POPULATION BIOLOGY AND ECOLOGY OF THE CRITICALLY ENDANGERED SUCCULENT *ADENIUM SWAZICUM*

A dissertation submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Masters of Science

By

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DECLARATION

This dissertation is submitted, in accordance with the regulations of the University of the Witwatersrand, Johannesburg in fulfilment of the requirements for the degree of Master of Science. The work described in this dissertation was carried out by me, except where otherwise acknowledged, and has not been submitted for any degree or examination to any other university or institution.

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ABSTRACT

Adenium swazicum is currently listed as Critically Endangered due to past and future population declines, which have been estimated to be 80% over three generations. Although 10-20% of the population is present in formal protected areas, no studies have been published on the species population biology and ecology, which are essential to ensure its effective conservation. The broad aim of this research was to investigate the population biology and ecology of *Adenium swazicum* by investigating factors such as current distribution, population structure, reproductive characteristics, germination, herbivory and current threats.

The current distribution of *Adenium swazicum* was determined by searching all herbarium records on the National Herbarium Pretoria Computerised Information System as well as additional suitable habitat through fieldwork, conducted during the flowering period (October to April) between 2009 and 2011. The current Extent of Occurrence (EOO) and Area of Occupancy (AOO) were determined by incorporating all confirmed localities of *Adenium swazicum.* To determine the population biology of *Adenium swazicum,* fifty plants in four representative populations were studied with regards to plant size, extent and intensity of herbivory, flower production as well as follicle and seed production. The reproduction of *Adenium swazicum* was determined through pollinator observations while the number of flowers and fruit (follicles) produced, as well as fruit and seed set were compared in the four representative populations. Seed viability was determined through tetrazolium staining, while germination experiments were used to determine minimum, maximum and optimum temperature ranges as well as mean germination time. Seedling emergence and establishment

were determined for various soil media, depth of seed planting, watering regimes and shading.

The current distribution of *A. swazicum* included 23 localities in South Africa, Swaziland and Mozambique, while the Extent of Occurrence (EOO) was approximately 8 392km^2 (839 246 ha) and the Area of Occupancy (AOO) was estimated to be 8.5km^2 (850ha). Although this indicates that *A. swazicum* might be more widespread than previously believed, the population sizes were all small (between 1 and 141) and most of the populations were still threatened, mostly by habitat destruction and harvesting for medicinal purposes.

The research found that adult *A. swazicum* plants were found to have a high tolerance to natural disturbance (fire, herbivory) and resprout from the underground tuber even if all above ground parts were destroyed. It is however unlikely that seedlings and juveniles will be able to withstand significant impact on the above ground parts since the underground tuber only develops in plants older than 24 months.

Despite big and bright floral displays which should attract insect pollinators, low diurnal insect activity was observed around *A. swazicum*. However, a fast flying *Sphingidae* (Hawk Moth), which is most likely a pollinator, was observed at *A. swazicum* flowers at dusk. Small population size and isolation might have been the cause of no reproduction (very few follicles and no seed) in at least one population in 2010. Continued reproduction failure as well as destruction of remaining adult plants by housing developments and collection for medicinal purposes might lead to the local extinction of this population.

With sufficient available moisture, high germination success (82 to 90%) for *A. swazicum* was achieved at temperatures between 20°C and 35°C, without any pre-treatment, and 'maximum' germination was reached in less than 90 hours. The cultivation of *A. swazicum* from seed is highly successful, with different soil media having no apparent influence on seedling emergence and establishment. Seedling emergence was highly dependent on water, and although shading did not influence seedling emergence, seedling establishment/survival was highly dependent on shading. Since seed release from *A. swazicum* coincides with the start of the rainy season (October), as well as short germination responses, it is highly unlikely that *A. swazicum* forms persistent soil seed banks.

The uncomplicated propagation of *A. swazicum* has resulted in a significant *ex situ* collection at the Lowveld National Botanical Garden, with more than 2000 plants (adults and seedlings) grown from seeds which were collected from four different populations over an eight year period. In addition, the Skukuza indigenous nursery in Kruger National Park has more than 250 plants (adults and seedlings) all of which were grown from seed collected in the KNP. These living *ex situ* collections provide a valuable source of plant material for future restoration projects.

Despite an increase in the number of known *Adenium swazicum* populations, the population sizes are small (1 to 141) and most populations are still threatened by habitat destruction and high levels of exploitation by medicinal plant harvesters. It is therefore recommended that *Adenium swazicum* remains listed as Critically Endangered (A4acd;B2cb). Criteria A4acd:

"An observed, estimated, inferred, projected or suspected population reduction (up to a maximum of 100 years) where the time period must include both the past and future, and where the causes of reduction may not have ceased or, may not be understood, or may not be reversible based on". This assessment was based on (a) the causes of reduction of *Adenium swazicum* populations have not ceased and may not be reversible, (c) there is a decline in AOO, EOO and habitat quality; and (d) there is actual, continuing exploitation of *A. swazicum*. *Adenium swazicum* also qualifies as CR under criteria B2b(iv,v): "Geographic range in the form of Area of Occupancy (AOO) of $<10km^2$ with (b) continuing decline in (iv) number of locations or subpopulations and (v) number of mature individuals".

No immediate management intervention is needed for populations which are located in formal protected areas, although it is recommended that these populations be monitored, especially with regards to reproduction to inform future management decisions. It is possible that low seed viability recorded in population C in 2009 and 2010 could be due to inbreeding depression caused by the destruction of surrounding populations due to sugarcane fields and harvesting of adult plants for medicinal uses. The Lowveld National Botanical Garden had collected parental material from populations surrounding population C since 2003; it is recommended that restoration of decimated populations be conducted in secure areas on for example private land and community schools. Lastly, seed collected from various populations should be banked at the Kew Millennium Seed Bank Project, England to ensure viable *ex situ* collections.

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DEFINITIONS

Alien species	"A species that is not an indigenous species; or an indigenous species translocated or intended to be translocated to place outside its natural distribution range in nature" (National Environmental Management Biodiversity Act).
Biodiversity	"Biodiversity is the variability among living organisms from all sources including *inter alia* terrestrial, marine and other aquatic ecosystems and ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems" (NEMBA).
Caudex (plural Caudices)	The thickened base of a stem or rootstock which is usually located underground, and from which new leaves and stems arise (Thain & Hickman 1994).
Colleters	Leaf or bud scale that produce a sticky secretion or a group of tuft usually found near the base of the leaf lamina and on the calyx (Thomas 1991).
Community	"Assemblage of populations living in a prescribed area or physical habitat, inhabiting some common environment" (Thain & Hickman 1994).
Conservation	The management of an area to ensure it yields the maximum sustainable benefit to present generation while at the same time maintaining the area's potential to meet the needs of future generations (Van Dyk 2003).
Conservation concern	"Species of conservation concern are species that have a high conservation importance in terms of preserving South Africa's high floristic diversity and include not only threatened species, but also those classified in the categories Extinct in the Wild (EW), Regionally Extinct (RE), Near Threatened (NT), Critically Rare, Rare, Declining and Data Deficient - Insufficient Information (DDD)" (Raimondo et al. 2009).
Conservation status	An indicator of the likelihood of that species persisting in the present day or the near future, or a measure of its extinction risk denoted by the species' Red List status (IUCN 2012).
Critically Endangered	"A species is Critically Endangered when the best available evidence indicates that it meets at least one of the five International Union for the Conservation of Nature (IUCN) criteria for Critically Endangered, indicating that the species is facing an extremely high risk of extinction" (IUCN 2012).
Data Deficient	"There is inadequate information to make a direct, or indirect, assessment of a taxon's risk of extinction based on its distribution and/or population status. Data Deficient is therefore not a category of

	threat. Listing of taxa in this category indicates that more information is required and acknowledges the possibility that future research will show that a threatened classification is appropriate" (Raimondo et al. 2009).
Declining	"A species is Declining when it does not meet or nearly meet any of the five IUCN criteria and does not qualify for Critically Endangered, Endangered, Vulnerable or Near Threatened, but there are threatening processes causing a continuing decline of the species" (Raimondo et al. 2009).
Disturbance	Any event, natural or human driven, that causes temporary and localized shifts in demographic areas (Van Dyk 2003).
Endangered	"A species is Endangered when the best available evidence indicates that it meets at least one of the five IUCN criteria for Endangered, indicating that the species is facing a very high risk of extinction" (IUCN 2012).
Ex situ	Off-site or outside a species' natural habitat (Havens et al. 2006).
Flora	The plant populations or plant life of a specified area (Thain & Hickman 1994).
Forb	A plant species which are not woody and usually live for only one or two seasons (Thain & Hickman 1994).
Genebank	A breeding colony (Havens et al. 2006).
Habitat	The area or home in which any plant or animal lives and include features which are essential to its survival (Low & Rebelo 1998).
In situ	Within a species' natural habitat (Havens et al. 2006).
Indigenous	A species that occurs naturally in South Africa (Thain & Hickman 1994).
Infundibular	Funnel shaped (Thain & Hickman 1994).
IUCN Red List	"The IUCN Red List is set upon precise criteria to evaluate the extinction risk of thousands of species and subspecies. These criteria are relevant to all species and all regions of the world" (IUCN 2012).
Least Concern	"A species is Least Concern when it has been evaluated against the IUCN criteria and does not qualify for any of the above categories. Species classified as Least Concern are considered at low risk of extinction. Widespread and abundant species are typically classified in this category (IUCN 2012).
Mitigation	The implementation of practical measures to reduce adverse impacts (Department of Environmental Affairs 2013).
Mucronate	Ending abruptly in a short point (Schmidt et al. 2002).

Natural distribution range	The area in which a species used to occur as determined through all available records and publications (NEMBA).
Near Threatened	"A species is Near Threatened when available evidence indicates that it nearly meets any of the IUCN criteria for Vulnerable, and is therefore likely to become at risk of extinction in the near future (IUCN 2012)".
Protected Plant	According to Provincial Nature Conservation Ordinances / Acts, no person may sell, buy, transport, or harvest this plant without a permit from the responsible authority. These plants are protected by the National Environmental Management: Biodiversity Act, No. 10 of 2004 (NEMBA) and other provincial legislation.
Sagittate	Shaped like the head of an arrow (Thain & Hickman 1994).
Strigose	Covered with appressed, rigid, straight hairs (Thain & Hickman 1994).
Threat	Any action that causes a decline in populations and compromises the future survival of a species or anything that has a detrimental effect on a species (NEMBA).
Threatened	"Threatened species are species that are facing a high risk of extinction. Any species classified in the IUCN categories Critically Endangered, Endangered or Vulnerable is a threatened species" (IUCN 2012).
Viable	The ability of a species or population to survive or persist and reproduce over multiple generations or a long time period (IUCN 2012).
Vulnerable	"Threatened species are species that are facing a high risk of extinction. Any species classified in the IUCN categories Critically Endangered, Endangered or Vulnerable is a threatened species" (IUCN 2012).

ABBREVIATIONS

BGCI	Botanic Gardens Conservation International
CBD	Convention on Biological Diversity
CITES	The Convention on International Trade in Endangered Species of Wild Fauna and Flora
CR	Critically Endangered
DEA	Department of Environmental Affairs
EX	Extinct
EKZNW	Ezemvelo KwaZulu-Natal Wildlife
EN	Endangered
EW	Extinct in the wild

EX	Extinct
GPS	Global Positioning System
GSPC	Global Strategy for Plant Conservation
IUCN	International Union for the Conservation of Nature
KNP	Kruger National Park
KZN	KwaZulu-Natal
MTPA	Mpumalanga Tourism and Parks Agency
NBG	National Botanical Garden
NEMBA	National Environmental Management: Biodiversity Act, No. 10 of 2004
NT	Near Threatened
QDGC	Quarter Degree Grid Cell
SANBI	South African National Biodiversity Institute
SANParks	South African National Parks
TCP	Thresholds of Potential Concern
TOPS	Threatened or Protected Species Regulations
TSP	Threatened Species Programme
TZ	Tetrazolium
VU	Vulnerable

CHAPTER 1: INTRODUCTION

1.1 GENERAL OVERVIEW

1.1.1 Adenium swazicum

The genus *Adenium* is closely related to *Pachypodium* and *Nerium* and belongs to the tribe *Nerieae* of the subfamily *Apocynoidae* in the family Apocynaceae. Although the genus *Adenium* originally consisted of six species, Plaizier (1980) in his revision of the genus reduced this number to five, namely *Adenium obesum* Roem & Schult, *A. multiflorum* Klotzsch, *A. boehmianum* Schinz, *A. oleifolium* Stapf and *A. swazicum* Stapf. With the exception of *A. obesum*, all species are restricted to small areas in southern Africa. All *Adenium* species occur in savanna or open forests on sandy or rocky soils, which are often brackish (Plaizier 1980). The type specimen for the genus is *A. obesum* in Arabia.

