30.1 to 100 cm	Large sapling
100.1 to 200 cm	Small shrub
200.1 to 400 cm	Medium shrub
400.1 to 600 cm	Large shrub
600.1 to 800 cm	Very large shrub

Table 3-2: Summary of the population sizes (Popn. size) and height ranges (m) of *S. lugardii* shrub populations surveyed at Mapungubwe and Morongwa, and the percentages (%) of shrubs smaller than 1m (<1m), smaller than 2m (<2m), larger than 2m (>2m) and larger than 4m (>4m) calculated for each population.

Location	Year	Popn. size (n)	Height range (m)	Shrubs < 1m	Shrubs < 2m	Shrubs > 2m	Shrubs > 4m
Mapungubwe	2005	150	0.7 to 6.5	3	13	87	28
Mapungubwe	2008	220	0.1 to 7.2	4	14	86	41
Mapungubwe	2014	49	0.4 to 5.4	6	16	83	30
Morongwa	2015	119	0.3 to 7.5	5	29	71	21

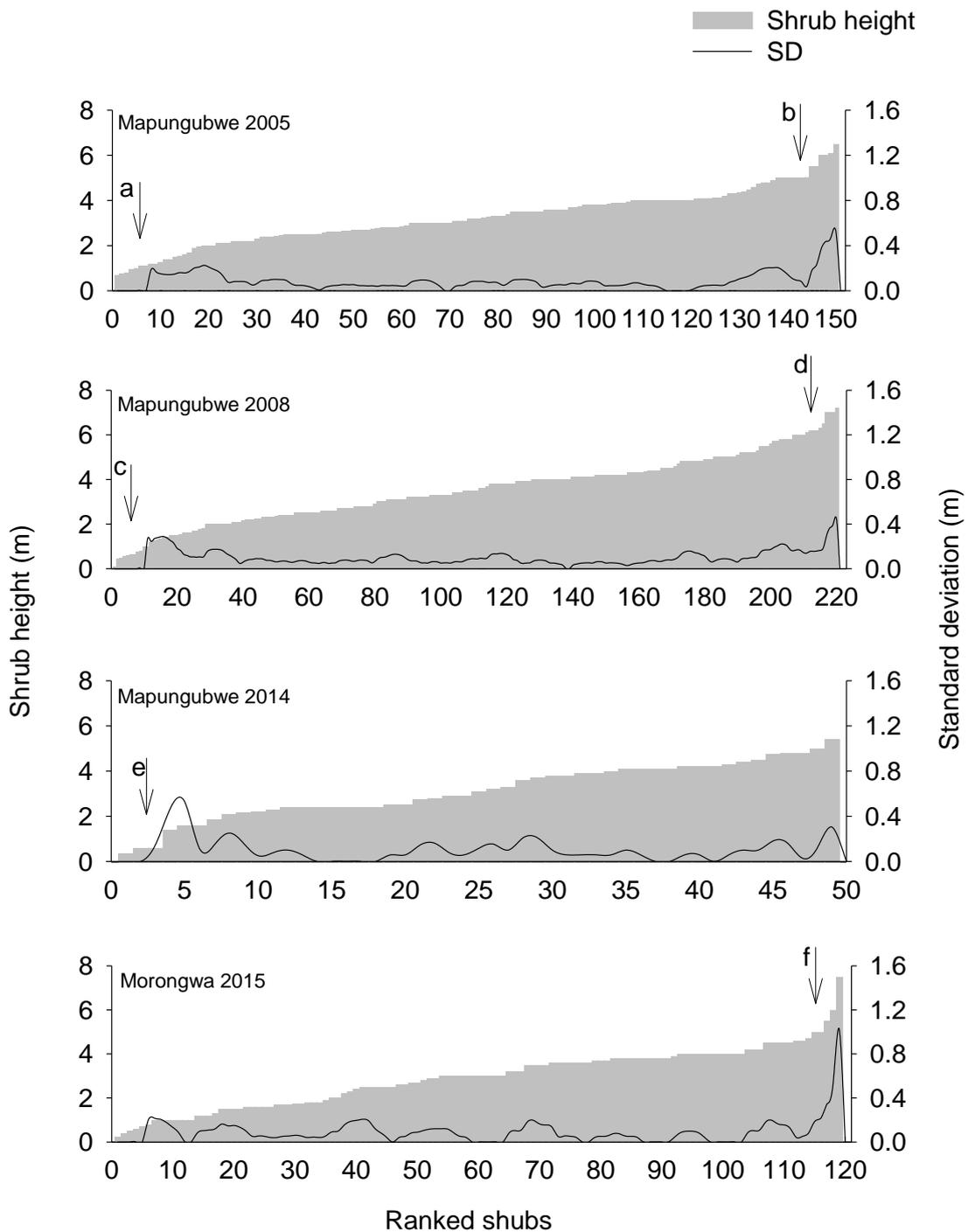


Figure 3-5: Histograms showing ranked distributions of shrubs (heights) for populations at Mapungubwe (2005, 2008 and 2015), and Morongwa (2015), displayed on primary Y-axes. Line graphs showing the standard deviations calculated for each population with moving window analysis, displayed on secondary Y-axes. Black arrows indicate peaks in standard deviation, indicative of large increases, or jumps, in shrub height in the ranked height profiles.

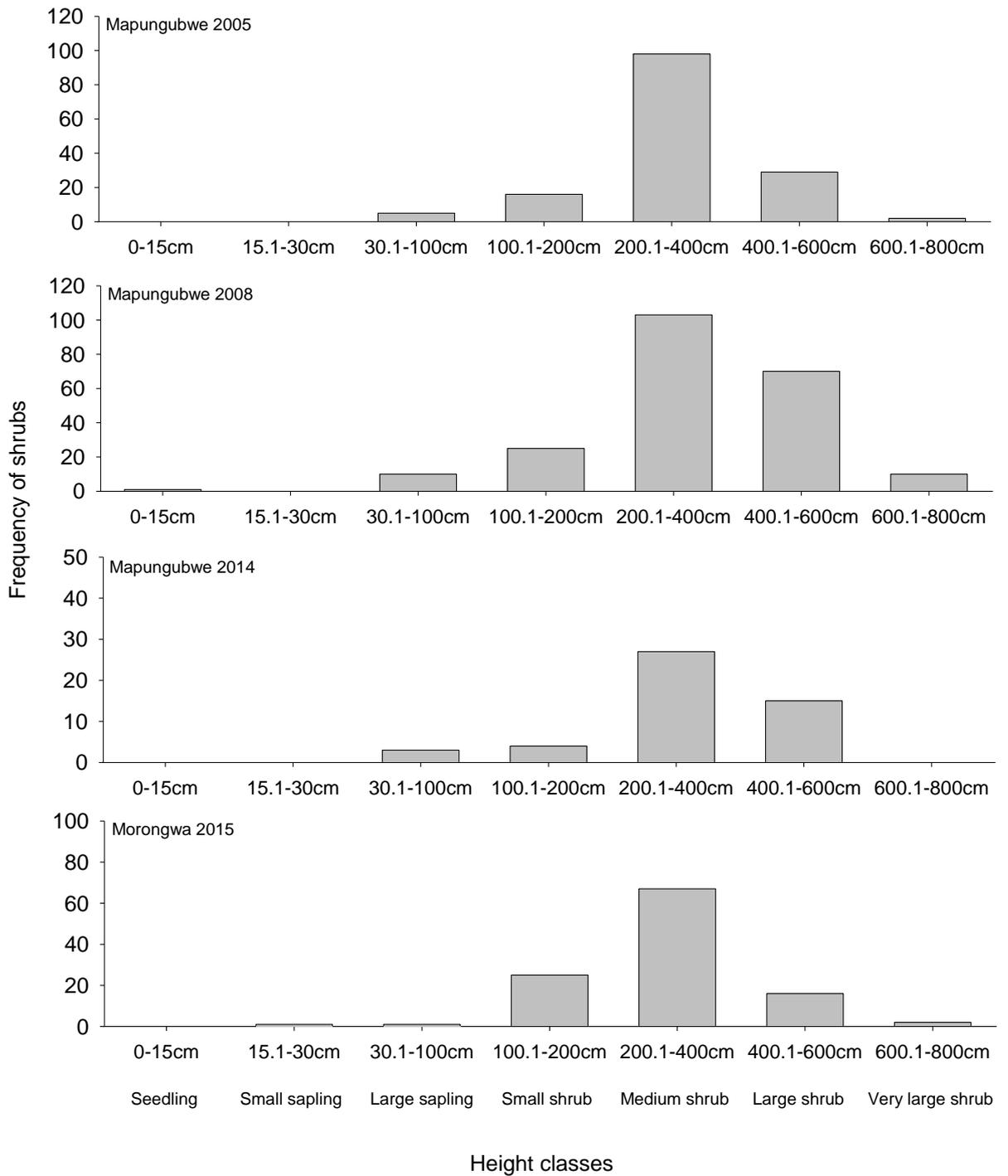


Figure 3-6: The height-class distributions of the populations of *S. lugardii* sampled at Mapungubwe (2005, 2008 and 2014) and at Morongwa (2015).

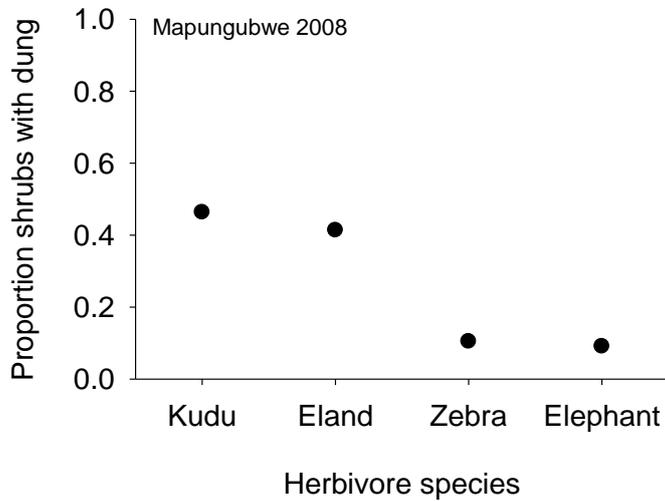
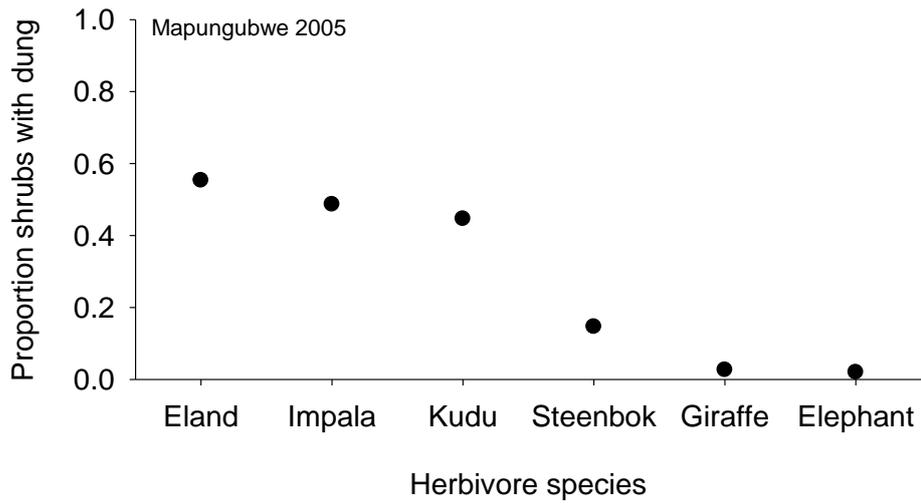


Figure 3-7: The proportion of *S. lugardii* shrubs that had herbivore dung present in close proximity, assessed at Mapungubwe (2005 and 2008). Dung was identified to herbivore species, and dung presence was used as a proxy for herbivore presence and residence time.

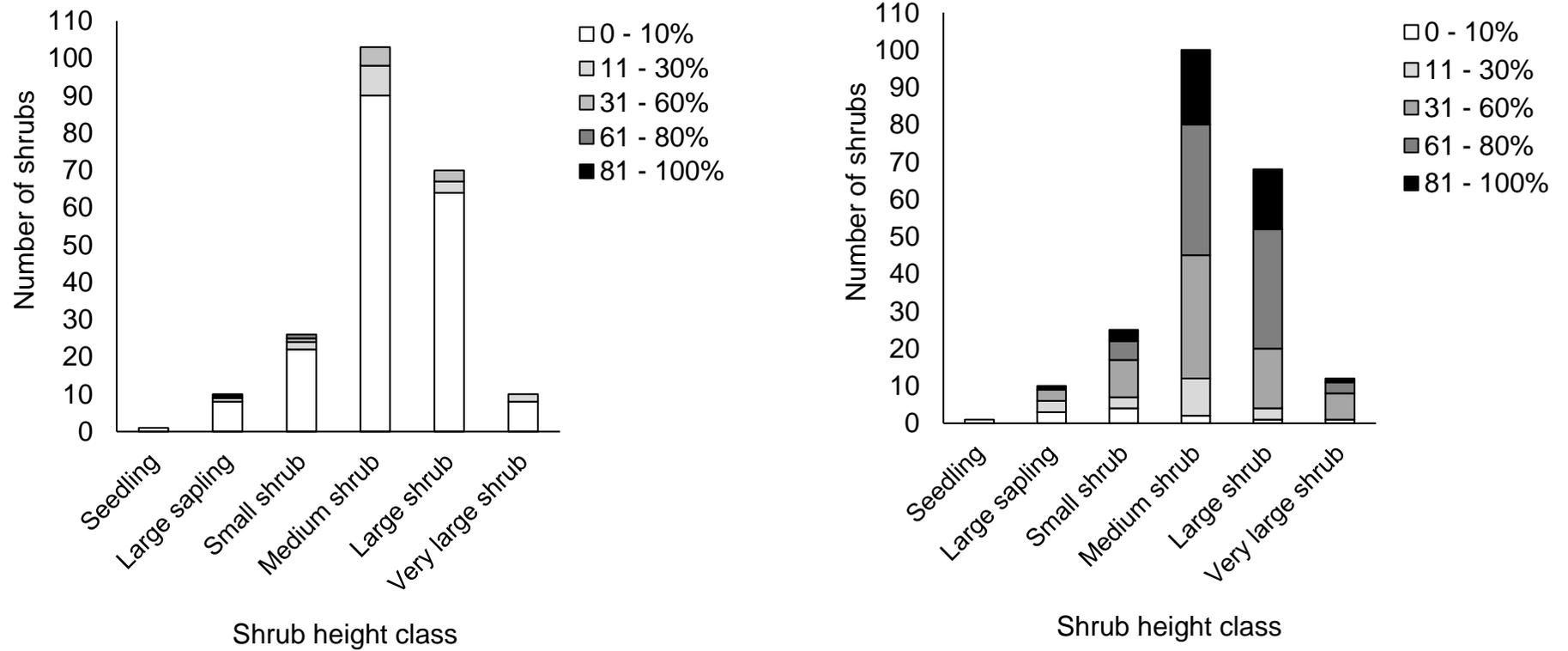


Figure 3-8: Canopy loss (%) and bark stripped (%) across shrub height-classes, for the *S. lugardii* population surveyed at Mapungubwe (2008).

Table 3-3: The mean percentage ( $\pm$  standard error) and range of damage (% of canopy loss and % of bark stripped) across the different height classes, surveyed in *S. lugardii* shrubs at Mapungubwe (2008).

Size class	n	Canopy loss		Bark stripped	
		Mean $\pm$ SE	Range	Mean $\pm$ SE	Range
Seedling	1	0	0	0	0
Large sapling	10	13.6 $\pm$ 9.4	0 - 95	30.5 $\pm$ 8.5	0 - 90
Small shrub	25	5.8 $\pm$ 3.2	0 - 75	45.3 $\pm$ 5.6	0 - 93
Medium shrub	103	5.8 $\pm$ 1.2	0 - 60	60.4 $\pm$ 2.4	Mar 98
Large shrub	70	4.3 $\pm$ 1.3	0 - 60	66.9 $\pm$ 2.4	Oct 95
Very large shrub	10	5.1 $\pm$ 3.1	0 - 30	52.5 $\pm$ 6.8	Jun 90

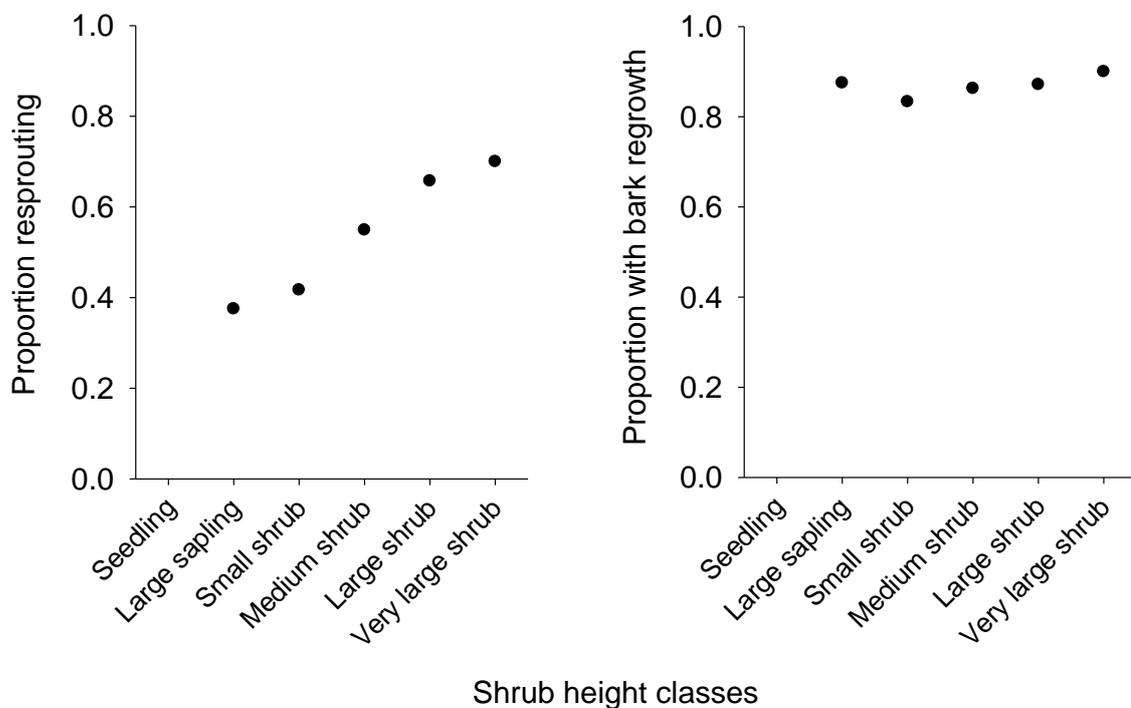


Figure 3-9: Scatter plots of the proportion of shrubs in each height class that have recovered from canopy loss (left) and bark stripping (right).