Adenium swazicum was originally described by Rowley (1980) as a variety of *A. boehmianum*, however *A. boehmianum* occurs in northern Namibia, southern Angola and Botswana while *A. swazicum* occurs in South Africa (Mpumalanga and Limpopo), Swaziland and Mozambique Plaizier (1980) therefore reinstated *A. swazicum* as a species. The type specimen for *A. swazicum* is located in Swaziland. *A. swazicum* is a succulent shrub of 0.2-0.7m tall with a carrot-like tuber, which can be up to 1m in diameter. The leaves are narrowly oblong, rounded and mucronate at the apex. The inflorescence is approximately 1.5-3.5 x 1-2.5cm in size and tinged with pink or red. Flowers cut vertically revealed two internal parts, the large outer chamber and the enclosed inner chamber (Figure 1.1). The large outer chamber allows free access to any insect while the small inner chamber protects the nectar and reproductive parts. The only entry to the inner chamber is through five narrow slits between the anthers and as described by Rowley (1980) only an insect with a proboscis length of at least 15mm can reach the nectar. The corolla is crimson, deep mauve, or pink to white while the tube is crimson to white. The fruit are follicles, which were not described in detail. During his revision of *Adenium*, Plaizier (1980) indicated that *A. swazicum* was considered to be widespread in savannas with sandy and often brackish soil and occurs in the eastern Mpumalanga (formerly the eastern Transvaal) and Northern Zululand as well as Swaziland and southern Mozambique.

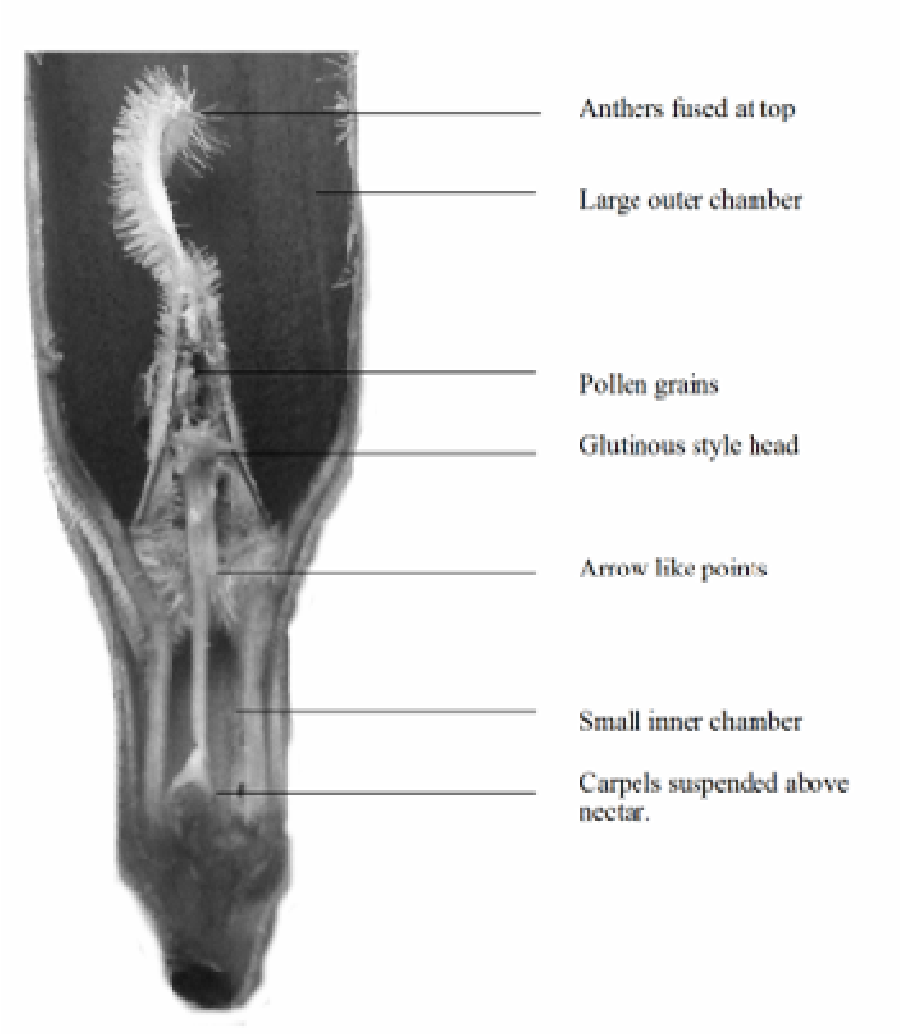


Figure 1.1: A mature *Adenium swazicum* flower cut vertically to reveal the large outer chamber as well as the closed inner chamber which houses the carpels and nectar.

1.1.2 Red List Assessments and Legislation

In the South African National scientific programmes report No. 45 of May 1980, *Adenium swazicum* was assessed according to the International Union for the Conservation of Nature (IUCN) Threatened Species committee criteria as being of an indeterminate status (Hall et al. 1980). This indeterminate status remained the same when the species was assessed again for the Red list in 1996 (Hilton-Taylor 1996). Indeterminate status means that although the taxa is known to be either Extinct (EX), Extinct in the Wild (EW), Critically Endangered (CR), Endangered (EN), Vulnerable (VU), Near Threatened (NT) or Rare (R), there is insufficient information available to decide which of the six categories is appropriate (Hilton-Taylor 1996). In 2002 *A. swazicum* was assessed as EN for Swaziland, but no assessment was done for South Africa (Golding 2002) or globally. In 2008 the South African National Biodiversity Institute (SANBI) through the Threatened Species Programme (TSP) assessed *A. swazicum* for the IUCN Red Data List and established that the species was Critically Endangered (CR:A4acd) based on the following criteria:

> (a) "The causes of reduction may not have ceased OR may not be understood OR may not be reversible based on direct observation;
>
> (c) There is a decline in Area of occupancy (AOO), extent of occurrence (EOO) and habitat quality; and
>
> (d) There are actual or potential levels of exploitation (TSP data SANBI)".

According to the assessments done to determine the threat status of *A. swazicum* for the 2008 IUCN Red List, it was estimated that 50% of the plants' past habitat has been transformed for sugarcane agriculture over the past three generations. Since the life history of *A. swazicum* was not known at the time of the assessment, three generations was estimated to be between 20-30 years. Past and future declines are estimated as an overall decline of 80% over three generations, with the Kruger National Park (KNP) indicated as a safe habitat representing an estimated 10-20% of the total population. This means that the population in the KNP is "safe guarding" the species against extinction.

Due to the continued decline and poor conservation status, *A. swazicum* was listed in 2007 as protected under the National Environmental Management: Biodiversity Act, 2004 (Act 10 of 2004) (NEMBA): Threatened or Protected Species Regulations (TOPS). These threatened or protected species were listed in the Government Gazette number 29657, Regulation Gazette number R.8638, Notice No R.151. The principle requirement of this Act states: "A person may not carry out a restricted activity involving a specimen of a listed threatened or protected species without a permit (section 57(1))". Restricted activities applicable to floral species are defined as follows:

- "Gathering, collecting or plucking any specimen of a listed threatened or protected species;
- Picking parts of, or cutting, chopping off, uprooting, damaging or destroying, any specimen of a listed threatened or protected species;
- Importing into the Republic, including introducing from the sea, any specimen of a listed threatened or protected species;
- Having in possession or exercising physical control over any specimen of a listed threatened or protected species, or causing it to multiply;
- Conveying, moving or otherwise translocating any specimen of a listed threatened or protected species;

- Selling or otherwise trading in, buying, receiving, giving or donating or accepting as a gift, or in any way acquiring or disposing of any specimens of a listed threatened or protected species; or
- Any other prescribed activity, which involves a specimen of a listed threatened or protected species."

This TOPS species list was expanded in 2013 and published for comment in the Government Gazette number 36375, Notice Number 389 of 2013. On the updated list, *A. swazicum* is listed under CR - medicinal flora and restricted activities are limited to wild specimens and therefore exclude artificially propagated specimens which are currently cultivated by nurseries. However, this list has not yet been implemented.

Despite the listing of *A. swazicum* as CR and the species being protected in terms of legislation, its conservation ecology has not been studied and gaps in the information available include:

- Historic and current distribution of *A. swazicum*;
- Population sizes including those in protected areas;
- The extent of medicinal and horticultural uses for the species;
- The ecology of the species; and
- Reproduction including pollination, seed production, germination, soil seed bank formation, seed dormancy and seedling establishment are unknown.

Hence there is a need for a detailed study on this species in order to facilitate its conservation both *in situ* and *ex situ*.

1.1.3 Biodiversity loss in South Africa

Despite various conservation efforts, loss of biodiversity continues on regional and global scales due to increasing intensity of disturbances such as overexploitation of species, habitat destruction, climate change and invasion by alien species (Witkowski et al. 1997; Mouillot et al. 2013). South Africa has the world's richest temperate flora (Germishuizen et al. 2006) consisting of more than 20 450 indigenous vascular plant species. Of the 20 450 species, 4 809 (24%) species are of conservation concern which include the IUCN categories (EX, EW, CR, EN, VU, NT) as well as the South African categories of Critically Rare, Rare and Declining (Raimondo et al. 2009).

Population size plays an important role in extinction probability of a species (Knowles & Witkowski 2000; Franzen & Nilsson 2009). It is generally accepted that the smaller the population, the greater the extinction risk from various causes such as demographic stochasticity, environmental stochasticity, genetic stochasticity and natural catastrophes (Shaffer 1981; van Dyke 2003). The number of plant species that are threatened with extinction has been increasing as habitats throughout southern Africa are transformed to meet the needs of people (Botha et al. 2004a). In addition to this, with the commercialisation of wild plants especially in instances where the demand is high and harvesting methods are destructive, the management of these species is particularly difficult (Botha et al. 2004a). Legislation has also not succeeded in preventing illegal trade in medicinal species in South Africa (Cousins et al. 2013a; Retief et al. 2014; Williams et al. 2014). Large portions of threatened plants in southern Africa are not represented in protected areas or nature reserves while the presence of a species within a protected area is also no guarantee that conservation will be effective (Witkowski et al. 1997; Knowles & Witkowski 2000; Pfab & Witkowski 2000).

The main threats affecting South Africa's plant taxa are habitat loss with the two main land uses driving habitat loss being crop cultivation and housing developments (Von Staden et al. 2009). After habitat loss, the second largest threat to plant species is habitat degradation with the main driver being overgrazing by livestock and deleterious fire regimes (Dovie et al. 2002; Von Staden et al. 2009). The same situation pertains to Swaziland, part of the range of *A. swazicum* (Witkowski et al. 2001). Approximately 405 plant species in South Africa are primarily threatened by plant collecting for the illegal horticultural trade and medicinal purposes, although only 20% of the 322 species regularly traded for medicinal purposes qualify as threatened according to the IUCN criteria (Von Staden et al. 2009). Rural communities are major contributors to the medicinal plant trade with the bulk of the traded plants collected from wild populations (Cunningham 1991; Mander 1998; Botha et al. 2004b). Many plant species, which are collected for medicinal purposes, are destructively harvested, with the plant parts most traded on the Witwatersrand being roots (38.4%), bark (25.6%), leaves and stems (13.5%) and bulbs (10.8%) (Williams et al. 2000). The harvesting of bulbs and roots in particular are more likely to result in plant mortality, and hence more rapid population decline. The trade in traditional medicines plays an important part of a multimillion-rand economy (Dold & Cocks 2002) with an estimated 27 million consumers of indigenous medicine in southern Africa (Mander 1998).