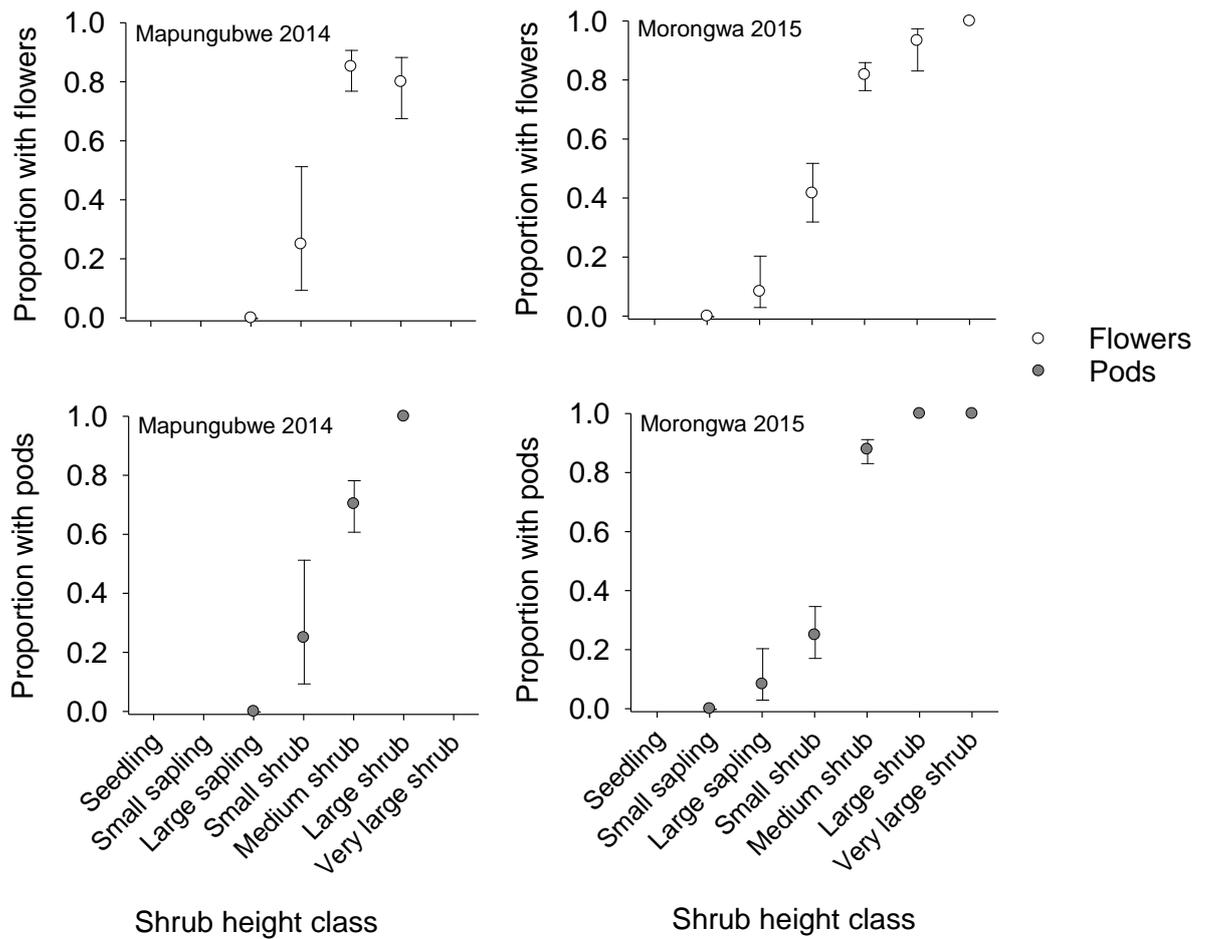


Figure 3-10: Scatter plots of the proportion of *S. lugardii* shrubs at Mapungubwe (2014) and Morongwa (2015) with flowers (white circles) and pods (grey circles) in each height class.

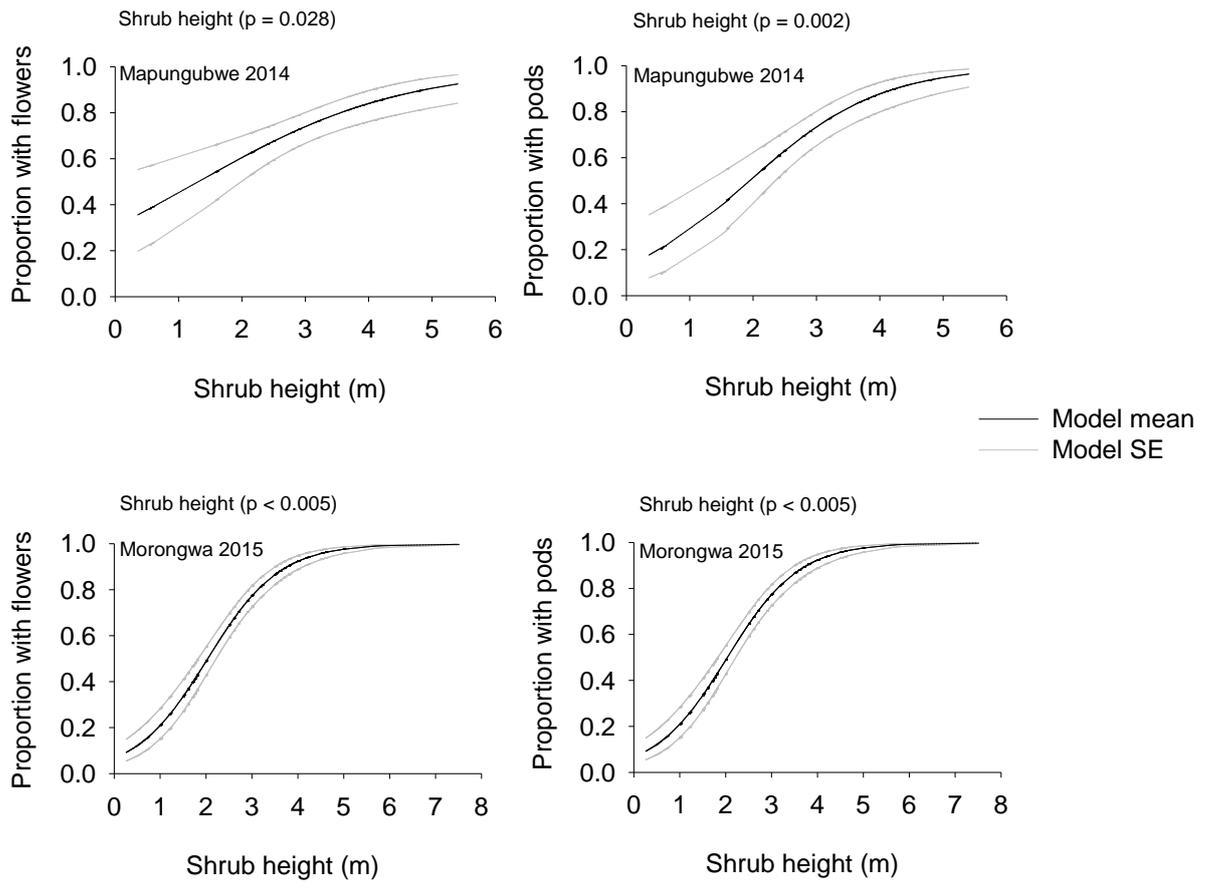


Figure 3-11: The modelled proportions of *S. lugardii* shrubs with flowers (left) and pods (right) at Mapungubwe (top) and Morongwa (bottom). Modelled mean proportions of shrubs with flowers and pods are indicated with black lines, and predicted standard errors are indicated with grey lines.

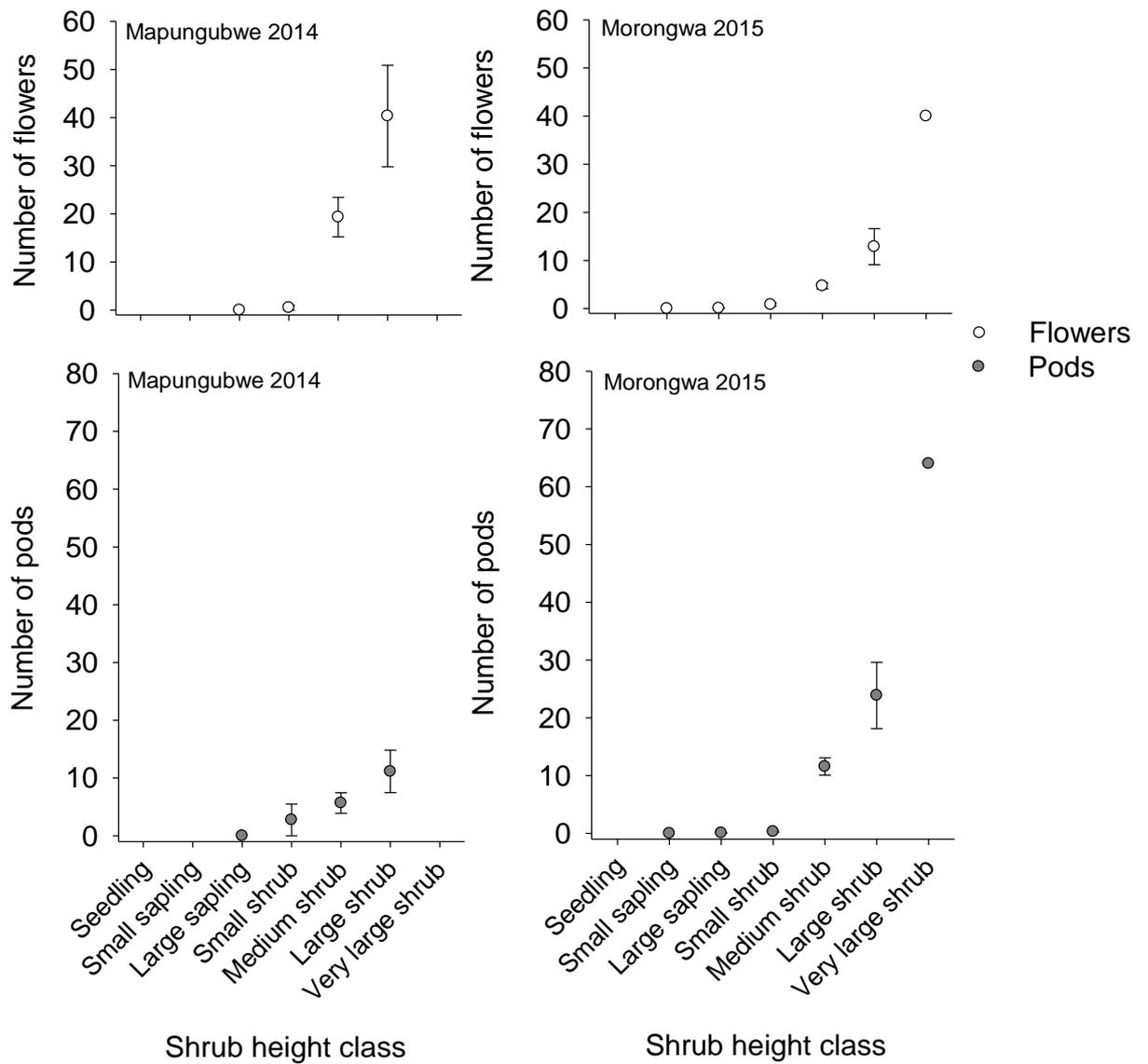


Figure 3-12: Scatter plots of the number of flowers (white circles) and pods (grey circles) in each shrub height class, assessed in populations of *S. lugardii* shrubs at Mapungubwe (2014) and Morongwa (2015).

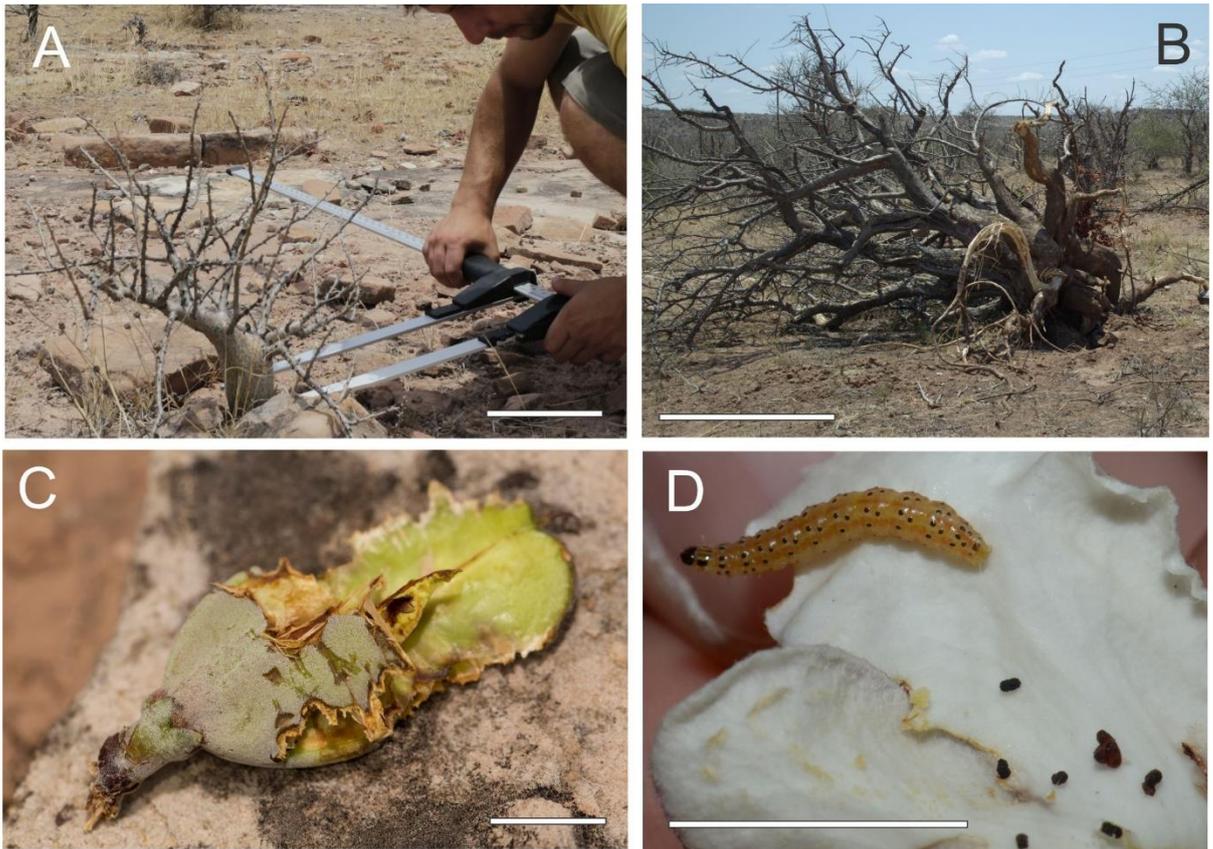


Figure 3-13: A small *S. lugardii* sapling found at Mapungubwe in 2014 (A). Scale bar = 10 cm. A toppled shrub (B). Scale bar = 1 m. A predated seed pod (C). Scale bar = 1 cm. A caterpillar found inside a flower (D). Scale bar = 1 cm. Photographs by Laurence Kruger (A, C), Alison Bijl (B) and Steven Johnson (D).

## CHAPTER 4:

### Conclusion

#### Outline

The aim of this thesis was to assess the breeding system, pollination relationship and demography of the Transvaal Sesame-bush, *Sesamothamnus lugardii* N. E. Br. Ex Stapf. (Pedaliaceae). The breeding system and pollination system of *S. lugardii* was explored in Chapter 2. In Chapter 3, the demography of *S. lugardii* was explored and the potential demographic bottlenecks that affect the study populations were identified. The vulnerability of *S. lugardii* to a pollination mutualism failure was assessed by determining whether the species had reproductive and demographic traits that render populations resilient to disturbance and reproductive failure. Additionally, the thresholds beyond which populations of *S. lugardii* become vulnerable to reproductive failure and local extirpation were considered. In this final chapter (Chapter 4) I summarise the key findings from each chapter, and use these to make predictions about the future of *S. lugardii* populations in South African savannas.

#### Chapter 2 synthesis

The floral traits of *S. lugardii* are convergent with the hawkmoth pollination syndrome (white, sweetly scented, long, narrow corolla tubes, evening anthesis and short-lived), and the extremely long corolla tubes of the *S. lugardii* flowers (*ca.* 10 cm) made *S. lugardii* a likely candidate for pollination by long-tongued hawkmoths. Visual and camera observations confirmed that *A. convolvuli* hawkmoths were visiting *S. lugardii* flowers, and observations of *S. lugardii* pollen on *A. convolvuli* hawkmoth proboscides confirmed that *A. convolvuli* hawkmoths were effective pollinators of *S. lugardii*. Additionally, hawkmoth scales were found on flower stigmas in association with pollen, which confirmed hawkmoth pollination. Flowers that attract convolvulus hawkmoths typically secrete large volumes of nectar and are known to contain the terpene alcohol, linalool, in their scent profiles, and both these traits were observed in *S. lugardii*.

flowers, further confirming specialisation for pollination by *A. convolvuli* hawkmoths. There was a mechanical fit between *S. lugardii* flowers and *A. convolvuli* hawkmoths. The lengths of *S. lugardii* flower corolla tubes and *A. convolvuli* hawkmoth proboscides matched. During the peak rate of flower opening in the *S. lugardii* populations, *A. convolvuli* hawkmoths were active, so there was also a temporal fit between flower anthesis and hawkmoth activity. These physical and temporal matches confirmed ultimately that *S. lugardii* flowers are adapted to, and specialised for pollination by *A. convolvuli* hawkmoths exclusively.

I tested whether *A. convolvuli* hawkmoths were imposing directional selection on *S. lugardii* corolla tube lengths, as described in Darwin's coevolutionary race model, but did not find sufficient evidence to confirm directional selection. This could be a novel and interesting component of future studies of *S. lugardii*.

I assessed the breeding system of *S. lugardii* to determine whether it was dependent on *A. convolvuli* hawkmoths for sexual reproduction, and found that *S. lugardii* flowers are self-incompatible and thus dependent on *A. convolvuli* hawkmoths for cross-pollination. I determined whether *A. convolvuli* were efficient pollinators by assessing populations for pollen limitation. At Morongwa, *A. convolvuli* hawkmoths were efficient pollinators as flowers were not pollen-limited, and were developing into pods. At Mapungubwe it was impossible to determine, because florivory appeared to be limiting pod development.

At Mapungubwe I explored the impact of melolonthine beetle florivory on plant fecundity. Melolonthine scarab beetles were seen eating flower petals and stigmas shortly after flowers had opened in the evenings, and I expected that predation by these scarab beetles would limit flowers available for pollination, and contribute to a reproductive bottleneck. I did not find sufficient evidence to confirm that beetle predation was responsible for limited pod development, because stigma damage was similar at Mapungubwe and Morongwa, where beetles were present and absent, respectively. Small caterpillars were occasionally observed inside the corolla tubes of *S. lugardii* flowers at both populations, and could also have contributed to the reproductive bottleneck. I was interested primarily in the pollination of *S. lugardii* flowers during

fieldwork periods, so chose to avoid sampling flowers that appeared damaged by caterpillars, and did not explore their role in flower predation. My bias in selecting undamaged flowers could have contributed to the unclear beetle predation findings. Melolonthine beetles and caterpillars may be imposing a demographic bottleneck, by limiting flower survival and pod development, and would be well worth considering in future assessments.

### **Chapter 3 synthesis**

I used the approach described by Bond (1994) to determine the vulnerability of *S. lugardii* to extirpation in the face of a mutualism collapse. *S. lugardii* flowers are highly specialised for pollination by a single hawkmoth species and are self-incompatible, and these high-risk breeding system traits could put populations at risk of extirpation if the pollination mutualism fail and the populations do not have low-risk demographic traits that offset the breeding system vulnerability. A demographic study of *S. lugardii* populations was thus necessary.

The physical characteristics of individual plants were assessed to understand the life history strategy of the species. The response of shrubs to herbivory was assessed to determine if *S. lugardii* shrubs are prone to or tolerant of disturbance. *S. lugardii* shrubs were found to be capable of epicormic resprouting and bark regrowth after strip damage. *S. lugardii* stems are also spiny. These traits make *S. lugardii* shrubs well adapted to herbivory once they are established (grown beyond the sapling stage).

The demographic profiles and height-class distributions were used to identify demographic bottlenecks in populations, assess the recruitment strategy of the species and identify if the populations stored reproductive potential. At a first glance *S. lugardii* appeared to be demographically vulnerable, because there was a clear lack of recruitment in populations at Mapungubwe, and to a lesser degree, at Morongwa. Small plants were missing from the populations, and only one seedling was found in all the surveys done at Mapungubwe over nine years. Recruitment bottlenecks imposed by florivores and herbivores were thought to be responsible for the absence of seedlings in populations.

Although populations of *S. lugardii* are affected severely by recruitment bottlenecks, *S. lugardii* shrubs were found to be very resilient to disturbance once they grow beyond the sapling stage, and the longevity of established shrubs creates a storage effect in the populations. The storage effect allows the Mapungubwe and Morongwa populations to persist through periods of reproductive failure, and greatly reduces the demographic dependence of these populations on seeds.

Longevity through resilience is the key low-risk demographic strategy that buffers *S. lugardii* populations from prolonged periods of reproductive failure and eventual extirpation. However, should some environmental change cause an increase in the currently very low rate of mortality of established *S. lugardii* shrubs in Mapungubwe and Morongwa, then the risk of extirpation would rapidly and seriously increase, because all the reproductive potential in the population is stored in the larger shrubs, and there are no smaller cohorts in the populations that could grow to replace the lost adults.

The life cycle diagram of *S. lugardii* (Figure 4-1) highlights the demographic bottlenecks that are most likely to affect shrubs growing in arid savanna ecosystems such as Mapungubwe. Recruitment failure can cause a major demographic bottleneck in *S. lugardii* populations. It can occur if shrubs fail to produce flowers (due to herbivores causing canopy loss or due to resource limitation in the form of a drought), but can also occur if flowers are pollen-limited (due to *A. convolvuli* hawkmoths being lost from the ecosystem). Recruitment failure can also be caused by flower and pod predators (such as scarab beetles, caterpillars, squirrels and antelope). A lack of sufficient germination cues and resources to support vulnerable seedlings can also cause recruitment failure (e.g. drought).

The remaining demographic bottlenecks that affect *S. lugardii* populations are those that limit size-class transitions (Figure 4-1). Seedlings are the most vulnerable to herbivory and environmental stress, as they have no capacity to store water and carbohydrates yet, cannot resprout epicormically, and do not have thick bark which can regrow. To survive, they must avoid disturbance and have sufficient resources to grow into larger, more resilient saplings. Saplings develop bark within a year and have been observed to be

multi-stemmed in nurseries, so are presumably capable of some degree of resprouting, and are therefore less susceptible than seedlings to the demographic bottlenecks that limit survival. Once shrubs are over 2 m tall, they are highly resilient and only major disturbances and extremely hostile environments will limit survival (*e.g.* intense herbivory and extreme drought) (Figure 4-1).

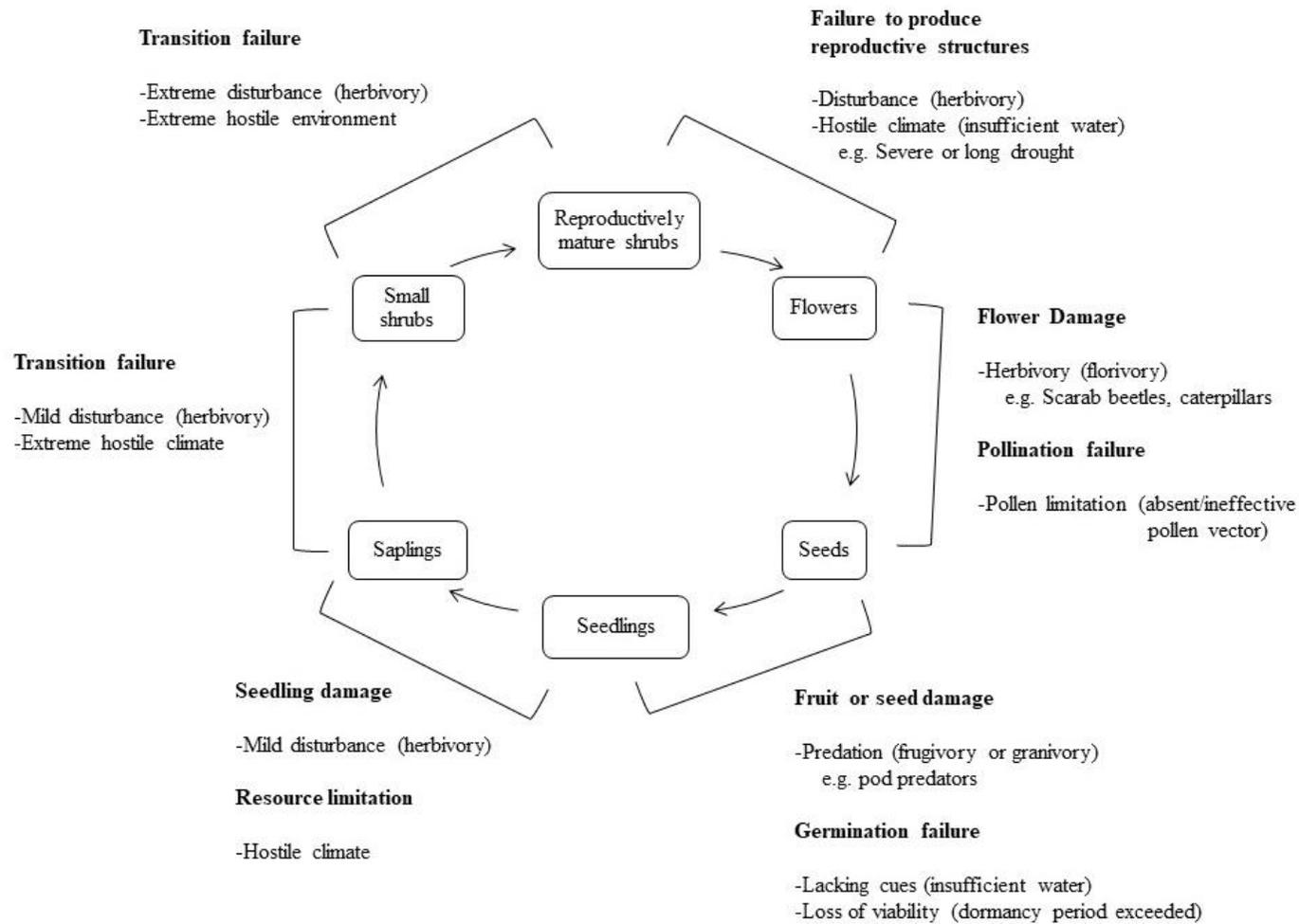


Figure 4-1: A scheme of the key disturbance processes and demographic bottlenecks that affect *S. lugardii* shrubs in arid South African savanna ecosystems such as Mapungubwe and Morongwa.

## Future concerns

Under current conditions, the existing *S. lugardii* populations at Mapungubwe and Morongwa are not at risk of extirpation. However, this does not mean that the risk of extirpation could not quickly become a threat in the future. Recruitment is limited by florivory and herbivory at Mapungubwe, and even in populations where antagonists are absent, such as Morongwa, recruitment from seed is rare. There are currently no smaller cohorts that could grow to replace the large shrubs which comprise the populations (and store all the reproductive potential), should some environmental change result in the increased mortality of large shrubs.

In South Africa, climate change is expected to cause temperature increases and rainfall decreases, and the frequency of extreme events like floods and droughts are predicted to increase (Nhemachena, 2008). If the Limpopo province becomes more arid and experiences more severe droughts in the future, then the established *S. lugardii* shrubs will not suffer from a lack of water, as their stem succulence makes them well-adapted for aridity, but drought-sensitive seedlings and small saplings may die. If germination is triggered by rainfall, then the chances of recruitment will decrease significantly. The recruitment bottlenecks created by herbivory, however, may lessen under drought conditions.

In Chapter 3, I found that eland, impala and kudu were most commonly associated with *S. lugardii* shrubs at Mapungubwe, and that elephants were rarely associated with *S. lugardii* shrubs. Under drought conditions, *S. lugardii* shrubs will likely experience less herbivory from meso-herbivores such as kudu, eland and impala, because their populations will decline. At Mapungubwe, between 2010 and 2014, several drought years caused losses of over 70 % in the kudu, eland and impala populations (Selier & Page, 2015). Elephant abundance, however, increased greatly in spite of the drought (Selier & Page, 2015). Elephant utilisation of *S. lugardii* was found to be low during the surveys done at Mapungubwe between 2005 and 2014. Only two shrubs were found toppled in 2014 (Figure 3-14, B), but it could increase under severe drought conditions.

At Mapungubwe, elephant utilisation of certain targeted species such as the Corkwoods (*Commiphora* species) is of great concern, because the populations have been severely damaged in recent years and the extirpation of these species, and other non-resprouting species, is possible (Coetsee & Wigley, 2016). At Mapungubwe, as a result of selective utilisation by elephants, entire thickets of *Commiphora* trees have been destroyed in the last decade, and over a third of all the *Commiphora* populations have been toppled (Coetsee & Wigley, 2016).

If elephants increasingly target and utilise *S. lugardii* shrubs, then populations of *S. lugardii* will suffer the same fate as the *Commiphora* populations at Mapungubwe. If the tree species that elephants currently prefer to utilise in Mapungubwe are lost, elephant impact on *S. lugardii* shrubs may also intensify quickly. In *S. lugardii* populations, increased elephant utilisation will have negative repercussions for recruitment, as canopy loss caused by elephant feeding will limit pod numbers on shrubs, and therefore reduce seed numbers. If *S. lugardii* shrubs are pollarded or toppled by elephants, this will threaten the longevity of populations. *S. lugardii* shrubs, though capable of epicormic resprouting, showed no signs of clonal reproduction or basal resprouting (A. Bijl, pers. obs.). Toppling by elephants can kill the shrubs and thus diminish the stored reproductive potential in the populations.

There is hope for re-establishing populations of *S. lugardii* if extirpation does occur (if this complies with the management strategy and conservation objectives of the area, and the processes that led to extirpation are mitigated), because the seeds can easily be germinated in nurseries, and the plants can be transplanted once they reach heights of one to two metres, where they are capable of epicormic resprouting and bark regrowth and can produce flowers and pods.

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## APPENDIX A

### **The breeding system and demography of *Sesamothamnus guerichii*: a pilot study**

#### **ABSTRACT**

*Sesamothamnus guerichii* shrubs were studied in the Kunene Region of Namibia, near Khorixas and Outjo. *S. guerichii* flowers have traits characteristic of hawkmoth pollination (pale flowers, long, narrow corolla tubes, evening anthesis and a sweet scent) and are attractive to a variety of noctuid moths and hawkmoths. The curved shape of the corolla tubes makes the flowers inaccessible to very long-tongued hawkmoths such as *A. convolvuli*. Hawkmoth pollination was not confirmed in this study, but Carpenter bees were found to be effective pollinators of *S. guerichii* flowers. *S. guerichii* may have a mixed pollination syndrome that includes moths and carpenter bees. *S. guerichii* populations assessed in this study experience recruitment bottlenecks, because no seedlings were observed in the demographic assessments. Whether the recruitment bottleneck is due to pollen limitation, flower predation, pod predation or germination failure due to water limitation was not explicitly tested. The storage effect buffers *S. guerichii* populations from reproductive failure and extirpation currently, so even if the pollination mutualism of *S. guerichii* collapses, the study populations are not at risk. However, if herbivory and harvesting intensify and threaten the established shrubs in the study populations (which currently retain all the reproductive potential), there will be no plants in smaller size classes to replace them.

#### **KEYWORDS**

*Sesamothamnus guerichii*, hawkmoth pollination, hawkmoths, recruitment bottlenecks, storage effect

## INTRODUCTION

### Study outline

In this study, I will assess whether one can determine the inherent risk of extinction (in the face of a mutualism collapse) for a given species based on a combination of breeding system traits and demographic traits, and assess whether one can determine the extirpation risk in populations of that given species using simple demographic assessments. Several methods and techniques described in Chapters 2 and 3 have been selected to use in this study, to create a simple “risk assessment strategy” that could be used in conservation planning and management. The Namibian species, *Sesamothamnus guerichii*, will be assessed. *S. guerichii* is selected, because the vegetative morphology is very similar to *S. lugardii*, and the floral traits suggest that it is also specialised for pollination (Figure A-1).

### The study species

*Sesamothamnus guerichii* (the Herero Sesame-bush, Pedaliaceae) is a perennial shrub found mainly around the Kaokoveld in northern Namibia, in very hot, dry climates and often on calcareous soils (Figure A-2A, D and F). *S. guerichii* shrubs are stem succulents, and have fat trunks relative to their height, with bark that is finely flaking and pale yellow to coppery in colour (Coates Palgrave, 1977) (Figure A-2A). *S. guerichii* shrubs are multi-stemmed and have spines on the secondary and tertiary branches (A. Bijl, pers. obs.). *S. guerichii* shrubs flower in November and December, and the flowers are bright yellow (Figure A-2B, C and E), have slightly shorter, and more curved corolla tubes than *S. lugardii* flowers (A. Bijl, pers. obs.) (Figure A-2, C and E), and *S. guerichii* flowers lack a distinct spur (Coates Palgrave, 1977) (Figure A-2E). *S. guerichii* flowers open in the evening and last only a single night. The corollas are then sloughed off the morning after anthesis (A. Bijl, pers. obs.). Where *S. lugardii* flowers have a slightly musky and sweet smell, *S. guerichii* flowers are also sweetly scented, but are less musky (A. Bijl, pers. obs.). The seed pods of *S. guerichii* are green and fleshy during development (Coates Palgrave, 1977), but harden and desiccate with maturation, and dehisce to release winged, wind dispersed seeds (A. Bijl, pers. obs.).