Neuwinger (1996) has conducted detailed studies on the traditional or medicinal uses for four *Adenium* species including *A. boehmianum* Schinz from which *A. swazicum* Stapf was described. *A. boehmianum* is the *Adenium* species which is most widely used as a hunting poison by the Ovambo people from Kaokoland, Namibia. Boehm did the first chemical investigation on *Adenium boehmianum* in 1889 (Neuwinger 1996), in which a highly toxic cardiac glycoside known as echujine was isolated. Large animals such as kudu, oryx, hartebeest and eland shot with poison arrows dipped in *Adenium* sap, died in less than ten hours depending on where on the body the animal was hit. In East Africa and Limpopo Province (formerly known as the northern Transvaal), *A. multiflorum* is used as a fish poison while in the central Kalahari, *A. oleifolium* is used as an ointment. The ointment is applied to snake and scorpion bites or an infusion is made from the roots which is taken as a tonic for gastric disturbance and as a remedy for fevers (Watt & Brandwijk 1962). In Somalia, a root decoction is used to treat rhinitis where a small piece is boiled with a glass of water and a drop of the extract is inserted in each nostril. Although glucosides have been isolated from tubers, roots, stems and seeds, only the roots and tubers are used for medicinal and hunting purposes (Neuwinger 1996).

A number of plant species have been harvested for their aesthetic value to such an extent that their populations have become very small and are becoming Critically Rare (Winter & Botha 1992). Plant species in the genus *Adenium* Roem & Schult. have proven to be very popular for ornamental purposes and landscaping in eastern countries such as China and Thailand. In the past decade, breeders from the United States of America (USA), India, Australia and Kenya have also been showing an increasing interest in *Adenium* cultivation (Dimmit & Hanson 1991). The most common *Adenium* hybrid in Thailand is *Adenium* "Arizona" which is a hybrid between *A. obesum* and *A. swazicum*. In this hybrid *A. swazicum* is the parent plant which contributes to flower colour and it is the only species in the genus which is cold and frost resistant (Dimmit 1998). *A. obesum* contributes to the strong and erect growth form and round flower shapes. The *Adenium* Arizona hybrid has a very large caudex and long flowering seasons. *A. swazicum* has been used for hybridization since 1985 (Dimmit et al. 2009) and the harvesting of wild *A. swazicum* was considered to be a contributing factor to past and future declines (TSP 2008).

1.1.4 Metapopulations, sources and sinks

Metapopulations are collections of smaller populations or subpopulations of a species in a specific area, occupying a suitable patch of habitat within a landscape of otherwise unsuitable habitat while still exchanging individuals or genes by way of dispersal (Brewer 1994; van Dyke 2003). This population structure can apply to species that live in a landscape where suitable patches are located in optimal habitats and therefore support large populations while some individuals disperse to other patches, which might be less than optimal and therefore hold smaller populations (Schemske et al. 1994). The term “sink” refers to habitats where population loss exceeds population increase while a “source” refers to habitats where surplus individuals are produced and these individuals are therefore available for dispersal (Brewer 1994; Runge et al. 2006). Metapopulations therefore persist because of the interactions between patches that will prevent the extinction of the whole metapopulation. According to Van Dyke (2003), the metapopulation theory has been an attractive paradigm for conservation for the following reasons:

- Many species have patchy distributions which fits the metapopulation paradigm;
- Habitat fragmentation tends to increase patchiness in distributions of populations, thereby creating metapopulations from populations that were contiguous;

- Metapopulation reinforces the hope of conservationists that disappearance of populations from local patches for fragments need not inevitably result in extinction; and
- A metapopulation normally has more genetic variation than single populations, and therefore the risk of extinction is lessened because all the populations are unlikely to be exposed to the same demographic events simultaneously.

The use of plants for examining metapopulation processes and predictions has lagged behind those studies that focussed on animals, while the few plant studies were biased towards annual and short-lived perennials (Husband & Barrett 1996), which are easier to study than perennial species such as shrubs and trees.

1.1.5 Understanding species ecology and population biology for threatened species

To evaluate the causes of endangerment and ensuring the continued survival of species in nature, as well as developing the criteria to determine when species recovery has been achieved, is a daunting task especially for plants (Schemske 1994). The lack of information on the biology of threatened plants has been highlighted almost two decades ago in South Africa (Witkowski et al. 1997). In order to determine the conservation ecology and status of threatened plant species in the northern regions of South Africa, up-to-date information on key parameters such as population size, rate of population decline and extent of occurrence is required (Baillie et al. 2004). The reasons for population decline are identified by an investigation of the species ecology, while the species population biology indicates / reveals the internal agents of decline including plant size reduction, reduced flowering, lack of pollinators, ineffective seed dispersal, fruit predation and germination failure (e.g. Pfab &

Witkowski 1999a; Knowles & Witkowski 2000; Witkowski et al. 2001). Growing seasons, flowering and fruiting sequences as well as insect interactions of plants are essential factors contributing to the total understanding of the population biology. According to Witkowski et al. (1997), studies of threatened species need to consider four major factors, which include population size and structure, physical and chemical environmental setting, regeneration capacity and disturbance regimes that impact on these threatened species. Limiting factors are the physical factors such as climate (including climate change), geology, geomorphology, soil type, soil chemical composition (i.e. pH and salinity), slope/aspect and fire regime that determine the zone in which life is possible for each organism (Witkowski et al. 1997). Soil properties have a large influence on the composition and structure of terrestrial vegetation (Medinski et al. 2010). Direct effects of soil on plant species include pH, while resource effects relate to the nutrients and water availability. There are obvious differences in species composition of vegetation on sodic sites, which may be related to the soil's physical and chemical characteristics (Medinski 2007). In acidic soils (especially soils with a pH <6), essential nutrients such as calcium, magnesium, potassium, phosphorus and molybdenum are depleted or not available in a form that is useable to plants (Medinski 2007). Individual sodic soil sites are usually small, have relatively uniform vegetation, are generally found next to drainage lines, and vary in size from a few square meters to several hectares (Khomo & Rogers 2005). The plant growth in these areas is hindered by the presence of the B-horizon at a shallow depth; this B-horizon possesses an unfavourable physical structure due to the presence of deflocculated clay (Dye & Walker 1980). Sodic sites develop because of subsurface water movement in duplex soils, and these soils are located in the footslopes of granitic catenas where the loss of the A-horizon follows the destruction of the protective grass cover (Dye & Walker 1980). Apart from clumps of *Euclea divinorum* trees and shrubs, woody vegetation is generally absent from eroded sodic sites. Furthermore, an important factor in the formation of sodic sites is the concentrations of wildlife within these areas due to the presence of permanent water (Chappell 1992). The high clay and silt content observed in sodic sites, tends to increase the crusting potential of soil and therefore decreases soil infiltration, thereby preventing the germination and emergence of seedlings (Mills et al. 2006; Medinski 2010).

Plant conservation biology has largely been based on decades of research into plant population dynamics and distributions and the factors that affect them (Harper 1977 in Ancheta & Heard 2011). The variation in population growth rates is caused by different underlying vital rates such as germination, seedling growth, reproduction and death while the vital rates are in turn influenced by multiple environmental factors including fire, herbivory and weather and the contribution of these factors to population dynamics depend on the life history of the species (Buckley et al. 2010). After rainfall and soils, fire and herbivory are two of the main determinants of savanna ecosystems with fire considered the major role player in distinguishing between vegetation types such as grassland, woodland and forests (Adams 2013; Masunga et al. 2013). Plants use their resources for growth, maintenance and reproduction (Stephenson 1981) and the amounts of these resources that are available will depend on factors such as rainfall, temperature, diseases, herbivory and fire. Although succulent plants are found to often grow in rocky areas, mortality rates of succulents after fire is highly variable and can be more than 50% (Thomas 1991; Pfab & Witkowski 1999a; Cousins et al. in press). Herbivory can have important effects on population dynamics of some rare plant species (Pfab & Witkowski 1999b; Louda 2001; Witkowski et al. 2001; Maron & Crone 2006). However, the impact of herbivory could be different on a host plant that has recently declined to rarity compared to one that has been rare through evolutionary time and has therefore evolved traits to survive herbivory (Ancheta & Heard 2011). Among insect feeding guilds, seed predation is generally considered to have strong effects on plant populations (Crawley 1990), while plants tolerate considerable damage to non-reproductive tissue such as leaves (Ancheta & Heard 2011). However, herbivory might hinder the maturation of flowers into fruit especially when herbivores destroy photosynthetic tissues such as leaves, which result in a depletion of inorganic nutrients essential for fruit formation (Stephenson 1981; Pfab & Witkowski 1999a). Witkowski (1990) found that resource allocation to reproduction might be altered by producing fewer and smaller sized seeds and aborting immature seeds in *Leucospermum parile.*

In order to understand community processes such as regeneration, establishment, succession, species survival strategies and causes of rarity it is important to have knowledge of a plant's reproductive ecology (Cousins et al. 2013b) including pollination. Pollination includes pollination strategies, flowering phenology and patterns of nectar, fruit and seed production (Lovett Doust & Lovett Doust 1990; Arena et al. 2013; Cousins et al. 2013b) while seed ecology deals with dispersal, dormancy and germination (Fenner & Thompson 2006; Cousins et al. 2013b). *Pachypodium* and *Adenium* have scent-less flowers displayed in colours of red, pink, yellow and white with nectar at the bottom of the tube (Rowley 1980). Flower colour functions as a long distance signal for pollinators, especially for actively flying diurnal insects (Kevan 1978). Floral guides are a form of close-in attractant, can be olfactory, structural or visual with some visual guides reflecting UV light, and are therefore not visible to the human eye. Hawkmoths (Sphinx moths) hover at flowers in the same fashion as hummingbirds, and suck nectar through their long proboscises (Kelber et al. 2003).

Hawkmoths are mostly nocturnal and have the potential for wavelength discrimination or trichromatic colour vision that functions at low light levels.

Species in the family Apocynaceae, that includes the genus *Adenium*, show a strong degree of self-incompatibility (Waddington 1976; Rowley 1980; Darrault & Schlindwein 2005; Araujo et al. 2013). Some succulents have extremely simple and unspecialised pollination systems with flat heads and open blooms in which the nectar can be seen and is therefore available to a range of insects (Rowley 1980). In contrast to this, species in the family Apocynaceae have evolved highly specialized flowers which are designed to make maximum use of every insect visit through the removal of pollen adhering to the visitor's mouth parts while at the same time ensuring that a large portion of its own pollen becomes attached to the retracting probiscus (Rowley 1980, 1999; Darrault & Schlindwein 2005). The wide corolla tube of *Adenium* species, as well as the red or pink colour, coupled with a lack of scent suggests a large bee with a proboscis length of at least 18mm is likely to be the pollinator (Rowley 1980). There are very few records of pollinators for *Pachypodium* or *Adenium,* with hawkmoths suggested for long narrow-tubed flowers and small green flies for *Pachypodium densiflorum* (Rowley 1999), while bees (Rowley 1999) pollinate *Adenium* species, which are cultivated in Hawaii. The proboscis is used to carry pollen for most butterfly-pollinated and moth-pollinated flowers. Long, slender proboscises are adapted for reaching nectar at the base of narrowly tubular flowers (Suguira & Yamazaki 2005). Flowers that are pollinated by animals have various features such as colour, scent and structure, which reflect the foraging preferences of their potential pollinators (Hargreaves 2004). Factors influencing successful pollination include the transfer of pollen between flowers/plants and the amount of pollen collected by the pollinator (Hargreaves 2004). Pollinators are usually rewarded with pollen or nectar but there are also plants that have “rewardless” flowers and these are being pollinated by deceit (Lin & Bernardello 1999; Arena et al. 2013). At the population level, many individual plants of a species flowering at the same time will be more attractive to pollinators compared to fewer plants producing less flowers (Willson & Price 1976; Echart 1991). Androgynous (bisexual) plants often produce excess flowers which do not produce fruit, hypotheses for the production of these excess flowers include pollen limitation, pollinator attraction, bet hedging and selective abortion (Sutherland 1987). Bet hedging allows the plant to compensate for variations in resource availability for fruit maturation as well as variations in pollinator visitation while selective abortion allows the plant to selectively abort fruit and mature only high quality fruit (Stephenson 1981; Sutherland 1987). Species in the family Apocynaceae are generally considered to have a low rate of fruit production (Woodson 1930 in Waddington 1976).