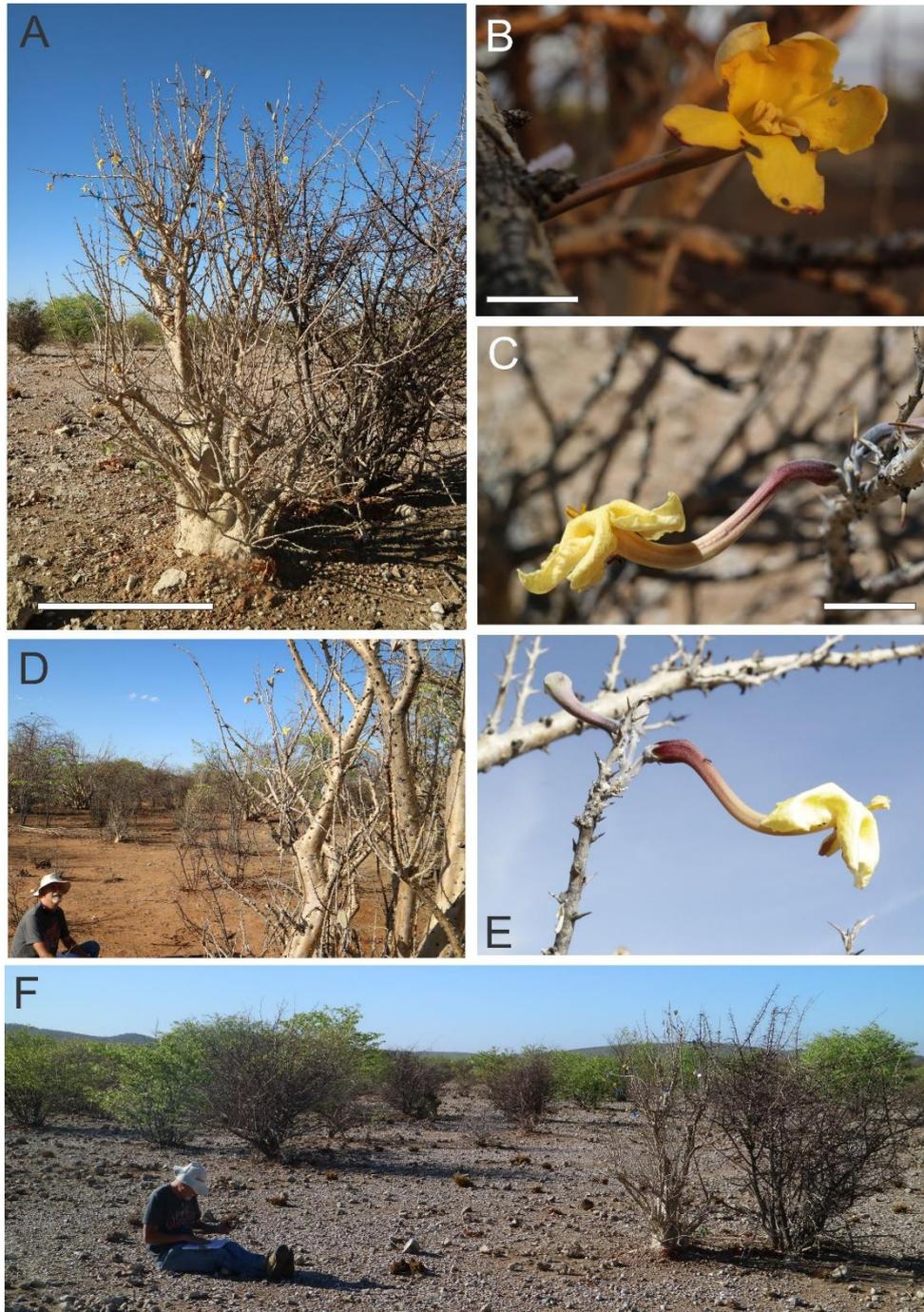


Figure A-1: A large *S. guerichii* shrub with highly ramified secondary and tertiary branches (A). Scale bar = 1 m. A bright yellow *S. guerichii* flower (B). Scale bar = 1 cm. *S. guerichii* flowers have curved corolla tubes and lack a distinct spur (C and E). Scale bar = 1 cm. The arid, heavily grazed and browsed landscape at Khorixas, Namibia (D and F). The calcareous soil at Khorixas (F).

### ***S. guerichii* populations at a glance**

Two populations of *S. guerichii* were studied in the Kunene Region of Namibia: one in an arid rangeland around the town of Khorixas, and one at a private game farm near the town of Outjo, called Sophienhof Guest Lodge.

At Khorixas, a large population of *S. guerichii* shrubs were found. Goats and cattle were herded through the study site daily and, as a result, the landscape was extremely barren, and most shrubs were heavily defoliated. The soil was thin, calcareous and rocky (Figure A-2A and F). The dominant shrubs in the landscape, which included *S. guerichii* and *C. mopane* were heavily browsed below the height of about two metres (A. Bijl pers. obs.). Very few small *S. guerichii* shrubs were noted during the initial observations of the population, and it appeared that the population was comprised mostly of large shrubs.

At Sophienhof, a small population of *S. guerichii* was found. Although herbivores such as eland (*Taurotragus oryx*), springbok (*Antidorcas marsupialis*), gemsbok (*Oryx gazella*), steenbok (*Raphicerus campestris*) and giraffe (*Giraffa camelopardalis*) were present on the farm, they were kept in a fenced camp, so the *S. guerichii* shrubs were, at the time of the study, excluded from herbivory and had been for several years (farm manager, pers. comm.). The soil at Sophienhof was less calcareous than observed at Khorixas, and in patches was deep, fine and red (Figure A-2D). The population of *S. guerichii* shrubs included many shrubs of around one metre tall, as well as larger shrubs, but small seedlings and saplings were not noted during the initial observations of the population.

***Questions addressed in this study***

1. Does *S. guerichii* have high-risk reproductive traits?
  - a. Are *S. guerichii* flowers specialised for pollination?
  - b. Are *S. guerichii* flowers self-incompatible?
  
2. Does *S. guerichii* have a vulnerable demography?
  - a. Do the populations of *S. guerichii* experience demographic bottlenecks?
  - b. Which environmental factors create demographic bottlenecks in *S. guerichii* populations?
  
3. If *S. guerichii* has high-risk traits reproductive traits or a vulnerable demography, does it also have compensation strategies that buffer populations from extirpation?

## **METHODS**

### **Field sites**

Two populations of *S. guerichii* were assessed in the Kunene Region of Namibia, one at Khorixas (-20.382483, 14.932549) and one at Sophienhof Guest Lodge (-20.099257, 16.095010) (Figure A-2). In this chapter, “Khorixas” refers to the plant populations in near the town of Khorixas, and “Sophienhof” refers to the plant population at Sophienhof Guest Lodge. The Sophienhof site is located in the Tree and Shrub Savanna biome, while the Khorixas site falls on the edge of the Tree and Shrub Savanna biome and the Nama Karoo biome (Digital atlas of Namibia, 2002). The vegetation structure at both sites is characterised as sparse shrubland, with grass cover comprising between 0.1 % and 10 % (Digital atlas of Namibia, 2002). At Khorixas the mean annual precipitation is about 233 mm, and at Outjo (Sophienhof) is 394 mm annually (Climate-data.org, S.a.), so both study sites are extremely arid. The Khorixas population was assessed from November the 23<sup>rd</sup> - 28<sup>th</sup> (2015), and the Sophienhof population was assessed from the 29<sup>th</sup> November - 4<sup>th</sup> December (2015).

### **Data collection**

#### **Flower traits**

At Khorixas (2015) and Sophienhof (2015), flower corolla tubes and styles were measured to the nearest millimetre. The mean corolla tube lengths and style lengths were then calculated for each study location and compared across locations.

To record flower opening time in the *S. guerichii* study populations, a sample of flowers that were due to open each night were marked with a unique identification tag prior to opening and were then observed at roughly ten-minute intervals over the course of the evening, between 16:00 and 21:00. During anthesis, petals unfurled sequentially, and the time at which the first petal was fully open was recorded as “open time” for each flower, because at this point the reproductive structures were exposed and flowers were sufficiently accessible to visitors.

Each flower was assigned to a 30-minute “open time” bin, and the cumulative number of flowers open per time bin was plotted for each sample population. An opening time distribution was created using a GLM with a binomial distribution and logit link function. The coefficients calculated in the logistic regressions for each location and year were substituted into Equation 1 (Chapter 2) to create a model flower-opening curve for each population, and the modelled curve was used to determine the peak flower opening time for each population.

### **Flower pollination**

To determine if flowers were receiving pollen at Khorixas (2015) and Sophienhof (2015), flower stigmas were inspected with a hand lens for pollen tetrads in the mornings ( $\pm 12$  hours after anthesis). If present, pollen tetrads were counted.

To assess whether the amount of pollen received was influenced by corolla tube length or the location of the study site, a GLM with a negative binomial distribution and log link function was used. Corolla tube length (cm), location (Khorixas or Sophienhof) and the interaction term of corolla tube length and location (T x L) were used as the predictor variables, and pollen tetrad count as the dependent variable.

The mean corolla tube length of flowers was calculated for each location, and then flowers with corolla tubes shorter than the mean value were classified as “short” and flowers with corolla tubes longer than the mean, classified as “long”. To assess the difference in pollen tetrad count between the long and short flowers at each of the two locations, a GLM with a negative binomial distribution and log link function was run for each population. Corolla tube length (short or long) was used as the predictor variable, and pollen tetrad count as the dependent variable.

### **Flower visitors and effective pollinator identification**

Bushnell Natureview motion-activated cameras (equipped with passive infra-red sensors and close up lenses (460 mm) were used at Sophienhof on the 22<sup>nd</sup>, 23<sup>rd</sup>, 24<sup>th</sup> and 26<sup>th</sup> of November (2015) and 16 flowers in total were recorded. Each flower was recorded for a single night. Each afternoon at 16:00, prior to flower anthesis, a camera

trap was directed towards a selected flower, configured to record a 30-second video when triggered, left on throughout the night, and manually switched off the following morning at 10:00. No cameras were used at Khorixas. Video footage was assessed to identify visitors, record the time and frequency of visits and determine how visitors were utilising flowers. Visitor behaviour associated with likely movement of pollen was recorded as “positive”, whereas behaviour that damaged the stigma or anthers, or reduced the attractiveness of flowers on the opening night, was recorded as “negative.”

Visitors were observed visually and opportunistically captured by hand or with a hand-held net at Sophienhof (2015), to determine whether they were picking up and transporting *S. guerichii* pollen on their bodies. *S. guerichii* pollen tetrads are large and distinct enough to identify to species.

### **Breeding system**

A sample of flowers due to open each night was identified, marked with unique metal identification tags and covered with fine-mesh drawstring bags. Once the flowers had opened, the flowers were either left untreated (testing for autogamy), hand-pollinated with pollen from a randomly selected conspecific plant’s flowers (cross-pollinated positive control), or pollinated with their own pollen (testing for self-incompatibility). The bags remained on for several weeks until fruit had begun to form.

To assess the difference in fruit set between treatments across the two study locations, an events by trials GLM with a binomial distribution and logit link function was run. The number of flowers sampled represented the trials, and the number of flowers that developed into pods, the events. The predictor variable was treatment (self, cross or autogamy).

### **Population demography and demographic bottlenecks**

For each population (Khorixas and Sophienhof) the height (m) of each shrub was recorded, and used to create demographic profiles and height-class distributions. Shrubs were categorised according to biologically meaningful height classes (Table 3-1).

Demographic profiles were created for populations by ranking all the shrubs according to height, and graphing the distributions using histograms. A moving window analysis was then done for each distribution, where, for each population, shrub heights were ranked and then the mean height and standard deviation of an initial fixed subset of heights (5) was calculated.

The mean numbers (and standard errors) of flowers on shrubs in each height class in the Khorixas and Sophienhof populations were calculated. For each population, a binomial logistic GLM with logit link function was run, with shrub height as the predictor variable and flowers present (yes or no) as the outcome variable. The shrub heights associated with the modelled peak rate of increase in the probability of having flowers were then calculated using the regression coefficients of the GLMs, substituted into Equation 1 (Chapter 2). Flower output could then be compared across height classes and between the populations.

#### **Notes on statistical analyses**

All statistical analyses were done using IBM's SPSS® Statistics, version 23 software, and for all generalised linear models (GLMs), the chi-square likelihood ratio ( $\chi^2$ ) was used as the test statistic to assess the significance (p) of the model effects.

## RESULTS

### Flower traits

*S. guerichii* flowers have fairly long, narrow corolla tubes ranging from 5 - 9 cm, and the style lengths ranging from 6 - 10 cm (Table A-1). At Khorixas, the mean corolla tube length and style length was greater than at Sophienhof: Khorixas mean corolla tube:  $7.0 \pm 0.9$  cm, and mean style:  $8.1 \pm 1.4$  cm ( $n = 68$ ) and Sophienhof mean corolla tube:  $6.7 \pm 0.7$  cm, and mean style:  $7.8 \pm 0.6$  cm ( $n = 62$ ) (Table A-1). Corolla tube lengths did not differ significantly between Khorixas and Sophienhof ( $t = 1.732$ ,  $p = 0.086$ ) and nor did style lengths ( $t = 1.313$ ,  $p = 0.192$ ).

*S. guerichii* flowers at Khorixas (2015) and at Sophienhof (2015) began opening between 18:00 and 18:30. The modelled peak flower opening time at Khorixas (2015), was at 19:45 (GLM  $\chi^2(9) = 559.081$ ,  $p < 0.005$ ), and at Sophienhof (2015), was at 19:42 (GLM  $\chi^2(9) = 474.642$ ,  $p < 0.005$ ) (Figure A-3).

Flowers were receiving pollen at Khorixas (2015) and Sophienhof (2015). Pollen deposited on flower stigmas at Sophienhof ranged from 0 - 90 tetrads, and at Khorixas, ranged from 0 - 95 tetrads (Figure A-4). The volume of pollen received (number of pollen tetrads deposited) was influenced by corolla tube length and the location of the study site (GLM  $\chi^2(3) = 10.435$ , Tube length  $p = 0.005$ , Location  $p = 0.019$ , T x L  $p = 0.022$ ).

At Khorixas (2015), flowers with corolla tubes longer than the population mean tube length (7 cm) received significantly more pollen than flowers with corolla tubes shorter than the population mean ( $\chi^2(1) = 10.395$ ,  $p = 0.001$ ), but at Sophienhof (2015) there was no significant difference in pollen receipt between short and long flowers ( $\chi^2(1) = 0.004$ ,  $p = 0.950$ ) (Figure A-5).

## Flower visitors and effective pollinator identification

A total of 85 flower visits were recorded on *S. guerichii* flowers (n = 16) at Sophienhof (2015) using Bushnell Natureview motion-activated cameras. Flowers were visited frequently at night after anthesis (48 visits), but also fairly frequently in the mornings after anthesis (38 visits). (Table A-2). A variety of insects were attracted to the flowers. The most common night time visitors were small settling moths (Noctuidae) (Supplementary video S 4-1), active between 21:00 and 03:30 (Figure A-6).

Unidentified hawkmoths were seen flying past flowers at night on six occasions, between 18:42 and 22:56, but they never probed the flowers (Table A-2). The most common positive morning visitors were Carpenter bees (*Xylocopa* spp.) (n = 2) active between 08:30 and 09:30 (Figure A-6), and their visits were positive, because the bees brushed against the anthers and stigmas of flowers when landing on the petals (Table A-2; Figure A-7), but the Carpenter bees (*Xylocopa* spp.) also had a negative impact on flowers because they robbed the flowers of nectar (Table A-2). White-bellied Sunbirds (*Cinnyris talatala*) were frequent, negative visitors (n = 10), as they robbed the flowers of nectar. White-bellied Sunbirds were active between 06:30 and 10:30 (Figure A-6).

Carpenter bees (n = 2) were captured and were found to be carrying *S. guerichii* pollen (Table A-3). A small cockroach (n = 1), Christmas beetles (n = 2), scarab beetles (n = 5), sugar ants (n = 5), cocktail ants (n = 5), bal-byter ants (n = 5) and small settling moths were captured when they visited *S. guerichii* flowers, but none were carrying pollen on their bodies (Table A-3): most were visiting the flowers to forage for nectar at the extra-floral nectaries (EFNs) or to forage for pollen (Table A-3).

Carpenter bee behaviour was observed visually on the mornings of the 26<sup>th</sup> and 27<sup>th</sup> of November (2015) at Sophienhof, between 07:33 and 07:42 (Figure A-7E to J). On both occasions, the Carpenter bee would visit several flowers on a shrub in succession, leave to visit another shrub, and then return to visit more flowers on the first shrub. The bees were too large to enter the corolla tube to access the nectar at the base, but would land on the petals, walk over them, brushing the stigma and anthers of the flower, and then move down the tube to the base of the flower to chew a hole in the tube and rob nectar from the flower (Supplementary video S 4-2).

## **Breeding system**

In the hand-pollination experiments, pods developed in 50 % of cross-pollinated flowers (8 out of 16), 0 % of self-pollinated flowers (0 out of 5) and 0 % of pollinator-excluded and unmanipulated flowers (0 out of 7). Treatment (cross-pollinated, self-pollinated and autogamy) was a significant predictor of pod development (GLM  $\chi^2(2) = 11.322$ ,  $p = 0.003$ ) (Figure A-8).

## **Population demography and demographic bottlenecks**

*S. guerichii* populations at Khorixas (2015) and Sophienhof (2015) were comprised mostly of large shrubs (Figure A-9). There appear to be recruitment bottlenecks in both populations, as very small plants were missing from the demographic profiles and height-class distributions of the populations (Figure A-9).

No seedlings were found at Khorixas (2015), and only a single small sapling (15 – 30 cm) was observed. Most shrubs fell within the medium (2 – 4 m) height class, and very few shrubs fell in the small (1 – 2 m) and large (4 – 6 m) height classes (Figure A-10).

No seedlings were found at Sophienhof (2015) either. One small sapling (15 – 30 cm) and two large saplings (30 – 100 cm) were found. There were, however, many small (1 – 2 m), medium (2 – 4 m) and large (4 – 6 m) shrubs. Even a few very large shrubs (6 – 8 m) were found (Figure A-10).

## **Reproductive output and height of reproductive maturity**

At Khorixas and Sophienhof, 75 % of the small shrubs and 97 % of the medium shrubs had flowers, where at Sophienhof, only 30 % of the small shrubs and 58 % of the medium shrubs had flowers (Figure A-11). At Khorixas, 100 % of the large shrubs had flowers, and at Sophienhof, 90 % of the large shrubs and 100 % of the very large shrubs had flowers (Figure A-11).

At Khorixas, the height at which shrub is more likely than not to have flowers is 1.6 m (GLM  $\chi^2(1) = 16.978$ ,  $p = 0.00038$ ), and At Sophienhof, is 2.6 m (GLM  $\chi^2(1) = 31.428$ ,  $p = 2.0695E^{-8}$ ) (Figure A-11).

## DISCUSSION

The stem shape, stem number, branching structure, bark texture and bark colour of *S. lugardii* and *S. guerichii* is extremely similar (Figure A-12). The flowers of these two *Sesamothamnus* species, however, are distinctly different: *S. guerichii* flowers are shorter and more curved than *S. lugardii* flowers (Figure A-12). *S. guerichii* flowers also lack a distinct spur, and are pale to bright yellow in colour (Figure A-12).

*S. guerichii* flowers have fairly long, narrow corolla tubes with slightly protruding styles (Table A-1). The corolla tube lengths and style lengths vary a little between the Khorixas and Sophienhof populations, but the differences are not significant. Local variation in floral tube length across a geographical range is not unusual, and may be the result of coevolution between the plants and available pollinators in distinct populations (Anderson & Johnson, 2008).

*S. guerichii* flowers open in the evenings between 18:00 and 18:30 (Figure A-3) and the peak rate of flower-opening were extremely similar across the study populations (at Khorixas it was 19:45 and at Sophienhof was 19:42). This is unlike what was observed for *S. lugardii*, where the difference in peak opening time of the two geographically separated populations was greater than an hour.

*S. guerichii* flowers at both the Khorixas and Sophienhof populations received pollen, and the amount of pollen received was similar (Figure A-4). At Khorixas, flowers with longer corolla tubes received significantly more pollen than flowers with shorter corolla tubes (Figure A-5). Interestingly, at Sophienhof, there was no significant difference in pollen receipt between short and long-tubed flowers (Figure A-5). In the case of *S. lugardii*, long flowers received no more pollen than short flowers, because *A. convolvuli* hawkmoths effectively transport large volumes of pollen on their proboscides, so long and short flowers receive similar volumes of pollen and enjoyed no greater fitness benefit. In the case of *S. guerichii*, at Khorixas, having longer corolla tubes increases fitness, by ensuring greater pollen receipt, where, at Sophienhof, there was no difference between the long and short flowers. The relationship between corolla tube length and pollen receipt would be worth exploring in greater detail in a future study.

Flowers were visited frequently at night, after anthesis, and almost as frequently the mornings after anthesis, by a variety of insects and White-bellied Sunbirds (Table A-2). The most common night-time visitors were small settling moths, which would hover around the flowers, or land on the petals. Flowers were also approached by hawkmoths (unidentified species), but the hawkmoths were never recorded probing the flowers for nectar (Table A-2). The curved shape of the *S. guerichii* corolla tubes, and upward facing orientation of the majority of observed flowers, would make them almost impossible for hawkmoths such as *A. convolvuli* to probe, as it would require the moths to feed upside-down or bend their proboscides “upwards” beyond 180° (Figure A-7C & G). The most common positive morning visitors were Carpenter bees (*Xylocopa* spp), which would land on the petals, walk over them and brush against the anthers and stigmas of flowers, and then move down the corolla tube to rob nectar. When doing visitor captures, it was found that only the Carpenter bees were carrying *S. guerichii* pollen, making them the only effective pollinators of *S. guerichii* noted in this pilot study.

Although the flowers of *S. guerichii* are long-tubed, and are attractive to noctuid and sphingid moths, no moths were found to be effective pollinators in this pilot study. As hawkmoth tongues cannot bend “upwards” the curved morphology and orientation of the *S. guerichii* flowers make it unlikely that they could be easily accessible, and therefore pollinated by, very long-tongued hawkmoths such as *A. convolvuli* (see Figure A-1 E), but perhaps other hawkmoth species could successfully probe the flowers. Carpenter bees were found to be visiting *S. guerichii* flowers and moving pollen, so they may be effective pollinators of *S. guerichii*.

Groman and Pellmyr (1999) found that flowers adapted to night-time pollinators may also be visited by diurnal species which contribute partially to their reproductive success, and tested this in *Manfreda virginica*, a nocturnally blooming species pollinated by medium and large hawkmoths. Groman and Pellmyr (1999) found that large bees play a minor role in pollination, and that the effectiveness of the bees was limited mostly because their activity time and the period of floral anthesis were asynchronous. The yellow-flowered Evening primrose (*Oenothera elata*) is another

example of a flower that appears specialised exclusively for hawkmoth pollination (it has typical hawkmoth traits), but which is effectively pollinated by both hawkmoths and evening-foraging Carpenter bees (*Xylocopa tabaniformis orpifex*) (Barthell & Knops, 1997). Bees have trichromatic colour vision, so can distinguish colours, and Carpenter bees (*Xylocopa* spp) seem to prefer yellow flowers, but are also attracted to purplish-white, creamy white and bluish white flowers (Raju & Rao, 2006). This may explain why pale “hawkmoth flowers” are attractive to Carpenter bees too. Another hawkmoth-pollinated Evening primrose (*Oenothera macrocarpa*), was also found to be effectively pollinated by diurnal visitors, specifically small bees (Moody-Weis & Heywood, 2001). Evening primroses are therefore better described as having a mixed pollination syndrome (Barthell & Knops, 1997). Perhaps *S. guerichii* also has a mixed pollination syndrome, and this would be worth exploring in future research.

The breeding system experiments confirmed that only cross-pollinated *S. guerichii* flowers develop into pods, so flowers are self-incompatible (Figure A-8).

The demographic profiles of *S. guerichii* populations showed a clear lack of recruitment (Figure A-9). Additionally, the height-class distributions of the populations approximated Gaussian distributions, and were skewed towards larger, older plants (particularly at Sophienhof). This trend is characteristic of populations of plants that are slow-growing and long-lived, with erratic recruitment. The *S. guerichii* study populations retain reproductive potential in the established medium and large shrubs, and therefore, these populations are buffered from extirpation during periods of reproductive failure.

It is interesting that at Khorixas there were almost no shrubs smaller than two metres tall, or larger than four metres tall. In conversation, a goat herder confided that the locals harvest *S. guerichii* branches to make carvings, so perhaps the shrubs are kept in these medium size classes by extensive harvesting of secondary and tertiary branches, and so transitioning into larger size classes is suppressed.

The absence of seedlings, saplings and small shrubs at Khorixas may be a consequence of the intense herbivory by goats and cattle on this communal rangeland. The

recruitment bottleneck experienced at Sophienhof may also be the result of selective herbivory by small antelope. Steenbok were often seen around the *S. guerichii* population, and the manager confirmed that small, wild antelope frequently entered and passed through the property.

The environment at Sophienhof and especially at Khorixas is extremely arid, so water limitation could be limiting germination rates. This was unexplored. Pollen limitation also does not appear to be affecting the populations, as most flowers were receiving pollen, but this was not tested experimentally. Although flowers in both populations were receiving pollen, many received fairly small quantities, and only a few flowers received large quantities of pollen (Figure A-4). A pollen supplementation experiment would confirm whether these populations are pollen limited, and is recommended for future research. Flower predation may limit recruitment of *S. guerichii*. Scarab beetles were recorded in low numbers preying flowers at Sophienhof (Table A-2) and may be responsible for limiting the numbers of flowers available for pollination.

At both locations, the large and very large shrubs all had flowers (Figure A-11). At Khorixas, shrubs were more likely to have flowers than not when they were over 1.6 m tall, and at Sophienhof, over 2.6 m tall. Perhaps the intense goat herbivory and harvesting of branches at Khorixas induces more branch ramification in the shrubs, resulting in more axillary buds from which more flowers can develop. This may explain why the small and medium shrubs at Khorixas have more flowers than same-sized shrubs at Sophienhof (where most herbivores are excluded).

The recruitment bottleneck affecting the populations of *S. guerichii* may be the consequence of pollen limitation, flower and pod predation, or due to a lack of sufficient germination cues and resources to support vulnerable seedlings (Figure A-1). It would be worth exploring the recruitment bottleneck in more detail in a future assessment.

## CONCLUSION

In this study, I aimed to assess whether one can determine the inherent risk of extinction (in the face of a mutualism collapse) for a given species based on a combination of breeding system traits and demographic traits, and determine whether the extirpation risk in populations of a given species could be predicted using simple demographic assessments.

Although *S. guerichii* flowers have traits characteristic of hawkmoth pollination (pale flowers, long, narrow corolla tubes, evening anthesis and a sweet scent) and are clearly attractive to a variety of noctuid moths and hawkmoths, their shape makes them inaccessible to long-tongued hawkmoths such as *A. convolvuli*. No evidence of *A. convolvuli* hawkmoth pollination was observed in this study, but Carpenter bees were found to be effective pollinators of *S. guerichii* flowers, so I expect that, unlike *S. lugardii* which is exclusively pollinated by *A. convolvuli* hawkmoths, *S. guerichii* has a mixed pollination syndrome that includes moths and carpenter bees. Like *S. lugardii*, *S. guerichii* flowers are self-incompatible, and the populations of *S. guerichii* studied, also experienced severe recruitment bottlenecks. Although pollen limitation, flower predation and pod predation and germination failure due to water limitation were not explicitly tested or explored, they may contribute towards failed recruitment in the *S. guerichii* populations, and would be worth exploring. The storage effect buffers *S. guerichii* populations from reproductive failure and possible extirpation, so even if the pollination mutualism, or mutualisms of *S. guerichii* collapse, the species is not currently at risk of extirpation. In Khorixas, there are extremely few small shrubs, so if herbivory and harvesting intensify and threaten the survival of the medium shrubs (the size cohort which comprises almost the entire population), there will be no plants to replace the lost plants which currently retain all the reproductive potential of the population.

This type of “risk assessment strategy” was useful for determining the extirpation risk of two populations of *S. guerichii* in Namibia. It provided a practical approach for assessing risk and could be useful in conservation planning and plant resource management.

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## TABLES AND FIGURES



Figure A-2: Study sites where populations of *S. guerichii* were assessed in the Kunene Region of Namibia, at Khorixas (-20.382483, 14.932549) and Sophienhof Guest Lodge (-20.099257, 16.095010).

Table A-1: The corolla tube length ranges (cm), style length ranges (cm) mean tube lengths ( $\pm$  standard deviation (n)) and mean style lengths ( $\pm$  standard deviation (n)) at Khorixas and Sophienhof (2015).

Location	Tube length range	Style length range	Mean tube length $\pm$ SD (n)	Mean style length $\pm$ SD (n)
Khorixas	5.2 - 9.0	7.1 - 10.0	7.0 $\pm$ 0.9 (62)	8.1 $\pm$ 1.4 (62)
Sophienhof	5.0 - 7.6	6.0 - 7.9	6.7 $\pm$ 0.7 (68)	7.8 $\pm$ 0.6 (68)

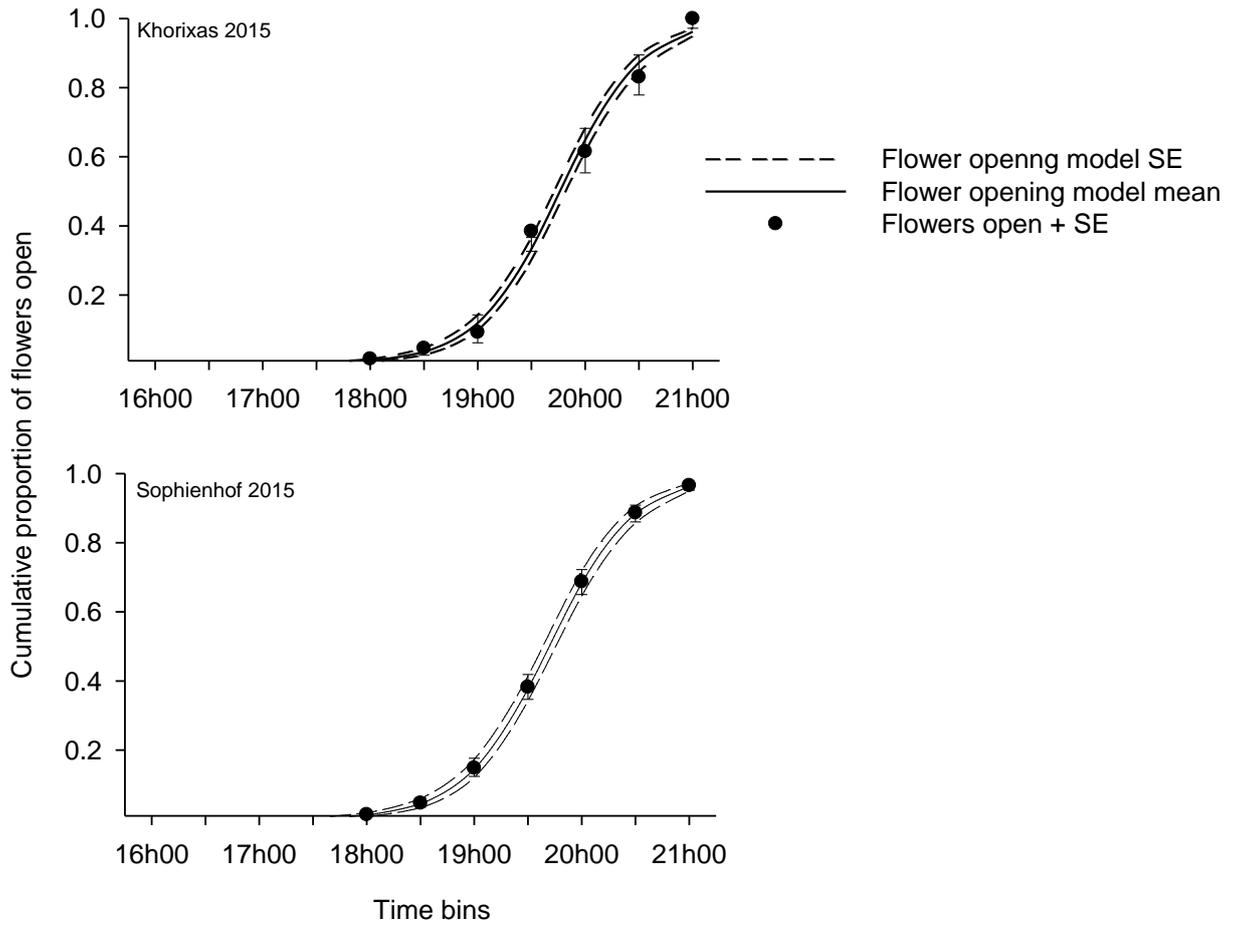


Figure A-3: The cumulative number of *S. guerichii* flowers open per 30-minute time bin, at Khorixas (2015) and Sophienhof (2015). The observed number of flowers open indicated by black circles, and the GLM binomial logistic regression model means and predicted standard errors indicated with dotted and solid black lines respectively.

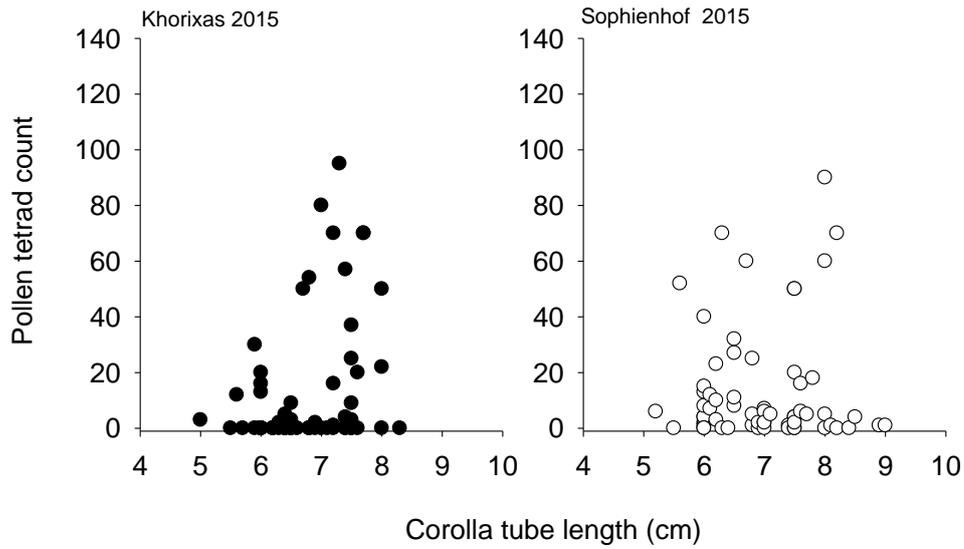


Figure A-4: The number of pollen tetrads on flowers at Khorixas (2015) and Sophienhof (2015).