The long-term persistence of a species depends on its regenerative capacity through seed or re-sprouting from vegetative organs (Weiersbye & Witkowski 1998). Studies by Stephenson (1981) revealed that many plant species produce a limited number of mature fruit from a small portion of the flowers, and it is common for these species to abort flowers and immature fruit because of limited availability of resources for reproduction, and hence low fruit set and low seed set. The resources that a plant needs to produce fruit are obtained from several sources such as vegetative organs and the leaves close to the developing fruit; it is usually the availability of these resources that determine the maximum number of fruit produced (Fenner & Thompson 2006; Cousins et al. 2013b). Resource availability varies amongst populations from year to year. Low seed set implies wastage in the reproduction process and seed that fail to develop comes at a cost to the plant (Fenner & Thompson 2006;

Cousins et al. 2013b). Fruit abortion is usually a result of high temperatures, abiotic damage to young fruit and pathogens transmitted by insects (Stephenson 1981; Witkowski 1990). It is however possible that the production of excess flowers represent ovaries that can be used if resources are plentiful but can be discarded with minimum cost if resources are scarce (Fenner & Thompson 2006). A germinated seed is highly vulnerable to various factors such as lack of moisture for growth, fire, herbivores, burial under litter, being washed away by rain, and heat on bare soil, and hence up to 90% of released seed will not make it past the seedling stage (Leck 2008). Sodic or brackish areas have added stress to seedlings due to high salt content of the soil (Medinski et al. 2010) as they are hard when dry and lack structure (hence poor aeration) when wet. Such extreme conditions cause some species to become specialized by forming corms, bulbs or tubers under the soil surface to avoid drought or heat.

Species survival often depends on reproduction, seed dispersal within the same community, expansion into new habitats and survival through times, which are unfavourable for growth (Vasquez-Yanes 1993; Cousins et al. 2013b). Areas under tree canopies in savannas have reduced soil temperature and higher nutrient levels when compared to adjacent open spaces, which improves the survival and growth of seedlings (Kos & Poschlod 2006). Temperature is considered the most important environmental factor governing the maximum germination percentage and rate of germination while germination is usually only possible within well-defined temperature limits (Garcia-Huidobro et al. 1982; Hardegree 2006; Mattana et al. 2010). This leads to a consideration of cardinal temperatures which include the maximum, minimum and optimum temperatures. The optimum germination temperature for a species is characterised by maximum germination in the shortest time, while no germination will occur beyond maximum and minimum temperatures (Probert 2000). Once a species' cardinal temperatures are reached, some species have a fast germination response to rain (Fennel & Thompson 2006). However, the availability of water is a major factor limiting early seedling success and establishment (Wilson & Witkowski 1998). The environment surrounding a seedling is critical and determines the dynamics of plant populations and composition of the plant community (Fowler 1988). In the succulent thicket biome, nearly all the endemic succulent species were recorded under *Euclea* shrubs (Moolman & Cowling 1994). Seedling establishment under or close to the mother plant could be indicative of localized seed dispersal and seed trapping (seed gets trapped in branches of mother plants or surrounding vegetation) rather than nurse effects *per se* on the seedlings (Haussmann et al. 2010; Soliveres et al. 2012).

1.1.6 *Ex situ* conservation and the role of communities

Worldwide, botanical gardens have moved away from economic botany and focussed more on plant conservation of native species, including stands of local native vegetation within their boundaries (Crane et al. 2009; Powledge 2011; Hulme 2014). Hardwick et al. (2011) indicated that disciplines in botanical gardens which are valuable in the field of ecology and restoration include plant taxonomy, DNA fingerprinting, geographical information systems, practical conservation skills (seed banking, collection of propagation material), and relevant expertise in conservation genetics, seed science, mycology and plant physiology. Living collections in botanic gardens play an important role in harbouring the majority of threatened species since not all species can be adequately conserved as seed (Piovan et al. 2011; Sharrock & Jones 2011), while the study of a single-species population ecology is a well-established activity in botanic gardens (Hardwick et al. 2011). The combined collections of botanic gardens worldwide hold up to one third of all known plant species, many of which have considerable conservation value (Donaldson 2009; Sharrock et al. 2011). It has been argued that living collections in botanic gardens are often of limited scientific and conservation value due to inadequate sampling, lack of documentation and long-term sustainability (Heywood 2011). However Crane et al. (2009) pointed out that there has been a shift in conservation policies for botanic gardens over the last two of decades. Many botanic gardens have historically acted as plant introduction centres and played an important role in the spread of germplasm of agricultural, industrial, forestry and ornamental plants around the world (Heywood 2011). The Botanic Gardens Conservation International (BGCI) is based in London and is the centre of botanic garden's global network with more than 700 members in 118 countries and have documented over 150 000 plants in cultivation, thousands of which are threatened (Powledge 2011). According to the IUCN, the number of plant species that are extinct in the wild would be 34% higher were it not for those conserved in botanic gardens (Sharrock et al. 2011). The Global Strategy for Plant Conservation (GSPC) was adopted under the Convention on Biological Diversity (CBD) in 2002 in response to the alarming decrease in plant diversity (Schulman & Lehvavirta 2011). The GSPC strategy up to 2020 was approved at the Conference of Parties to the CBD in Nagoya (CBD 2010). Botanic gardens worldwide are tasked with implementing and mainstreaming the GSPC.

In South Africa's National Botanical Gardens, there has been an effort to conserve threatened indigenous plants since the establishment of Kirstenbosch National Botanical Garden in 1913 (Willis & Van Wyk 2006). The Lowveld National Botanical Garden (LNBG), which was established in 1970, collected 120 rare and endangered plants in 40 years (Botha et al. 2000). According to W. Froneman (pers. comm. 2014), the total number of rare and endangered plant species currently in LNBG is approximately 150. One of these is *A. swazicum,* which was first collected as seed from wild populations in 2003 with more than 100 of these adult plants now growing in field gene banks in the LNBG.

A number of botanic gardens have engaged their local communities in the cultivation and use of local, underutilized or medicinal plants (Sharrock 2011). The cultivation of medicinal plants to alleviate the pressure from traditional healers and harvesters on wild populations has been suggested in the early 1900s (Miller 1914; Havenhill 1921) and practised worldwide (Selvakumar et al. 2001) as well as within South Africa (Gentry 1961; Mander et al. 1996; Jager & Van Staden 2000; Ndawonde et al. 2007; Kowalski & Van Staden 2007; Konz et al. 2009; De Beer 2010; Cousins & Witkowski 2015). Outreach nurseries established in communities have been popular in South Africa since the 1980's although it was found that of the 65 outreach/community nurseries in South Africa, 54% no longer existed, 33% were just surviving while only the remaining 13% were considered viable at the time of the study in 2006 (Botha et al. 2006). The LNBG was involved in one community outreach project to train community members (traditional healers, schoolchildren and other adults) namely Project M.G.U – Useful Plants Project between 2009 and 2010 (see Appendix A and B). This project was funded by a Spanish philanthropist and managed by the Kew Millennium Seed Bank Project with the aim of enhancing the *ex situ* conservation of native useful plants for human wellbeing by building the capacity of local communities to successfully conserve and use these species sustainably. The project was initiated in 2007 in five countries, Botswana, Kenya, Mali, Mexico and South Africa and the main components of the project included:

- Targeting and prioritising useful plants with local communities;
- *Ex situ* conservation of useful plants through seed banking;

- Propagation and conservation of useful plants in local communities;
- Research to enable conservation and sustainable use of plants;
- Sustainable use and income generation from useful plants; and
- Supporting *in situ* conservation of useful plants.

Project M.G.U. prioritized 120 medicinal plants within the Lowveld of Mpumalanga for propagation and community education, one of these species was *A. swazicum.* Through this project, traditional healers as well as schoolchildren in the Nkomazi Municipality (where *A. swazicum* occurs) received training in various propagation techniques. In addition to this, twenty adult *A. swazicum* plants were planted at the Mvagatini Primary School in the Figtree district, which was adjacent to an existing *A. swazicum* population (population P). These adult plants were propagated from wild seeds which were collected in 2003 from the population in the natural areas surrounding the school.

1.2 AIM AND OBJECTIVES OF THE STUDY

Adenium swazicum is currently listed as CR and it is estimated that KNP is "safe guarding" 10-20% of the population. However, during the planning stages of this MSc, an interview conducted with Mr N. Zimbatis from KNP scientific services, revealed that there has been no official assessment or monitoring of *A. swazicum* in the KNP and the size of the population was therefore unknown. In addition, KNP had not yet set any Thresholds of Potential Concern (TCP) for *A. swazicum*. TPC's include a set of operational goals that are defined as upper and lower levels along a continuum of change for a selected environmental variable or species (Biggs and Rogers 2003).

Despite the extensive cultivation of *A. swazicum* for ornamental purposes, no research has been done on the dormancy and germination requirements for *Adenium* (pers. comm. Kevin Liu, Seed Information Database Coordinator – Royal Botanic Gardens Kew, Seed Conservation Department). In addition to this, no study has been published on the reproductive biology of *A. swazicum.*

The broad aim of this study was to investigate the conservation biology and ecology of *A. swazicum* with the intention that the results will be used to develop *in situ* and *ex situ* management recommendations for the conservation of this species.

The specific objectives of the study were:

i. Determine the current distribution of *A. swazicum* in South Africa and where possible verify populations in Swaziland and southern Mozambique;
ii. Determine the population structure of selected, representative populations of *A. swazicum*;
iii. Analyse the reproductive characteristics of the species by studying its phenology, seed production and seed viability;
iv. Determine the optimum germination cues, soil media for *A. swazicum* under controlled conditions*;*
v. Determine the effect of herbivory on population structure, reproduction and seedling establishment; and
vi. Determine current and future threats to *A. swazicum.*

1.3 STRUCTURE OF THE DISSERTATION

This dissertation is constituted by six chapters (Figure 1.2). A general literature overview of a range of topics covered in the dissertation as well as the rationale and aims for the study are covered in Chapter 1. Chapters 2-5 deal with various study objectives while the final chapter (chapter 6) articulates the ecological and theoretical implications of the study's results. Due to this type of dissertation structure, repetition between chapters, although minimised, is unavoidable.

Chapter 2 describes the study area with special reference to the vegetation types and land use associated with populations. Four representative populations are identified and described. The historic distribution, current distribution and subsequent Extent of Occurrence and Area of Occupancy were determined for *A. swazicum*. Historic, current and potential threats were assessed for all populations.

In *Chapter 3,* the population biology of *A. swazicum* was investigated through examining the population structure of four representative populations. Vertebrate and invertebrate impact on the leaves, flowers, stems and branches was determined for plants in the wild, while these impacts were simulated in *ex situ* collections to monitor recovery of damaged plants. The impacts of invertebrates on fruit follicles and seed were determined. The effect of fire on *A. swazicum* was studied.

Chapter 4 and *Chapter 5* investigated all aspects related to reproduction. *Chapter 4* investigates the floral structure, flower production, pollination and seed set, while *Chapter 5* examined the parameters for germination, seedling emergence and seedling establishment.

In *Chapter 6*, the findings of each chapter are synthesized and this chapter serves as the general discussion and conclusion of the dissertation.