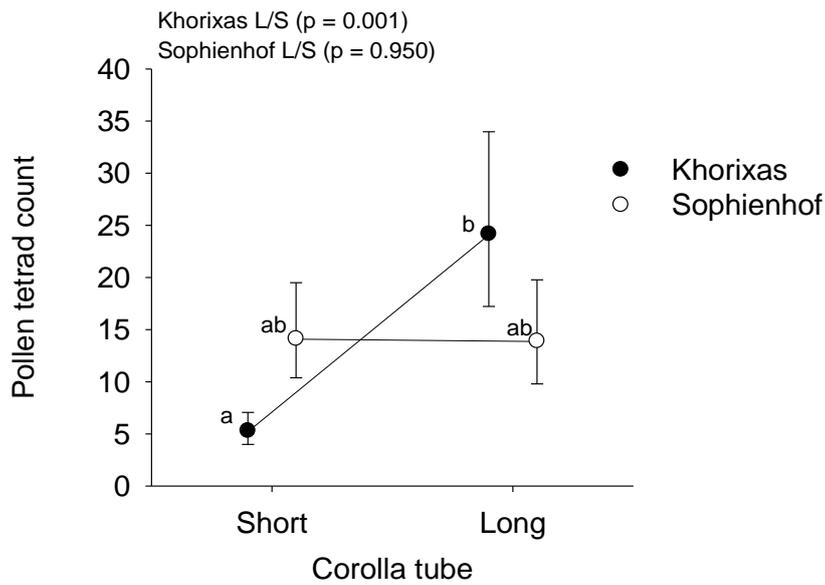


Figure A-5: Scatter plot of the mean number of pollen tetrads present on flower stigmas across the study locations. For each population, the significance values of the predictor (Long or short corolla tube (L/S)). Means that share letters are not significantly different.

Table A-2: Table indicating the total number of flower visitors and the number of visits in each impact category (positive or negative) on the opening night of flowers and during the mornings after the opening night, recorded at Sophienhof guest lodge, in Outjo, Namibia (2015).

Visitor	Flower opening night interactions			Morning after flower opening interactions			Most common behaviour
	Total	Positive	Negative	Total	Positive	Negative	
Small settling moths (multiple spp)	17	4		3	1		Walking around on petals
Sugar Ant ( <i>Camponotus</i> spp)	9			1			Foraging for nectar at EFN
Christmas beetle ( <i>Anoplognathus</i> spp)	7	2					Walking around on petals
Bal-byter ant ( <i>Camponotus</i> spp)	4			1			Foraging for nectar at EFN
Hawkmoth (unknown spp)	6						Flying around shrubs
Small cockroach ( <i>Blatella</i> spp)	1			2			Walking around on petals
Grasshopper ( <i>Chortophaga</i> spp?)	1						Flower predation
Scarab beetle ( <i>Phyllophaga</i> spp)	3		3				Flower predation
Carpenter bee ( <i>Xylocopa</i> spp)				8	2	2	Nectar robbing + Foraging for nectar
Solitary bee (multiple spp)				3	1		Foraging for nectar
Cocktail Ant ( <i>Crematogaster</i> spp)				2	1		Walking around on petals
White-bellied Sunbird ( <i>Cinnyris talatala</i> )				10		10	Robbing nectar from spur
Ichneumon wasp (Family <i>Ichneumonoidea</i> )				8			Walking around on petals
<b>Total</b>	<b>48</b>	<b>6</b>	<b>3</b>	<b>38</b>	<b>5</b>	<b>12</b>	

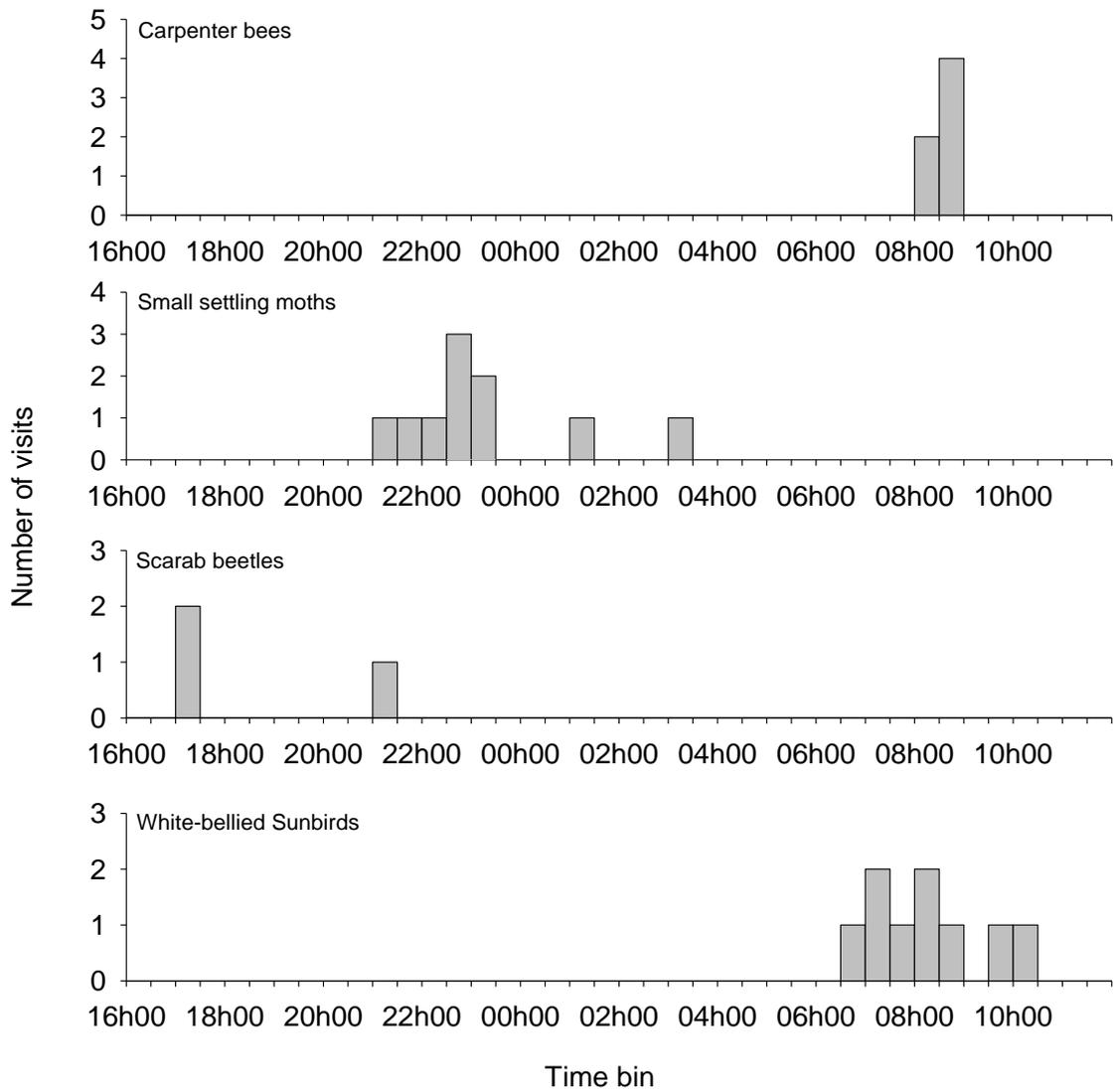


Figure A-6: The frequency distributions of most common positive and negative visitors of *S. guerichii* flowers, recorded with Bushnell Natureview motion-activated cameras at Sophienhof (2015).

Table A-3: *S. guerichii* flower visitors opportunistically captured at Sophienhof (2015). Visitor locations on the flowers and behaviours were recorded. Sample size captured and presence or absence of *S. guerichii* pollen on the visitor was recorded.

Visitor	Location	Behaviour	Captured (n)	Pollen present
Small cockroach ( <i>Blatella</i> spp)	Petals + corolla tube	Unknown	1	No
Carpenter bee ( <i>Xylocopa</i> spp)	Petals + corolla tube	Nectar robbery/pollen foraging	2	Yes
Christmas beetle ( <i>Anoplognathus</i> spp)	Petals	Unknown	2	No
Scarab beetle ( <i>Phyllophaga</i> spp)	Petals, stigmas, anthers	Predation	5	No
Sugar ant ( <i>Camponotus</i> spp)	Petals + corolla tube	Nectar foraging at EFN	5	No
Cocktail ant ( <i>Crematogaster</i> spp)	Petals + corolla tube	Nectar foraging at EFN	5	No
Small settling moths (multiple spp)	Petals + corolla tube	Nectar foraging	5	No
Bal-byter ant ( <i>Camponotus</i> spp)	Petals + corolla tube	Nectar foraging at EFN	5	No
Solitary bee (multiple spp)	Petals, stigmas, anthers	Nectar/pollen foraging	0	NA
White-bellied Sunbird ( <i>Cinnyris talatala</i> )	Corolla tube	Nectar robbery	0	NA



Figure A-7: A bright yellow flower of *S. guerichii* (A). Scale bar = 1 cm. A pale-yellow flower of *S. guerichii*, with a twisted and curved corolla tube (B). Scale bar = 2 cm. Curved and upward facing flowers of *S. guerichii* (C and D) Scale bar = 5 cm. A Carpenter bee landing on the petals of a *S. guerichii* flower, and moving over the reproductive structures (E, F, G and H). Scale bar = 1 cm. Carpenter bees robbing nectar from the base of *S. guerichii* corolla tubes (I and J). Scale bar = 1 cm.

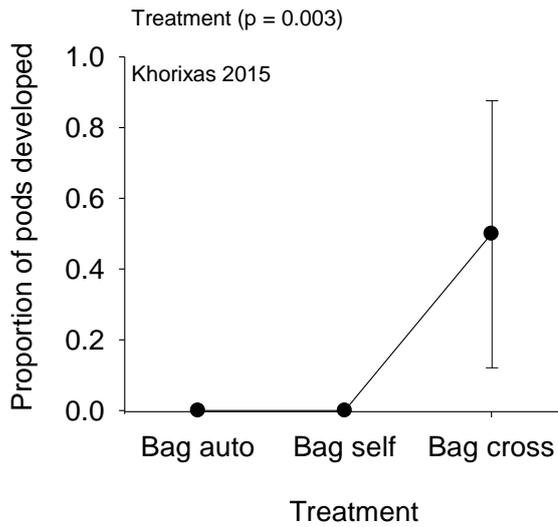


Figure A-8: The mean ( $\pm$ SE) proportion of pods that developed in hand-pollination experiments, where flowers were left untreated and bagged (Bag auto), pollinated with own pollen and bagged (Bag self) or pollinated with a conspecific plant's pollen and bagged (Bag cross).

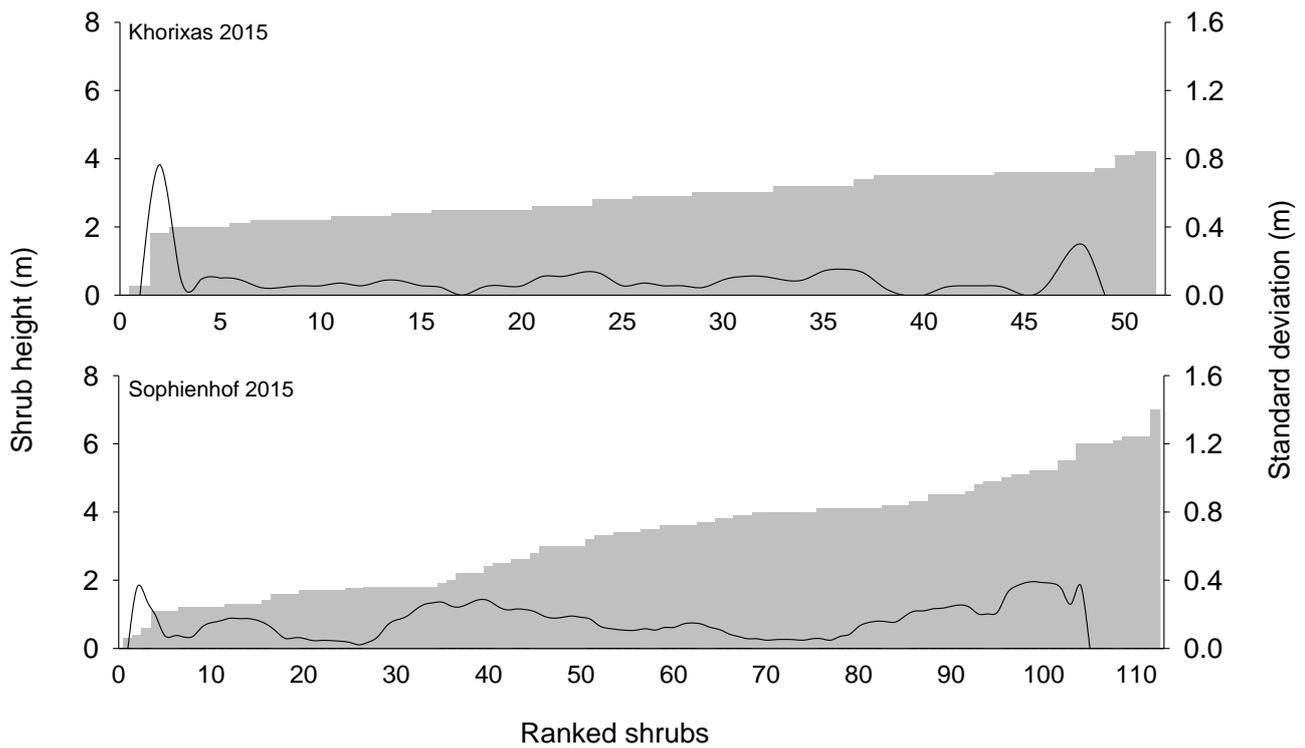


Figure A-9: The ranked distributions of *S. guerichii* shrub heights of populations in Khorixas (2015) and Sophienhof (2015) with moving window analyses displayed on secondary Y-axes.

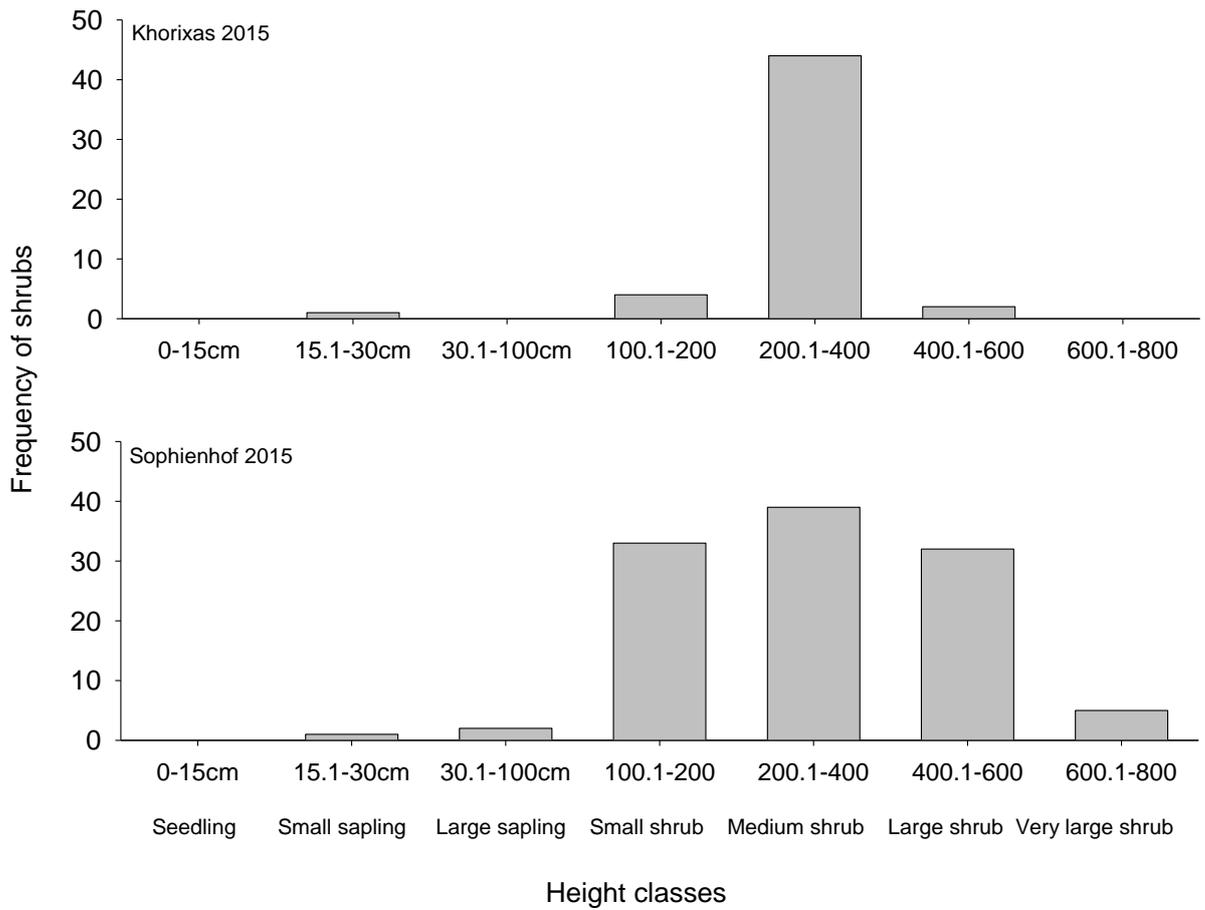


Figure A-10: The height-class distributions of the populations of *S. guerichii* of populations in Khorixas (2015) and Sophienhof (2015).