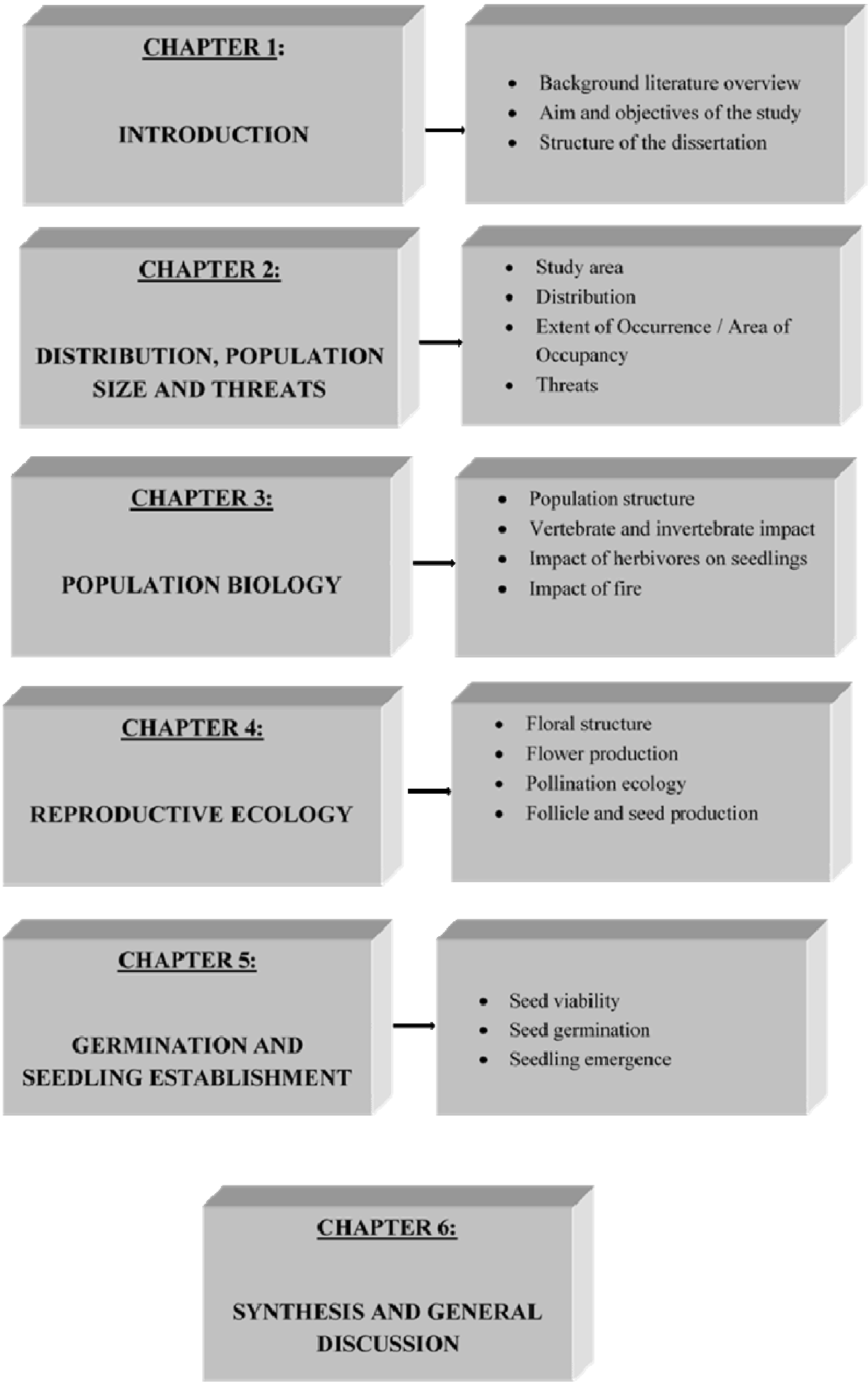


Figure 1.2: Study (dissertation) structure for investigating the population biology and ecology of *Adenium swazicum*.

2 CHAPTER 2: DISTRIBUTION, POPULATION SIZE AND THREATS OF THE CRITICALLY ENDANGERED SUCCULENT, *ADENIUM SWAZICUM* STAPF

Abstract

Historic distribution records from PRECIS prior to 2009 indicated that *Adenium swazicum* has been recorded from 20 localities in 11 Quarter Degree Grid Cells (QDGC's), eight of these QDGC's were in Swaziland. However, no recent or more accurate location records existed on the occurrence of *A. swazicum.* To determine the current distribution of *A. swazicum*, the PRECIS localities in South Africa were investigated during the flowering period for *A. swazicum* between March 2009 and April 2011. *A. swazicum* was confirmed from 23 localities through fieldwork in South Africa, as well as records submitted by botanists in Swaziland and Mozambique. This indicated that *A. swazicum* was still present in eight of the original PRECIS localities (in four QDGC's) as well as seven new QDGC's within South Africa, Mozambique and Swaziland. Four of the original localities have been transformed by agriculture (sugarcane fields) while eight localities could not be verified due to incomplete records. The current Extent of Occurrence of *A. swazicum* was calculated to be approximately 8 392km^2 (839 246ha) while the Area of Occupancy is estimated to be 850ha. Although some populations were located in formal protected areas and wildlife sanctuaries, most populations were still threatened by habitat destruction and developments such as residential areas (houses and lodges) and. Populations, which were located on communal land, were severely impacted by destructive harvesting for medicinal purposes with more than 80% of the plants in one population destroyed between 2008 and 2010. Although the current distribution records indicate that *A. swazicum* might be more widespread than

previously believed, the small population sizes (between 1 and 141 per population) and threats affecting most of the populations, the species is still considered to be highly threatened.

Keywords: *Adenium swazicum,* Area of Occupancy, distribution, Extent of Occurrence, habitat destruction, legislation, medicinal plants, population size.

2.1 INTRODUCTION

It is imperative to understand the status and distribution of rare species and relate this to the extent of decline and possible shifts in the geographic range over time (Farnsworth & Ogurcak 2006). For more than 30 years, conservation assessments have indicated that *A. swazicum* is in need of conservation (Hall et al. 1980; Hilton-Taylor 1996; Golding 2002 and TSP 2008), yet no formal study has been conducted to determine the population size or distribution of this species. Within South Africa, *A. swazicum* was listed as Critically Endangered in 2008 (TSP) based on an estimated past and future population decline of 80%, a decline attributed to habitat destruction and exploitation for horticultural and medicinal purposes. *Adenium swazicum* favours low lying areas in bushveld or savannas with sandy or brackish soil or sparsely wooded grassy areas (Plaizier 1980; Onderstall 1984; Pooley 1998; Schmidt et al. 2002), which are also favoured for the establishment of large sugarcane fields. Rural communities are major contributors to the medicinal plant trade with the bulk of the traded plants collected from wild populations (Cunningham 1991; Mander 1998; Botha et al. 2004b). Many plant species, which are collected for medicinal purposes, are destructively harvested, with the plant parts most traded on the Witwatersrand being roots (38.4%), bark (25.6%), leaves and stems (13.5%) and bulbs (10.8%) (Williams et al. 2000). It is known that

Adenium swazicum is harvested for medicinal purposes (Botha et al. 2004a), although the levels of harvesting of wild plants has not been quantified.

The aim of this chapter is to study the current distribution of *A. swazicum* and determine the population size and current threats through the following objectives:

- Determine the current distribution of *A. swazicum* through verification of historical records and fieldwork to determine new localities;
- Determine the population size of *A. swazicum;*
- Determine the current Extent of Occurrence and Area of Occupancy of *A. swazicum;*
- Determine the historic, current, and future threats on *A. swazicum* populations.

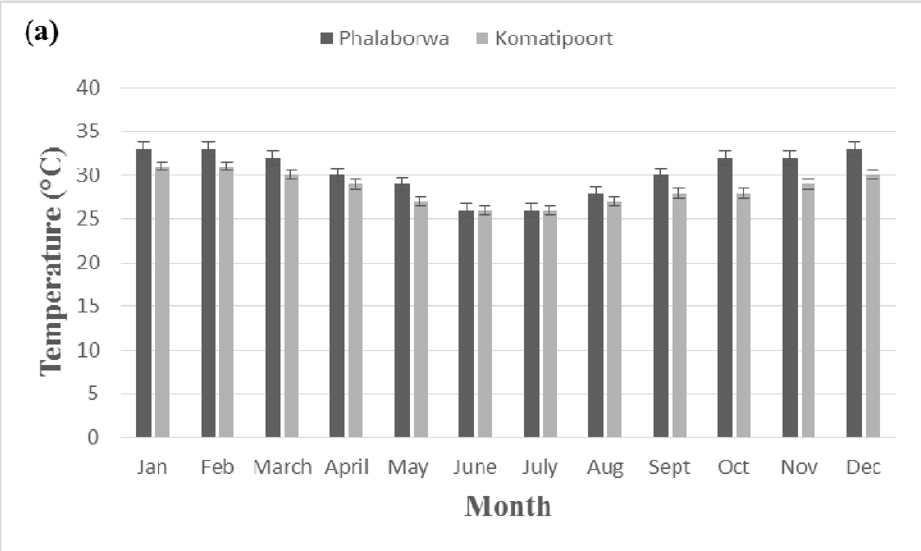
2.2 STUDY REGION

2.2.1 Study area, biophysical characteristics and land use description

The area investigated to determine the distribution of *A. swazicum*, ranged from the Kruger National Park (KNP) and Timbavati Private Nature Reserve in the north, through the Lowveld of Mpumalanga and Swaziland to Mkuze, KwaZulu-Natal in the south. The area was located between 24°19’15.21” - 27°38’02.76” south and 31°24’41.65 - 32°04’29.97” east. Four representative populations were selected for monitoring based on land use (protected areas and private land) and size of the population (minimum of 50 plants were needed per population). The four representative populations were located in the Lowveld of Mpumalanga between 25°19’51.86” - 25°56’45.37” south and 31°44’30.36 - 31°48’44.14” east.

The distribution area of *A. swazicum* was characterised by predominantly mid-summer rainfall, receiving mean annual precipitation of 400mm to 1000mm (Mucina & Rutherford

2006). Mean monthly maximum temperatures for Komatipoort (relatively cooler) in the south and Phalaborwa (relatively warmer) in the north range from 24°C to 31°C and 26°C to 33°C respectively, while minimum temperatures range from 8°C to 18°C for Komatipoort and 9°C to 23°C for Phalaborwa (Figure 2.1). Average annual rainfall for Komatipoort and Phalaborwa is 449 ± 30.47 mm and 351 ± 25.96 mm respectively. Climatic data was collected between 2000 and 2009 (worldweatheronline.com).



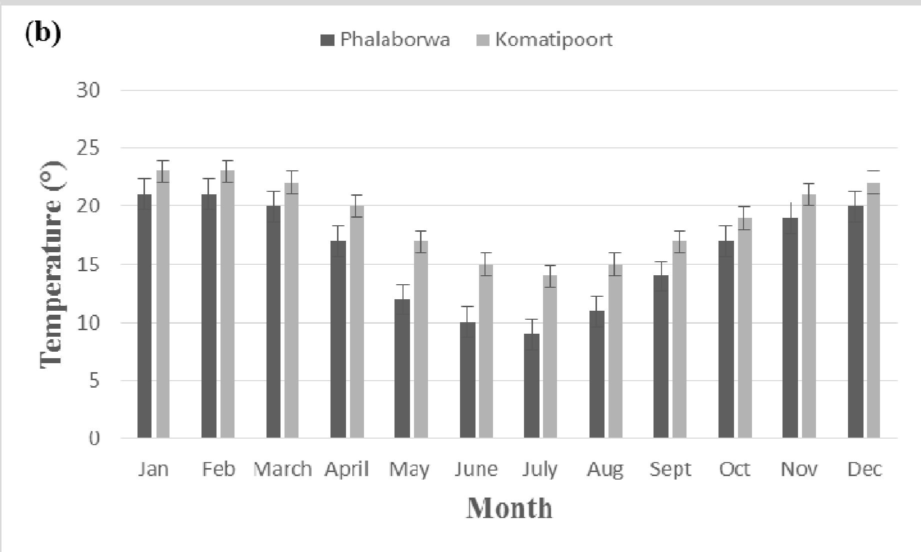


Figure 2.1: Mean (±S.E.) (a) maximum and (b) minimum monthly temperatures (°C) for Komatipoort, Mpumalanga in the south and Phalaborwa, Limpopo in the northern portion of the *Adenium swazicum* distribution in South Africa, excluding Mkuze in KwaZulu-Natal.

2.2.2 Vegetation types associated with distribution of *Adenium swazicum*

Adenium swazicum occurs within the Savanna Biome (Rutherford & Westfall 1994). The Savanna Biome is the largest biome in southern Africa, occupying over one-third of the surface area of South Africa (Mucina & Rutherford 2006) and is characterised by a grassy ground layer and a distinct upper layer of woody plants. Where this upper layer is near the ground the vegetation may be referred to as Shrubveld, and where the tree layer is dense, it is known as woodland, while the intermediate stages are locally known as Bushveld (Mucina & Rutherford 2006). The Savanna Biome comprises a number of vegetation types. The most recent, national vegetation classification by Mucina & Rutherford (2006), describes six broad vegetation types within the study area, namely, (a) Delagoa Lowveld, (b) Gabbro Grassy Bushveld, (c) Granite Lowveld, (d) Malelane Mountain Bushveld, (e) Tshokwane-Hlane Basalt Lowveld, and (f) Zululand Thornveld (Figure 2.2).

The Delagoa Lowveld vegetation type is mostly confined to Mpumalanga and Swaziland, although it narrowly extends into KwaZulu-Natal (includes the Mkuze location) and consists of a dense tree or tall shrub layer dominated by *Acacia welwitschii*. The soils are rich in sodium and are very susceptible to erosion and are dominated by species such as *Acacia senegal* var. *rostrata, A. welwitschii, Albizia petersiana, Schotia capitata, Spirostachys africana, Pappea capensis* and *Euclea divinorum.* Herbaceous species include *Abutilon austro-africanum, Justicia flava, Blepharis integrifolia* and *Kyphocarpa angustifolia* while the graminoid layer includes species such as *Chloris virgata, Panicum coloratum, Panicum maximum, Sporobolus nitens* and *Tragus berteronianus.* Mucina & Rutherford (2006) classify Delagoa Lowveld as Vulnerable with 18% conserved in KNP and more than 33% transformed by cultivation.

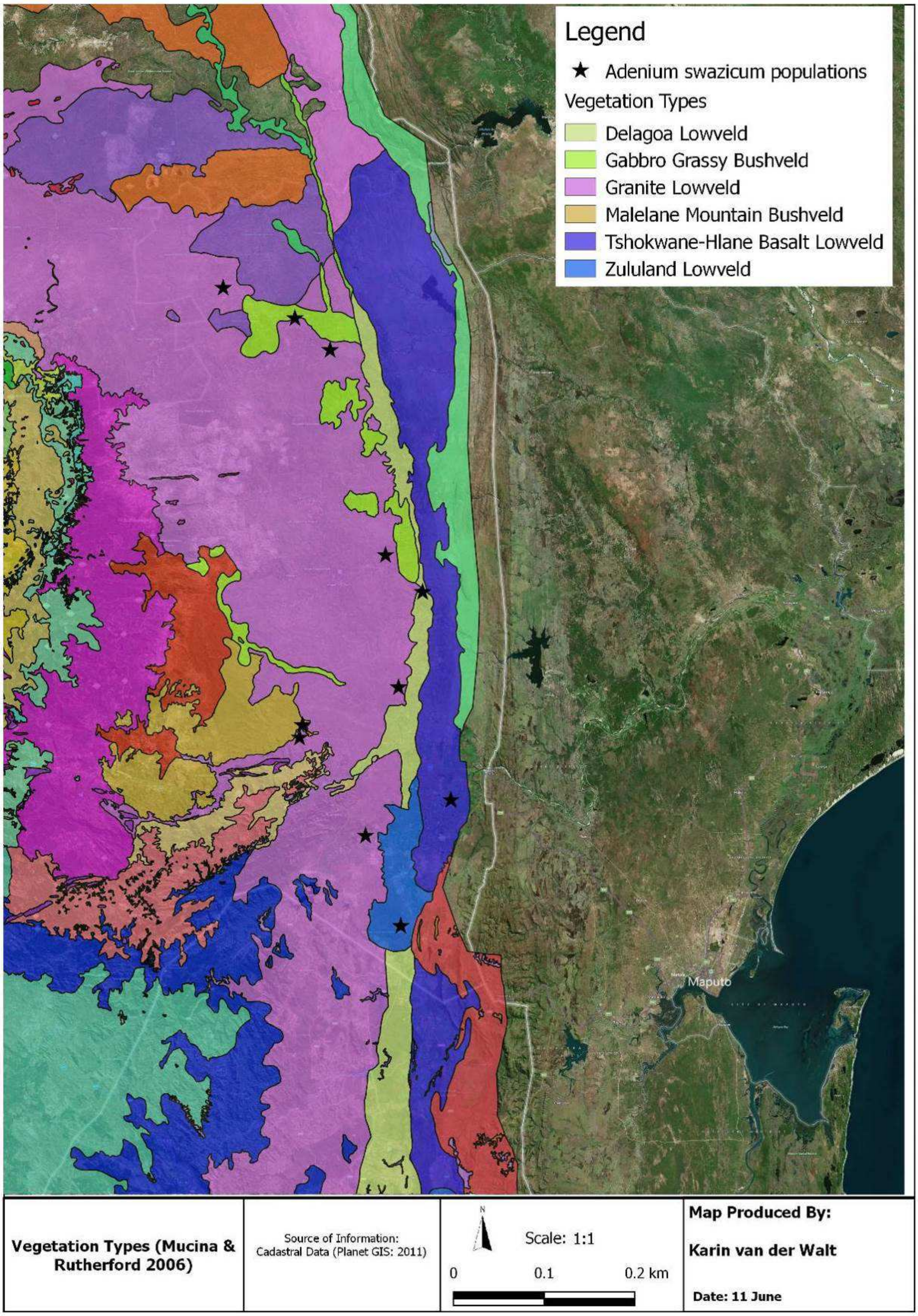


Figure 2.2: Vegetation types in which *Adenium swazicum* occurs.

The Gabbro Grassy Bushveld only occurs in Mpumalanga and consists of flats and hills in open savanna where it consists of dense grass cover with scattered trees and shrubs. Soils are mostly dark vertic clay soils and dominated by graminoid species such as *Chloris virgata, Setaria incrassata, Themeda triandra, Cymbopogon pospischilii, Digitaria eriantha, Eragrostis superba, Panicum maximum* and *Sorghum versicolor*. Scattered trees include species such as *Acacia nigrescens, Sclerocarya birrea, Acacia tortilis, Ziziphus mucronata, Ormocarpum trichocarpum* and *Grewia bicolor.* Gabbro Grassy Bushveld is classified as Least Threatened (Mucina & Rutherford, 2006) with more than 95% conserved in KNP.

Granite Lowveld occurs in the Limpopo and Mpumalanga Provinces as well as Swaziland where it consists of tall shrubland with a few trees to moderately dense low woodland and deep sandy uplands. Important trees include *Acacia nigrescens, Sclerocarya birrea* subsp. *caffra, Acacia caffra, Combretum apiculatum, C. imberbe, C. zeyheri* and *Gymnosporia glaucophylla.* Graminoid species include *Brachiaria nigropedata, Digitaria eriantha, Melinis repens, Aristida congesta, Enneapogon cenchroides, Heteropogon contortus, Leptochloa eleusine* and *Urochloa mosambicensis.* Although 17% of the Granite Lowveld vegetation type is conserved in KNP, it is still classified as Vulnerable (Mucina & Rutherford 2006).

Malelane Mountain Bushveld is restricted to Mpumalanga and consists of open savanna on mountains and higher-lying slopes with open to dense, short mountain Bushveld and low-lying areas. The soils are shallow, coarse and sandy and dominated by trees such as *Pterocarpus angolensis, Acacia caffra, A. davyi, Combretum molle, Dombeya rotundifolia* and *Heteropyxis natalensis*. Graminoid species include *Bothriochloa radicans, Enneapogon* *scoparius, Eragrostis rigidior, Heteropogon contortus, Themeda triandra* and *Urochloa mosambicensis*. Mucina & Rutherford (2006) classify Malelane Mountain Bushveld as Least Threatened with 39% conserved in KNP and approximately 4% transformed.

The Tshokwane-Hlane Basalt Lowveld vegetation type is located in the Mpumalanga province as well as Swaziland and consists of flat plains with open tree savanna. The soils are usually black, brown or red clay with trees such as *Acacia nigrescens, Sclerocarya birrea, Philenoptera violacea, Acacia borleae, Acacia gerrardii, Albizia harveyi, Combretum hereroense, Combretum imberbe, Gymnosporia maranguensis* and *Searsia gueinzii.* The graminoid layer consists of species such as *Bothriochloa radicans, Digitaria eriantha, Panicum coloratum, Panicum maximum, Themeda triandra, Aristida congesta, Heteropogon contortus* and *Eragrostis superba.* According to Mucina & Rutherford (2006), Tshokwane-Hlane Basalt Lowveld is classified as Least Threatened with 64% conserved in the Kruger National Park (KNP) and approximately 17% transformed by cultivation.

Zululand Thornveld occurs in Swaziland as well Mpumalanga and KwaZulu-Natal provinces in South Africa. It is a slightly undulating landscape that supports various Bushveld regions ranging from dense *Dichrostachys cinerea* and *Acacia* thickets to open *Acacia tortilis* savanna. The soils are black clay soils as well as duplex soils and dominated by species such as *Acacia burkei, A. nigrescens, Sclerocarya birrea, Schotia brachypetala* and *Spirostachys africana.* Succulent trees such as *Aloe marlothii, Euphorbia grandidens* and *E. ingens* are common while the graminoid component includes species such as *Enteropogon monostachyus, Eragrostis capensis, E. curvula, E. racemosa, Heteropogon contortus, Panicum maximum, Themeda triandra, Aristida bipartita* and *Digitaria natalensis.* Zululand

Thornveld is classified as Vulnerable by Mucina & Rutherford (2006) with 18% conserved in the Hluhluwe-iMfolozi Park and Phongolapoort nature reserve and more than 26% transformed, mostly by cultivation.

2.2.3 Land use

Land use in the study area included formal protected areas, communal areas, sugarcane farms and a holiday township/wildlife sanctuary which is classified as natural, degraded and under cultivation according to the latest South African Land Use Map (Figure 2.3). It is important to note that due to the sensitive nature of the distribution data, localities of *A. swazicum* populations are an approximation. In addition, symbols indicated on the map might include more than one population within the area. For the purpose of this study, protected areas included state or privately owned game reserves, nature reserves or hunting concessions. These protected areas varied considerably in size as well as faunal species assemblages, but were all fenced. Communal areas included unfenced rural areas, used for subsistence farming and stock grazing (cattle and goats), which are in the possession of a community rather than an individual. The majority of households in communal areas are dependent on resources from the local woodlands (Botha et al. 2002a; Dovie et al. 2002; Dovie et al. 2006) and due to the high number of livestock (cattle and goats) in this area, as well as frequent fires, the vegetation was considered to be more frequently and/or intensively disturbed than the protected area. Sugarcane farms included large commercial and private sugarcane farms with restricted access. This area could be termed as a sugarcane plantation land matrix with patches of natural vegetation. The holiday township/wildlife sanctuary included a fenced area of approximately 3000ha with numerous individual, unfenced houses.