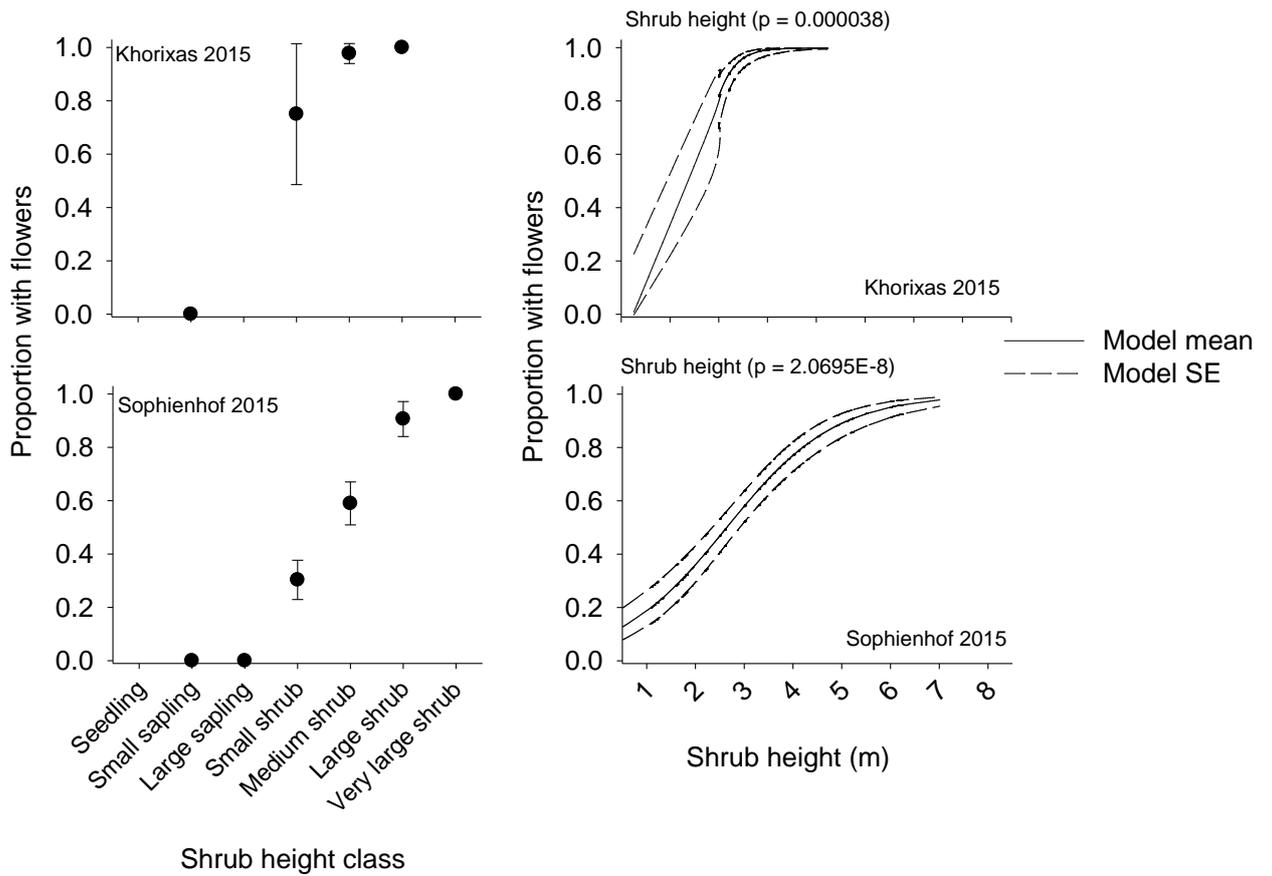


Figure A-11: Left: The proportions of *S. guerichii* shrubs with flowers, in each height class, at Khorixas (top) and Sophienhof (bottom) in 2015. Right: The modelled proportions of shrubs with flowers, across shrub heights, at Khorixas (top) and Sophienhof (bottom).

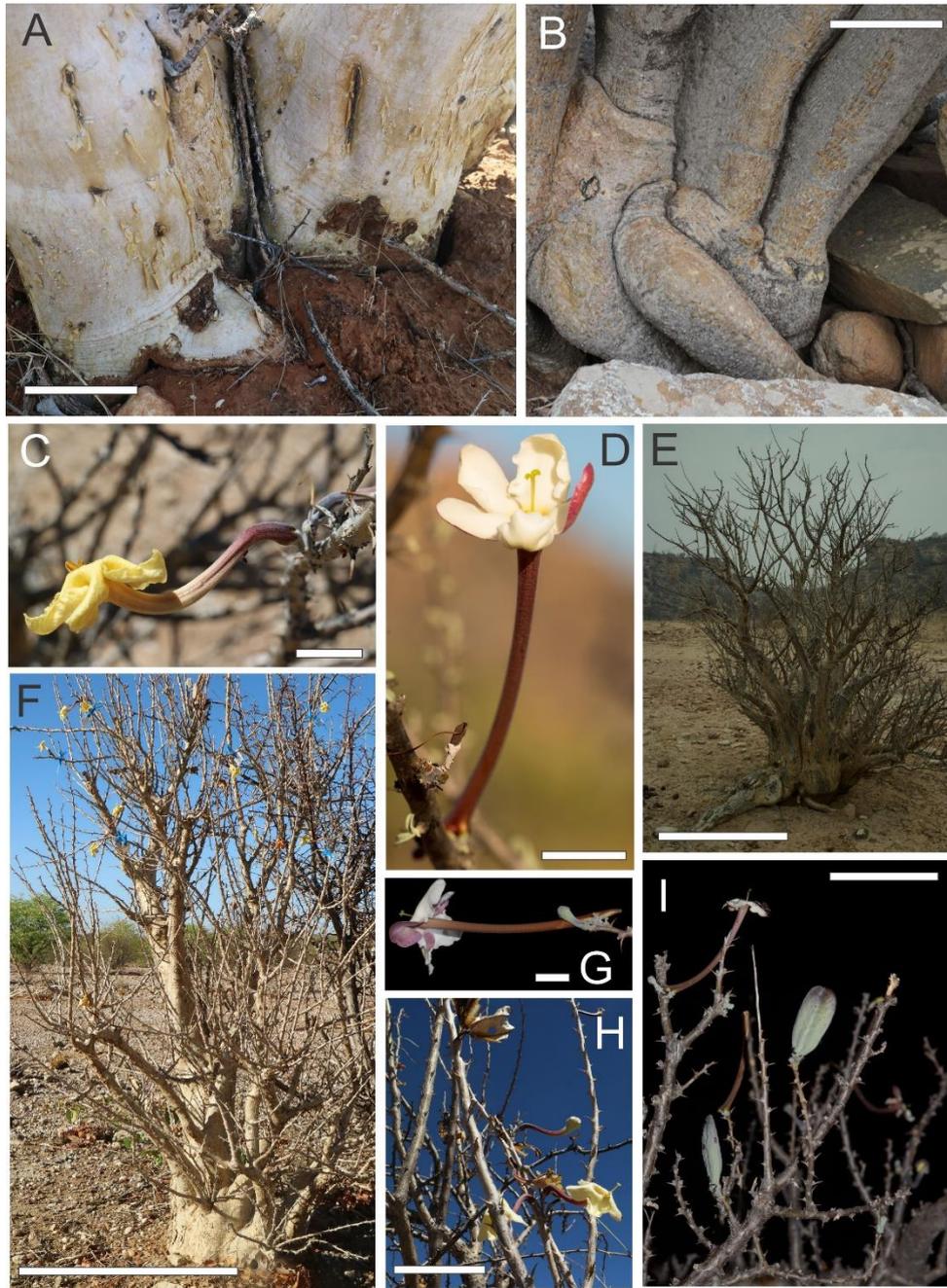


Figure A-12: The succulent stem with pale yellow and flaking bark of *S. guerichii* (A), Scale bar = 5 cm, and *S. lugardii* (B). Scale bar = 10 cm. A yellow, long-tubed, curved *S. guerichii* flower without a spur (C). Scale bar = 1 cm. A white, straight, long-tubed *S. lugardii* flower with a spur (D). Scale bar = 1 cm. An *S. lugardii* flower with distinct spur (G). Scale bar = 1 cm. A typical, multi-stemmed *S. lugardii* shrub (E). Scale bar = 1 m. A typical, multi-stemmed *S. guerichii* shrub (F). Scale bar = 1 m. The ramified tertiary branches of *S. guerichii* (H), Scale bar = 10 cm, and *S. lugardii* (I). Scale bar = 10 cm.

## **APPENDIX B**

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No edits to the original manuscript have been made in this thesis submission

# From dusk till dawn: camera traps reveal the diel patterns of flower-feeding by hawkmoths

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**Abstract.** 1. Diel rhythms of foraging activity by animal flower visitors can reflect niche partitioning and are considered an important component of selection on floral traits. However, it has been notoriously difficult to obtain objective information on the patterns of flower visitation by crepuscular and nocturnal insects.

2. We used motion-activated cameras for field-based studies of hawkmoth foraging behaviour on six African plant species.

4. Our results showed that short-tongued hawkmoth species forage mainly around dusk and then sporadically throughout the night, while long-tongued hawkmoth species feed consistently throughout the night, with a peak shortly before midnight.

5. These results provide the first quantitative estimates of diel patterns of interactions between multiple hawkmoth and plant species and, when combined with qualitative reports from other studies, suggest that differences in diel activity between the two main

hawkmoth functional groups (short- and long-tongued) are consistent across the Old and New Worlds.

**Keywords.** motion-activated camera, niche, Noctuidae, pollination, Sphingidae, tongue length

## **Introduction**

Information on the diel activity patterns of flower-visiting animals is essential for understanding the ecology of pollination mutualisms ([Broadhead et al., 2017](#); [Hoballah et al., 2005](#); [Stone et al., 1996](#)). However, there is little verified information on the activity patterns of nocturnal flower-visiting insects, such as hawkmoths, as it is notoriously difficult to observe them in a manner that does not affect their behaviour and because human stamina for all night observations is limited.

Hawkmoths are key pollinators of plants in the tropics and warm temperate regions of the world ([Haber & Frankie, 1989](#); [Johnson et al., 2017](#)). Studies have shown that there are distinct functional hawkmoth guilds that are distinguished by differences in proboscis length ([Johnson et al., 2017](#)). Most hawkmoths have short proboscides (<8 cm), but there are also distinct guilds of species with much longer proboscides (8-28 cm).

It has generally been assumed that hawkmoths forage for nectar at dusk, but there have been some intriguing reports of late-night activity by hawkmoths ([Baum, 1995](#); [Gregory, 1963](#)) and even dawn foraging ([Baum, 1995](#); [Janzen, 1984](#); [Martins & Johnson, 2013](#); [Murcia, 1990](#)). It has not been clear whether these represent primary or secondary peaks in activity and whether there is variation in activity patterns among different hawkmoth functional groups. There are also some data on general activity rhythms of hawkmoths from laboratory studies ([Broadhead et al., 2017](#); [Hoballah et al., 2005](#)) and light-trapping ([Beck & Linsenmair, 2006](#); [de Camargo et al., 2016](#)), but it has been uncertain whether these data reflect actual patterns of periodicity of flower-feeding in the wild.

The aim of this study was to document the diel rhythms of flower-feeding by hawkmoths to test whether there is variation among functional groups. We also tested the efficiency of motion-activated cameras for recording moth activity, and conducted a survey of previous qualitative reports on the timing of hawkmoth activity at flowers.

## **Materials and methods**

We used Bushnell Nature View cameras (model 119740, Bushnell Corporation, Overland Park, Kansas) to record visitation by moths to flowers of six plant species: *Satyrium longicauda* (Orchidaceae), *Habenaria clavata* (Orchidaceae), *Bonatea antennifera* (Orchidaceae), *Gardenia thunbergii* (Rubiaceae), *Crinum macowaniana* (Amaryllidaceae) and *Sesamothamnus lugardii*

(Pedaliaceae) (Details in Table S2). These cameras use passive-infrared detection (PIR) and are widely used to study vertebrates, but their utility for recording insect behaviour has not previously been verified. As the thoracic temperature of hawkmoths is typically 31-36 degrees C in flight ([Heinrich, 1993](#)), we predicted that these insects would be detected by these sensitive PIR cameras. Lenses supplied with Bushnell 119740 cameras allow close focusing either 460 mm or 250 mm from the subject. We used 4-8 cameras per night and spaced them 30-400 m apart to minimize the chance of repeat sampling of individual moths. We used 460 mm lenses, except in the case of *Satyrium longicauda* for which we used both 460 mm and 250 mm lenses as this species is visited by hawkmoths and smaller noctuid moths. Cameras were set at maximum sensitivity, with infrared illumination at the lowest setting and shutter speed at the highest possible

setting. Video duration was typically 40s.

For each foraging bout recorded, we calculated the decimal time after dusk (TAD) when a particular moth began to feed. This was done by converting the local time in hours and minutes to decimal time after dusk (sunset values according to place and date were obtained from [timeanddate.com](http://timeanddate.com)). Median TAD values were compared between short- and long-tongued hawkmoths (which are easily distinguished in the videos using patterning and the ratio of body size to tongue length) using Mann Whitney U tests as the data were either skewed or bimodal and could not be normalized using transformations. These analyses were done using data for all plant species and, separately, for each plant species that was visited by more than one moth group.

To test the reliability of motion-activated cameras for recording flower visitation by hawkmoths and noctuid moths, we set up the cameras as described above and, through direct observations with a flashlight equipped with a red filter to minimize disturbance, kept a record of the exact time of each moth visit. The mean proportion of visits captured on camera was compared among different focal distances and among moth types using logistic generalized linear models implemented in SPSS 26 (IBM Corp.).

We searched for studies in which authors made remarks about the timing of hawkmoth activity on flowers in natural systems (Table S1). We used logistic generalized linear models to assess whether tongue length of short- versus long-tongued hawkmoths (< 8 cm or > 8 cm) and landmass (Old versus New World), and their interaction, were significant predictors of remarks about crepuscular versus nocturnal activity.

## Results

We obtained 351 motion-activated videos of moths foraging on flowers of the six study species (c.f. video S1). Flower foraging by short-tongued hawkmoths peaked shortly after dusk, while that of long-tongued hawkmoths continued steadily through the night with a peak around midnight (Fig 1). The overall median foraging time in decimal hours after dusk (TAD) was 0.38 for short-tongued hawkmoths and 3.61 for long-tongued hawkmoths ( $Z = 9.08$ ,  $P < 0.0001$ ). These patterns were also evident for *B. antennifera* which is visited by both hawkmoth groups (Fig 1c). Median TAD values for this species were 0.42 and 2.98 for short- and long-tongued hawkmoths, respectively ( $Z = 3.57$ ,  $P < 0.001$ ). In the case of *S. longicauda* (Fig 1a) which was also visited by noctuid moths, the median TAD values were earlier for short-tongued hawkmoths than they were for noctuid moths (0.33 vs 1.41,  $Z = 5.52$ ,  $P < 0.001$ ).

Camera traps placed 460 mm from flowers of *B. antennifera* recorded 17 (80.9%) of the 21 hawkmoths that we observed directly. In the case of *S. longicauda*, camera traps placed 250 mm from flowers recorded 23 (72%) of the 32 noctuid moths that we observed directly, but when placed 460 mm from flowers, cameras recorded just 9 (41%) of the 22 noctuid moths that we observed directly on these plants ( $\chi^2 = 5.2$ ,  $P = 0.02$ ).

For studies that include qualitative remarks about the foraging times of hawkmoths (Table S1), reports of crepuscular (as opposed to nocturnal activity) were made in 93% of 28 studies of short-tongued hawkmoths and in 59% of 17 studies of flower-foraging by long-tongued hawkmoths ( $\chi^2 = 7.44$ ,  $P = 0.006$ ). This pattern was nearly identical across 23 case studies in the Old World and 22 case studies in the New World (tongue

length by landmass interaction:  $\chi^2 = 0.058$ ,  $P = 0.81$ ).

## Discussion

Proboscis length is a key functional trait in flower-feeding insects. Studies of hawkmoth assemblages in both the New and Old Worlds have revealed bimodal or multimodal distributions of proboscis length with one mode consisting of species with short proboscides (< 8 cm) and one or more additional modes consisting of species with long proboscides (> 8 cm) ([Johnson et al., 2017](#); [Martins & Johnson, 2013](#)). [Gregory \(1963\)](#) hypothesized that short-tongued hawkmoths feed before or at dusk, while long-tongued species feed in “deep dusk or darkness”. This idea was subsequently overlooked in the hawkmoth literature, perhaps on account of having been published in a botanical taxonomic monograph. The data obtained in this study support Gregory’s original hypothesis. It is evident that foraging by short-tongued hawkmoths tends to peak shortly after dusk, with occasional activity later in the night until shortly before dawn (Fig 1a-c), while that of long-tongued hawkmoths is mostly continuous throughout the night and peaks around midnight (Fig 1d-f). Similar qualitative observations have been made by other field researchers (Table S1) and laboratory studies have shown that the short-tongued hawkmoth *Hyles lineata* exhibits crepuscular flight rhythms ([Broadhead et al., 2017](#)) in contrast to the long-tongued hawkmoth *Manduca sexta* which exhibits flight activity throughout the night ([Broadhead et al., 2017](#); [Hoballah et al., 2005](#)).

The ultimate reasons for differences in the timing of flower foraging among hawkmoth functional groups are elusive. It is not yet possible to determine whether these observed diel differences are related to proboscis length or some other fundamental differences in the biology of these moths. [Heinrich \(1993\)](#) argued that thermoregulation was an

important determinant of hawkmoth foraging, noting that the common hawkmoth *Hyles lineata* flies after dusk in warm deserts and sometimes during the day in cooler mountainous regions. Janzen (pers. comm. to Heinrich) observed that activity of large *Cocytius* hawkmoths in Costa Rica is restricted to the early morning hours before sunrise. Since longer-tongued hawkmoths tend to have larger bodies (Agosta & Janzen, 2005) with more thermal loading, this may explain why longer-tongued hawkmoths tend to fly in the cooler hours of the night. It may also be significant that diurnal hawkmoths which can tolerate high temperatures (Herrera, 1992) are usually small-bodied. Broadhead et al. (2017) suggested that circadian rhythms could result from selection for synchronized sexual activity of males and females, but this is a principle that would apply to flight patterns in general and does not specifically explain the correlation between foraging times and tongue lengths. There is a possibility that short-tongued crepuscular hawkmoths in the Macroglossinae were ancestrally diurnal, as there are several lineages of diurnal species in this subfamily, including the earliest diverging clade (Kawahara et al., 2009). If so, they may require more light for efficient foraging than do the (mostly) longer-tongued Sphinginae (cf. Kelber et al., 2006). Another factor that may influence the timing of hawkmoth activity is that short-tongued hawkmoths often compete vigorously for the small amounts of nectar in short-tubed flowers (Hodges, 1987), while longer-tongued hawkmoths have access to a wider range of nectar sources and do not have to compete with short-tongued hawkmoths for access to the larger amounts of nectar in long-tubed flowers (Agosta & Janzen, 2005; Johnson et al., 2017; Martins & Johnson, 2013).