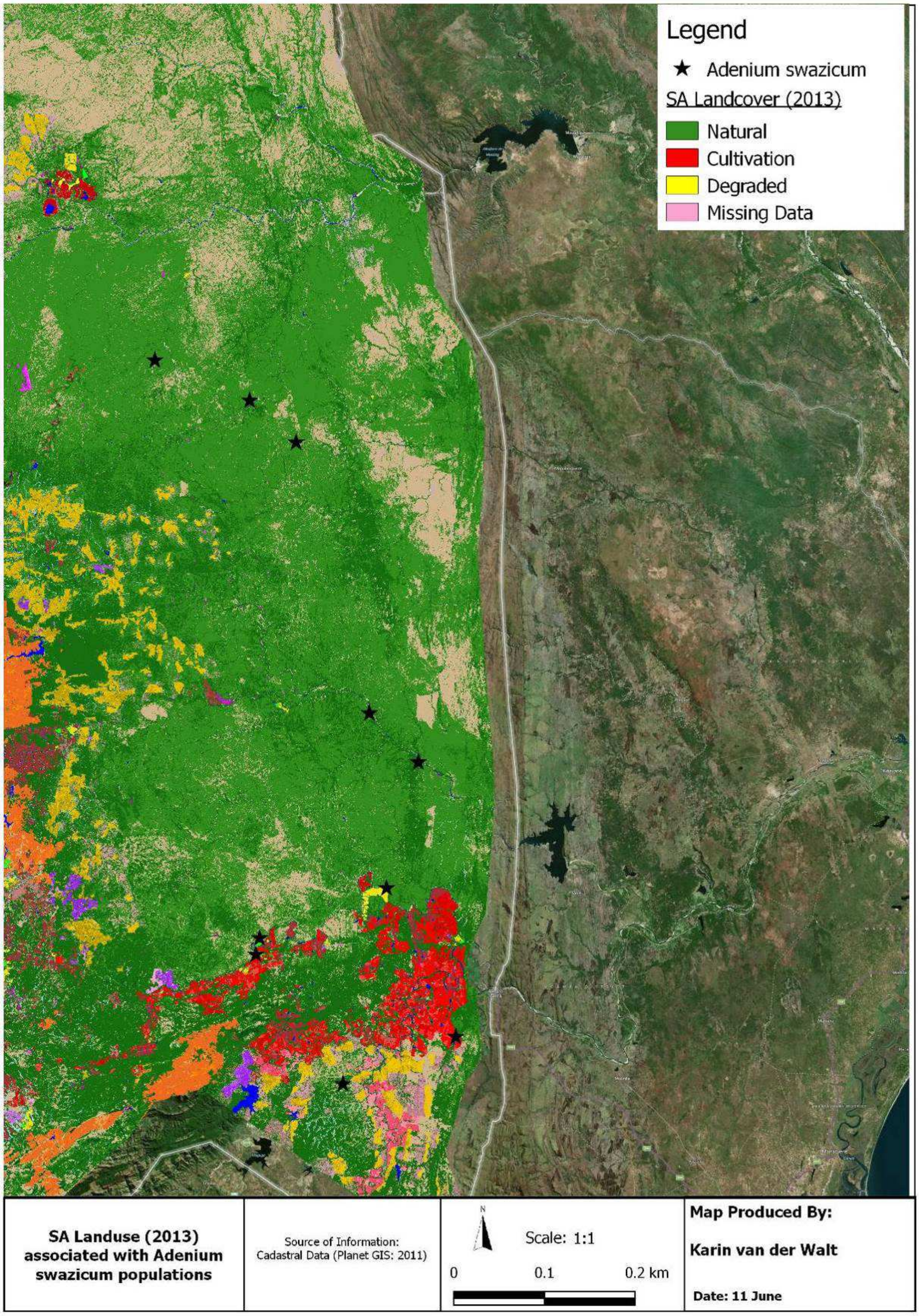


Figure 2.3: Land use in relation to the approximate location of *Adenium swazicum* populations.

The grass layer within the wildlife sanctuary was severely impacted by large numbers of herbivore species such as *Equus quagga* (Burchell's Zebra), *Aepyceros melampus* (Common Impala) and *Connochaetes taurinus* (Common Wildebeest) which has resulted in an increased occurrence of alien plant species such as *Zinnia peruviana*, *Bidens pilosa* and *Opuntia ficus-indica* (personal observations).

Population size and structure, regeneration (i.e. flowering, fruiting, seed production, germination) and herbivory were determined and compared between four populations (but only population size is included in this chapter). Two populations (A and B) were located in a formal protected area, one population (C) on a private sugarcane farm in the Komatipoort area, and one population (D) on a wildlife sanctuary within the Nkomazi municipal area. These four representative populations are described below:

Population A

Population A was located in a formal protected area within Quarter Degree Grid Cell (QDGC) 2531BC. This population was located on a sodic site, characterized by large bare soil patches and low grass species cover (Figure 2.4a). Dominant woody species included *Euclea divinorum, Diospyros mespiliformis, Combretum hereroense, Acacia nigrescens,* and *Schotia capitata.* Only one alien plant species, *Zinnia peruviana,* was recorded during the survey period between 2009 and 2011.

Population B

Population B was located in the same protected area as population A, also within QDGC 2531BC. This population occurred on a sodic site but with a denser graminoid basal cover observed for population A (Figure 2.4b). The woody component was dominated by *Euclea* *divinorum* and *Grewia flava.* The herbaceous layer consisted of species such as *Euphorbia knuthii, Aloe* sp., *Barleria elegans*, *Justicia flava*, *Momordica balsamina* and *Plectranthus tetensis* while the grass layer consisted of *Digitaria eriantha, Heteropogon contortus, Panicum maximum*, and *Themeda triandra.* Only one alien plant species, *Zinnia peruviana* was observed at the time of the surveys.

Population C

Population C was located on a private sugarcane farm close to Komatipoort, Mpumalanga within QDGC 2531DB. The habitat associated with population C had a well developed woody component which was dominated by various *Acacia* species as well as *Euclea divinorum* and *Spirostachys africana* while *Aloe marlothii* was frequently recorded (Figure 2.4c). No cattle were observed within this area during the survey period and thus the grass layer was well developed in summer although it was dominated by only a few species such as *Digitaria eriantha, Eragrostis superba*, *Panicum maximum* and *Themeda triandra*.

Population D

Population D was located in a 3000ha holiday township/wildlife sanctuary under the management of the Nkomazi Municipality in QDGC 2531BD. Unlike for the other three populations, the vegetation layer of this population was dominated by *Aloe chabaudii* while the grass layer was completely absent during the survey years (Figure 2.4d). This could possibly be attributed to the grazing by high number of game species such as *Aepyceros melampus* (Common Impala), *Equus quagga* (Zebra), *Phacochoerus africanus* (Warthog) and *Connochaetes taurinus* (Blue Wildebeest) which were present in the area. Local residents in the wildlife sanctuary supplied supplemental feeding to these species to prevent starvation (pers. obs.). The woody component consisted of species such as *Euclea divinorum,* *Rhigozum zambesiacum* and *Spirostachys africana.* Furthermore, dense infestations of weedy alien species such as *Bidens pilosa* and *Zinnia peruviana* were also observed during the summer periods.

Land use and habitat characteristics of the four populations studied between 2009 and 2011 are summarized in Table 2.1.

Figure 2.4: Habitat features of *Adenium swazicum* populations in January 2010 included (a) dense stands of *Euclea divinorum* with sparse graminoid layer in population A, (b & c) well developed graminoid layers in population B and C, and (d) a dense herbaceous layer in population D.

Table 2.1: Population characteristics of the four more intensively studied populations of *Adenium swazicum* in Mpumalanga Province, South Africa.

Population characteristic	Population A	Population B	Population C	Population D
Province	Mpumalanga	Mpumalanga	Mpumalanga	Mpumalanga
Vegetation type (Mucina & Rutherford 2006)	Malelane Mountain Bushveld	Granite Lowveld	Tshokwane-Hlane Basalt Bushveld	Granite Lowveld
Altitude (meters above sea level)	278	276	183	195
Land use	Protected area (Natural)	Protected area (Natural)	Private sugarcane farm (Cultivated)	Holiday township/ wildlife sanctuary (Degraded).
Associated vegetation	*Combretum hereroense* *Diospyros mespiliformis* *Euclea divinorum* *Grewia bicolour* *Grewia flava* *Schotia capitata*	*Acacia borleae* *Combretum hereroense* *Euclea divinorum* *Grewia bicolor* *Schotia brachypetala* *Xanthocercis zambesiaca*	*Aloe marlothii* *Elaeodendron transvaalense* *Euclea divinorum* *Gymnosporia senegalensis* *Pappea capensis*	*Aloe chabaudii* *Euclea divinorum* *Euphorbia schinzii* *Pappea capensis* *Rhigozum zambesiacum* *Spirostachys africana*
Alien vegetation	*Zinnia peruviana*	*Zinnia peruviana*	None seen	*Bidens pilosa* and *Zinnia peruviana*
Distribution of plants within population	Clumped	Clumped	Clumped	Clumped
Surface area of population (ha)	0.95	2.10	0.37	1.57
Total number of *A. swazicum* individuals in 2010	121	137	141	70

2.3 MATERIALS AND METHODS

2.3.1 Distribution

Distribution records were obtained from the National Herbarium Pretoria Computerised Information System (PRECIS) which is a database system on southern African plants from south of the Limpopo and Kunene Rivers (Namibia). Also included in this database is the tropical Africa collection, records from Compton Herbarium (NBG) and South African Museum (SAM). Over 900 000-specimen records are stored on PRECIS (posa.sanbi.org). Records obtained from the National Herbarium at Kew Royal National Botanical Gardens, are also reflected in the PRECIS database.

All the PRECIS localities were investigated between March 2009 and April 2011 to verify whether the locality has been transformed and to determine if *A. swazicum* was still present within the area. Since *A. swazicum* is deciduous, surveys were conducted during the flowering period (October to April) between 2009 and 2011. Where possible, areas found to support suitable habitat but with no specimens, were searched later in the flowering season or in subsequent years.

Statistical analyses were conducted using Analyse-it for Microsoft Excel (version 2.30) as well as XLStat (version 2014.6.04). Student t tests was used to compare the number of plants growing in formal protected areas (national park, provincial reserve and private reserve) and areas which were not formally protected (sugarcane farms, communal land, municipal land).

The Animal Demographic Unit (ADU) through its citizen science projects such as the South African Birds Atlas Project 1 (SABAP1) has proven the value of data contribution records collected by 'citizen scientists'. Various project posters in which citizens were requested to submit any localities where they observed *A. swazicum* were designed and placed at the Skukuza nursery and Malelane entrance gate of the Kruger National Park from 2009 to 2011.

2.3.2 Extent of Occurrence, Area of Occupancy and population size

The IUCN defines Extent of Occurrence (EOO) as: "the area contained within the shortest continuous imaginary boundary, which are drawn to encompass all the known, inferred or projected sites of present occurrence, but exclude cases of vagrancy" (IUCN 2014). The Extent of Occurrence (EOO) for *A. swazicum* encompassed all the localities confirmed during this study and expressed the affected area as km^2 and hectares. The historical EOO could not be determined since records obtained from PRECIS mostly referred to the QDGC, which is too broad to effectively determine the EOO. The Area of Occupancy (AOO) is the area within the EOO that is occupied by a taxon and excludes cases of vagrancy. The AOO is much smaller than the EOO because a taxon will not usually occur throughout the EOO but only occupies suitable habitats within the EOO. The AOO of each population was determined using a Garmin Quest 2 Global Positioning System (GPS) by walking in a circle around the population and recording all the individuals on the boundary of the population. GPS readings of these plants on the edges were connected to determine the AOO. For populations that were not personally verified but relied on records and photos provided by rangers and botanists, the AOO was determined by using aerial imagery such as Google Earth to determine suitable habitat.

Population size refers to the number of plants recorded during the survey. To determine the number of plants per population, total counts were conducted during the growing season (October to April) between 2009 and 2011 when plants were easier to see due to the presence of leaves and/or flowers. Line transects were conducted by two or more people walking six meters apart counting all the plants within a three meter radius. Transects were repeated until no additional plants were observed and once the neighbouring habitat was considered unsuitable for *A. swazicum*. In addition, a GPS location and number of plants recorded in the summer months of 2010 and 2011 by field rangers in the Kruger National Park using a Cybertracker GPS device were also included in this study.

2.3.3 Threats to populations

Based on the detailed species report for the Threatened Species Programme (TSP 2008), *A. swazicum* is listed as Critically Endangered due to harvesting for medicinal purposes and cultural, scientific or leisure activities. More than 50% of suitable habitat for this species has been degraded or transformed due to agricultural activities such as sugarcane growing. Threats and impacts were recorded throughout the survey period from 2009 to 2011 to determine the extent of these impacts on *A. swazicum.* Where possible, plants removed or harvested during the survey period were recorded.

2.4 RESULTS

2.4.1 Distribution

The PRECIS data for *A. swazicum*, obtained from the National Herbarium included 21 records from 20 localities in 11 Quarter Degree Grid Cells (QDGC), within South Africa and Swaziland, with no distribution records for Mozambique (Table 2.2). Eight of these localities in four QDGCs were confirmed during fieldwork conducted between March 2009 and April 2011. Four localities could not be confirmed due to vague descriptions such as “Barberton District” or “10 miles north of border gate”, that failed to indicate which border gate. Based on aerial imagery such as Google Earth, a further four localities were found to have been destroyed by cultivation (sugarcane fields). Despite the presence of suitable habitat in QDGC 2531BC, searches conducted in October 2009 and November 2010 failed to locate any *A. swazicum* plants. Data collected recorded *A. swazicum* from seven new localities, five of which were in South Africa, one in Swaziland and one in Mozambique. Information on localities not within South Africa were collected on an *ad hoc* basis, however three localities in Swaziland could not be verified although aerial imagery indicates that suitable habitat is still present. Table 2.2 summarizes data obtained from PRECIS while Table 2.3 summarizes new localities confirmed between 2009 and 2011 through fieldwork and records submitted by botanists and rangers. Specific names and geographic locations of populations are not given to protect the remaining populations of *A. swazicum.*

Table 2.2: PRECIS data for *Adenium swazicum*, including the PRECIS number, record date, QDGC and country in which it was confirmed, as well as whether these records were confirmed during fieldwork conducted between 2009 and 2011.