These results provide new perspectives on hawkmoth foraging behaviour and show that

there are marked differences in diel rhythms and thus temporal niche occupation between short- and long-tongued hawkmoths. From this finding, we can make the testable prediction that floral specialization for pollination by one of these hawkmoth functional groups will involve adjustment of flower opening, scent emission and nectar secretion patterns to match the diel rhythms of the moths.

This study shows that commercially available PIR cameras are sensitive enough to record the behaviour of hawkmoths and even noctuid moths. Unlike cameras using video motion detection (VMD) which have previously been used to record insect behaviour ([Steen, 2012](#); [Steen, 2017](#)), the Bushnell PIR cameras are relatively inexpensive, commercially available, and can be left at remote sites for long periods as they do not require constant illumination of the subject for activation. The videos we obtained using PIR cameras not only give insights into diel rhythms of activity and other behaviours, but in most cases also enable identification of individual moth species and the location of pollen loads on the body (video S1). We therefore foresee extensive use of these cameras in future studies of plant-animal interactions, particularly in circumstances where it is not feasible to make direct observations over the full 24 hours of each day.

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SDJ developed the study, analysed the data and led manuscript preparation. All authors contributed to data collection and writing.

### **Supporting information**

**Table S1.** Published reports commenting on the timing of hawkmoth flower-feeding activity.

**Table S2.** Details of study species, localities, dates of camera trapping, and sample sizes of moths

**Video S1.** Samples of hawkmoth foraging behaviour captured on motion-activated cameras

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## Figure legends

**Fig 1.** Diel rhythms of nectar-foraging activity by hawkmoths (Sphingidae) and settling moths (Noctuidae) on flowers of six plant species. Symbols represent the total number of moths recorded on motion-activated video on an hourly basis and are shaded according to functional group. See text for statistical analysis.

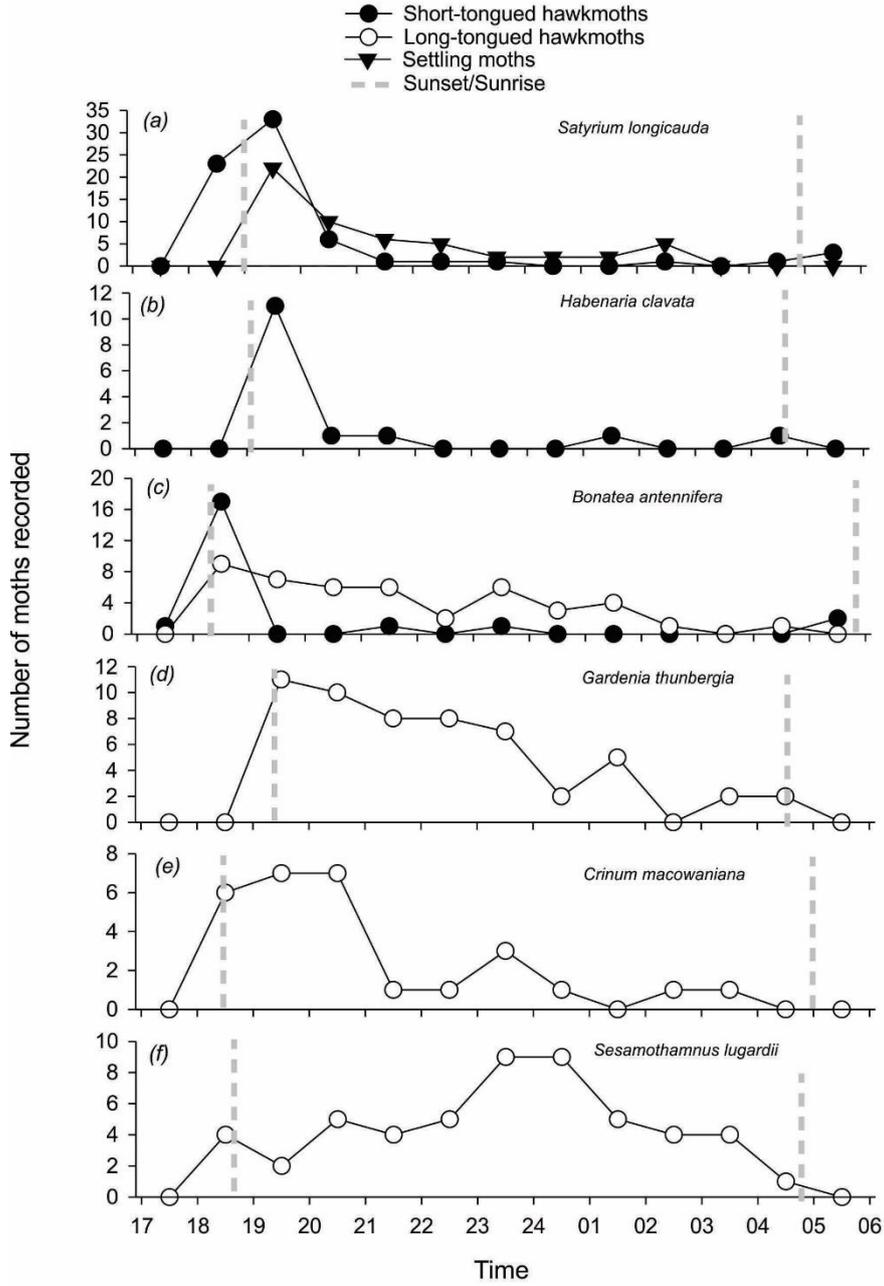


Table S1. Published reports commenting on the timing of hawkmoth flower-feeding activity. Reports are arranged alphabetically by continent and then by plant species. Scoring S1 = short-tongued crepuscular, S2 – short-tongued nocturnal, L1 – long-tongued crepuscular, L2 – long-tongued nocturnal. Long-tongued defined as proboscis > 8 cm. Foraging was scored as crepuscular if authors explicitly mentioned that it was confined to the first two hours after sunset, and as nocturnal if authors mentioned that it occurred throughout the night.

Plant species	Hawkmoth species (proboscis length in cm)	Continent	Remarks	Scoring	Observer
<i>Bonatea speciosa</i>	<i>Theretra capensis</i> (3.3)	Africa	Moth activity took place during a 40 minute period (19h20-20h00) around dusk	S1	( <u>Johnson and Liltved, 1997</u> )
<i>Crinum variable</i>	<i>Hyles lineata</i> (3.0)	Africa	The moths appeared for a brief window of 20 minutes after nightfall	S1	( <u>Manning and Snijman, 2002</u> )
<i>Delphinium leroyi</i>	<i>Hippotion celerio</i> (3.7)	Africa	The activity was confined to a short period from 18h00 to 18h30	S1	( <u>Johnson, 2001</u> )
<i>Disa cooperi</i>	<i>Basiothia schenki</i> (4.3) <i>Hyles lineata</i> (3.0) <i>Hippotion celerio</i> (3.9)	Africa	almost exclusively during the first 30 min after dusk	S1	( <u>Johnson, 1995;</u> <u>Johnson et al., 2005</u> )

<b><i>Mystacidium venosum</i></b>	<i>Nephele accentifera</i> (6.2) <i>Hippotion eson</i> (5.2)	Africa	Hawkmoth activity was confined to a short period after dusk	S1	( <a href="#">Luyt and Johnson, 2001</a> )
<b><i>Rangaeris amaniensis</i>, <i>Aerangis brachycarpa</i></b>	<i>Agrius convolvuli</i> (10.8)	Africa	remarkably restricted period of time	S1, L1	( <a href="#">Martins and Johnson, 2007</a> )
<b><i>A. confusa</i></b>	<i>Coelonia fulvinotata</i> (10.3)		from 1846 hours		
<b><i>A. thomsonii</i>,</b>	<i>Hippotion celerio</i> (4.2)				
<b><i>A. kotschyana</i></b>	<i>Daphnis nerii</i> (4.1)				
<b><i>Satyrium hallackii</i></b>	<i>Basiothia schenkii</i> (4.1) <i>Hippotion celerio</i> (3.9) <i>Agrius convolvuli</i> (8.9)	Africa	during late afternoon and evening (c. 16:00 to 19:00)	S1, L1	( <a href="#">Johnson, 1997</a> )
<b><i>Satyrium longicauda</i></b>	<i>Basiothia schenkii</i> (3.7)	Africa	exclusively in a short period of ca. 15 min after dusk.	S1	( <a href="#">Johnson et al., 2011</a> )
<b>Various plant species (n = 25)</b>	Principally <i>Hippotion celerio</i> (4.1) <i>Agrius convolvuli</i> (10.8) <i>Basiothia medea</i> (2.3) <i>Nephele comma</i> (4.2)	Africa	Hawkmoth foraging. during a short  period of c. 20 min around dusk...[long tongued <i>Agrius convolvuli</i> ] hawkmoths foraging at dawn on <i>Conostomium quadrangulare</i> ...	S1, L1	( <a href="#">Martins and Johnson, 2013</a> )

	<i>Daphnis nerii</i> (4.1)		[short-tongued hawkmoths] on <i>Lantana camara</i> between 05:45 and 05:55 h		
<b><i>Habenaria davidii</i></b>	<i>Cechenena lineosa</i> (6.8) <i>Agrius convolvuli</i> (10.9)	Asia	[ <i>Agrius convolvuli</i> ] usually appeared during 21:00 to 22:00h which was later than <i>C. lineosa</i>	S1,L2	( <a href="#">Zhang and Gao, 2017</a> )
<b><i>Habenaria glaucifolia</i></b>	<i>Hyles sp.</i> (2.4) <i>Macroglossum variegatum</i> (2.6) <i>Macroglossum sp</i> (3.5)	Asia	visits were before full dark, between 18:30 and 20:30 h	S1	( <a href="#">Xiong et al., 2015</a> )
<b><i>Habenaria limprichtii</i></b>	<i>Deilephila elpenor</i> subsp. <i>lewisii</i> (2.7)	Asia	visited orchid flowers from 19:30 to 21:00	S1	( <a href="#">Tao et al., 2018</a> )
<b><i>Stachytarpheta frantzii</i></b>	<i>Erinnyis</i> , <i>Xylophanes</i> , <i>Enyo</i> , <i>Eumorpha</i> , <i>Pachyloides</i> and <i>Perigonia</i>	Central America	...can be collected at the pre-dawn darkness while they drink nectar..	?	( <a href="#">Janzen, 1984</a> )
<b><i>Platanthera chlorantha</i></b>	<i>Hyloicus pinastri</i> (2.9)	Europe	Most of the... visits took place around midnight	S2	( <a href="#">Steen, 2012</a> )

<b><i>Adansonia grandidieri</i></b>	<i>Nephele comma</i> (4.2)	Madagascar	...reliable visitors in the first 30 minutes after anthesis and the 30 minutes before dawn	S1	(Baum, 1995)
<b><i>Adansonia perrieri</i></b>	<i>Coelonia solanii</i> (18.1) <i>Xanthopan morgani</i> (19.6)	Madagascar	Most visits by hawkmoths occur just after dusk, with many fewer later in the night	L1	(Baum, 1995)
<b><i>Adansonia za</i></b>	<i>Coelonia solanii</i> (18.1)	Madagascar	visited the flowers at a steady rate throughout the night	L2	(Baum, 1995)
<b><i>Adansonia za</i></b>	<i>Coelonia brevis</i> (c. 10) <i>Panogena jasminii</i> (6.6)	Madagascar	visited the flowers just after dusk and just before dawn	S1	(Baum, 1995)
<b><i>Adansonia rubrostipa</i></b>	<i>Coelonia solanii</i> (18.1)	Madagascar	..visited throughout the night .... with a peak of activity soon after anthesis	L2	(Baum, 1995)
<b><i>Cynorkis uniflora</i></b>	<i>Panogena lingens</i> (8.8) <i>Hippotion geryon</i> (4.6)	Madagascar	Visitation was concentrated to a very short period after dusk 18.30-19.15 hours, but it cannot be ruled out that at least stray visitation occurred later at night. We conclude that <i>C. uniflora</i> can be classified as a	S1	(Nilsson et al., 1992)

			shortly-after-dusk flower.		
<b><i>Aquilegia caerulea</i></b>	<i>Hyles lineata</i> (4.0)	North America	moth activity  restricted to around dusk.. or.. working the population  throughout the day with a peak of activity  at dusk.	S1	( <a href="#">Miller, 1981</a> )
<b><i>Aquilegia chrysantha</i></b>	<i>Eumopha achemon</i> (4.4)  <i>Sphinx chersis</i> (5.6)	North America	Early evening (20h15-21h55), single record of pre-dawn foraging by <i>Sphinx chersis</i>	S1	( <a href="#">Miller, 1985</a> )
<b><i>Datura discolor</i></b>	<i>Hyles lineata</i> (3.0)	North America	visited for 30 min after anthesis	S1	( <a href="#">Raguso and Willis, 2003</a> )
<b><i>Datura wrightii</i></b>	<i>H. lineata</i> (3.0)  <i>Agrius cingulatus</i> (9.9) <i>Manduca</i> . <i>quinquemaculata</i> (11.5) <i>Manduca rustica</i> (12.2) <i>Manduca sexta</i> (9.6)	North America	beginning with <i>H. lineata</i> ... All visits ceased after 40 min, at which point floral nectar and pollen likely had been depleted	S1,L1	( <a href="#">Raguso and Willis, 2003</a> )
<b><i>Hymenocallis coronaria</i></b>	<i>Dolba hyloeus</i> (3.2)	North America	Visited at dusk, consistent with previous observations	S1	( <a href="#">Graham, 2010</a> )

			that this moth is a dusk flier		
<b><i>Hymenocallis coronaria</i></b>	<i>Paratreia plebeja</i> (?) <i>Manduca rustica</i> (12.2)	North America	began 1h after dark .. and continued as late as 0100 h the following morning	L2	( <a href="#">Graham, 2010</a> )
<b><i>Hymenocallis occidentalis</i></b>	<i>Manduca rustica</i> (12.2)	North America	1–2 h after dark, and no visits were recorded by any moths after this time at these plants.	L1	( <a href="#">Graham, 2010</a> )
<b><i>Merremia palmeri</i></b>	<i>Hyles lineata</i> (3.0) <i>Eryinnyis ello</i> <i>Manduca sexta</i> (9.6) <i>Manduca quinquemaculata</i> (11.5) <i>Agrius cingulatus</i> (9.9)	North America	Hawkmoths began to arrive at dusk with <i>Hyles lineata</i> usually being the first species to appear. Visits continued through the period of observation [18h00 – 24h00]	S1,L2	( <a href="#">Willmott and Búrquez, 1996</a> )
<b><i>Mirabilis multiflora</i></b>	<i>Hyles lineata</i> (3.0) <i>Sphinx chersis</i> (5.6) <i>Manduca quinquemaculata</i> (11.5)	North America	Peak visitation by <i>Hyles lineata</i> is between 8:15 and 8:30 pm, while <i>Sphinx chersis</i> and <i>Manduca quinquemaculata</i> show peak visitation near 8:45 pm	S1,L1	( <a href="#">Hodges, 1987</a> )

***Oenothera albicaulis***      *Hyles lineata* (3.0)      North America      abundant for at least an hour      S1,L1      (Gregory, 1963)  
*Manduca quinquemaculata* (11.5)

***Oenothera biennis***      *Manduca rustica* (12.2)      North America      Large hawkmoths      L2      (Graham, 2010)  
*Manduca sexta* (9.6)      (*M. rustica* and *M. sexta*) arrived at *O. biennis* as soon as the first flowers opened (□2100 h), and *P. plebeja* begin visiting shortly thereafter. *Paratrea plebeja* and *M. sexta* were also captured visiting *O. biennis* shortly before sunrise, suggesting that visitation occurs intermittently throughout the night in this species.

***Oenothera caespitosa***      *Hyles lineata* (3.0)      North America      regular visitor      S1,L1      (Gregory, 1963; Hodges, 1987)  
*Manduca quinquemaculata* (11.5)      during the first hour or two after the flowers opened and occasional during the later evening (*Hyles lineata*). Visitation peaks between 8:15 and 8:30 pm (*Manduca*

<i>quinquemaculat)</i>					
<b><i>Oenothera greggii</i> and <i>O. hartwegii</i></b>	<i>Hyles lineata</i> (3.0) <i>Manduca quinquemaculata</i> (11.5)	North America	...peak of <i>Celerio</i> about one half hour after sunset and of <i>P. quinquemaculata</i> shortly after dark.	S1,L1	( <a href="#">Gregory, 1963</a> )
<b><i>Cereus peruvianus</i></b>	<i>Agrius cingulata</i> (9.6) <i>Manduca rustica</i> (13.5)	South America	visits occurred 1-8 hours after start of anthesis.	L2	( <a href="#">Silva and Sazima, 1995</a> )
<b><i>Habenaria johannensis</i></b>	<i>Manduca rustica</i> (13.5) <i>Manduca sexta</i> (10.3)	South America	crepuscular–nocturnal hawkmoths.  recorded at the flowers from 19:00 to 21:35 h	L1	( <a href="#">Pedron et al., 2012</a> )
<b><i>Habenaria macronectar</i></b>	<i>Eumorpha labruscae</i> (5.9) <i>Manduca lucetius</i> (6.9)	South America	19:00 to 21:35 h	S1	( <a href="#">Pedron et al., 2012</a> )
<b><i>Habenaria megapotamensis</i></b>	<i>Manduca lucetius</i> (6.9)	South America	19:00 to 21:35 h	S1	( <a href="#">Pedron et al., 2012</a> )

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