PRECIS No.	Record date	QDGC	Country	Locality confirmed in present study	No confirmation: Reasons
PRE342106	1947	2531BC	South Africa	Yes	None
PRE342107	1953	2531BC	South Africa	Yes	None
PRE342108	1929	2531BD	South Africa	Yes	None
PRE342109	1962	2431AD	South Africa	Yes	None
PRE342110	1926	2531BC	South Africa	No	Suitable habitat exists and it is therefore likely that *Adenium swazicum* is still present within the area
PRE342111	No date	2531CB	South Africa	No	Area transformed by sugarcane
PRE342112	1962	2431AD	South Africa	Yes	None
PRE342113	1962	2531DB	South Africa	Yes	None
PRE342114	1929	2531BC	South Africa	No	Suitable habitat exists and it is therefore highly likely that it is still present within the area. Same locality as PRE342110
PRE342115	1934	2531CB	South Africa	Yes	None
PRE342116	1911	2531DA	South Africa	No	Area transformed by sugarcane
PRE342117	1973	2531BC	South Africa	Yes	None
PRE342118	1930	Unknown	Unknown	No	Locality information very vague and could therefore not be verified

PRE342119	1948	2631BD	Swaziland	No	No fieldwork conducted in Swaziland
PRE342120	1972	2631BB	Swaziland	No	Locality within a game reserve and therefore still likely to be present
PRE353449	1970	2531DC	Swaziland	No	Based on aerial imagery the area is transformed and it is therefore considered unlikely that the plants will still be present at the locality
PRE532962	1970	2531DC	Swaziland	No	Based on aerial imagery the area is transformed and it is therefore considered unlikely that the plants will still be present at the locality
PRE717543	1957	2631BD	Swaziland	No	The coordinates for the locality does not correspond with the locality description. The elevation is much higher than other localities and it is therefore assumed that the locality information is not correct
PRE718776	1962	2631AD	Swaziland	No	The coordinates for the locality does not correspond with the locality description. The elevation is much higher than other localities and it is therefore assumed that the locality information is not correct
PRE719672	1961	2631DA	Swaziland	No	Large rural areas are still present in Stegi district and it is therefore likely that the localities still exist

PRE719209	1957	2631BD	Swaziland	No	The coordinates for the locality does not correspond with the locality description. The elevation is much higher than other localities and it is therefore assumed that the locality information is not correct

Table 2.3: Additional Quarter Degree Grid Cells (QDGC) in which *Adenium swazicum* was confirmed during fieldwork conducted between 2009 and 2010 in South Africa.

Date confirmed	Quarter Degree Grid Cell	Country	Province/District
2009	2631DD	Swaziland	Lubombo
2009	2531DB	South Africa	Mpumalanga
2010	2531BA	South Africa	Mpumalanga
2010	2531BB	South Africa	Mpumalanga
2010	2431DD	South Africa	Limpopo
2010	2431BC	South Africa	Mpumalanga
2010	2432AB	Mozambique	Gaza Province

Fieldwork conducted between March 2009 and April 2011, as well as records obtained from amateur botanists, rangers in protected areas and the general public (verified through photographs or fieldwork by the author) confirmed the occurrence of *A. swazicum* in 23 different localities (hereafter referred to as populations) in 16 QDGCs (Table 2.4). The populations were recorded in South Africa, Swaziland and Mozambique, between 24°19’15.21” - 27°38’02.76” south and 31°24’41.65 - 32°04’29.97” east. Within South Africa, this species was recorded in Mpumalanga and Limpopo provinces, and although it is suspected to occur in KwaZulu-Natal, no plants were confirmed during field surveys conducted in November 2010. Population sizes were only determined for South African localities, and of these only seven populations had more than 100 individuals between 2009 and 2011 (Figure 2.5). There was no difference between the number of plants/population in protected areas compared to non-protected areas ($t_{1,15} = 0.50$; $p = 0.6268$; $n = 17$).

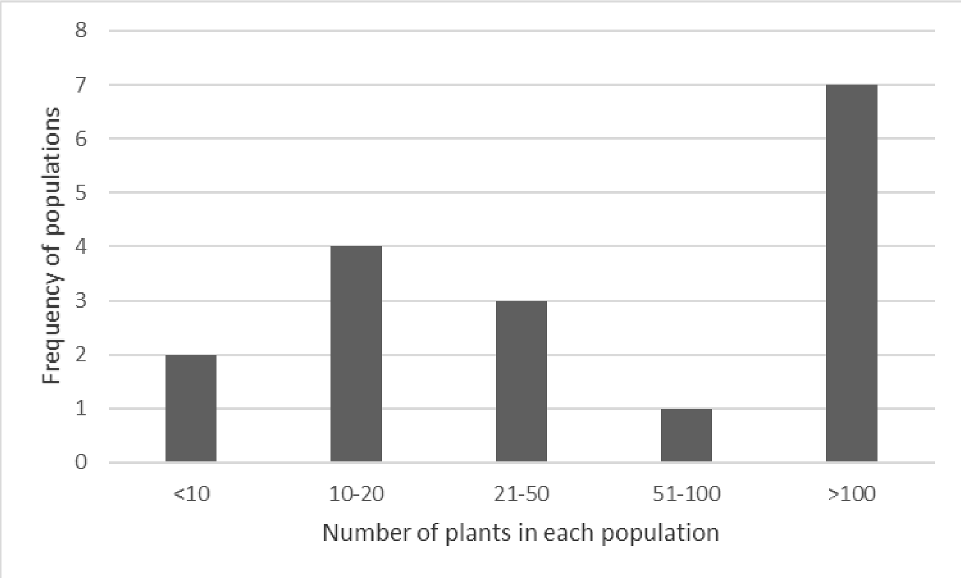


Figure 2.5: Population sizes of *Adenium swazicum* in South Africa, determined between 2009 and 2011 (n = 17).

Table 2.4: Summary of the number of populations, approximate number of plants in each population, region and country in which the populations were confirmed through fieldwork. Population sizes were not determined for localities in Swaziland and Mozambique as there was no fieldwork in these countries.

Population name	Region	Country	Number of plants counted in population
A	Southern Mpumalanga	South Africa	**121**
B	Southern Mpumalanga	South Africa	**137**
C	Southern Mpumalanga	South Africa	**141**
D	Southern Mpumalanga	South Africa	**70**
E	Southern Mpumalanga	South Africa	**21**
F	Southern Mpumalanga	South Africa	**110**
G	Southern Mpumalanga	South Africa	**140**
H	Southern Mpumalanga	South Africa	**25**
I	Southern Mpumalanga	South Africa	**120**
J	Southern Mpumalanga	South Africa	**40**
K	Southern Mpumalanga	South Africa	**20**
L	Central Mpumalanga	South Africa	**2**
M	Limpopo	South Africa	**55**
N	Limpopo	South Africa	**1**
O	Central Mpumalanga	South Africa	**20**
P	Southern Mpumalanga	South Africa	**12**
Q	Southern Mpumalanga	South Africa	**122**
Total number of plants in South Africa			**1167**
R	Swaziland	Swaziland	**Unknown****
S	Swaziland	Swaziland	**Unknown****
T	Swaziland	Swaziland	**Unknown****
U	Swaziland	Swaziland	**Unknown****
V	Swaziland	Swaziland	**Unknown****
W	Gaza province	Mozambique	**Unknown*****

* Uncertain if plants were planted or occur naturally
** Data obtained from Linda Lofter (2010), amateur botanist in Swaziland
*** Locality confirmed by John and Sandie Burrows (2010), botanists, South Africa

2.4.2 Extent of Occurrence (EOO), Area of Occupancy (AOO) and population sizes

Based on the distribution of *A. swazicum* as determined during this project, the current EOO is 8 392 km² or 839 246 ha (Figure 2.6) while the AOO is estimated to be 850ha. This information is considered an up to date assessment of the distribution EOO and AOO of *A.*

swazicum in South Africa, but it is likely that additional populations could be present in Swaziland and Mozambique.

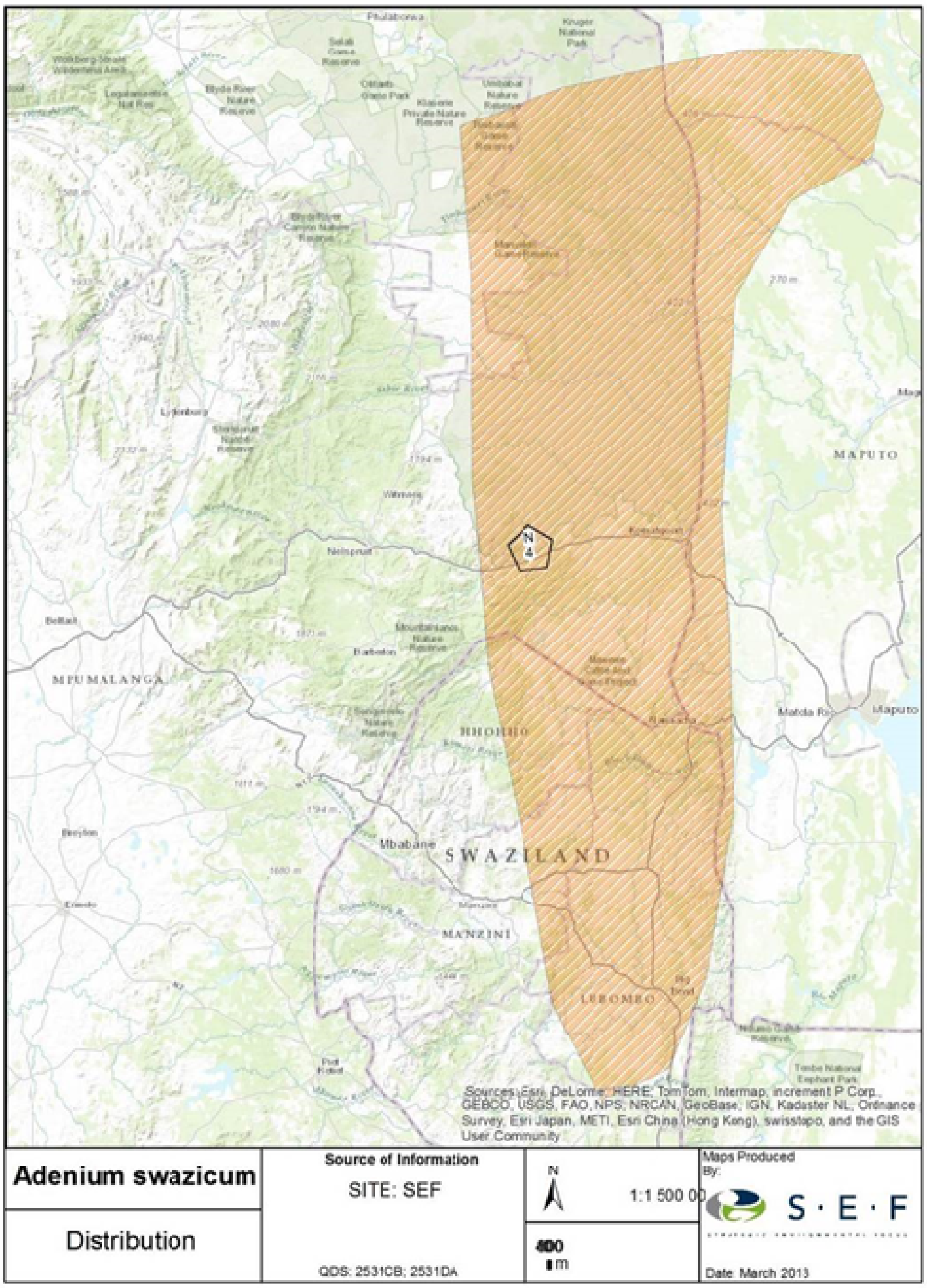


Figure 2.6: The current Extent of Occurrence (EOO) of *Adenium swazicum* in South Africa, Swaziland and Mozambique.

2.4.3 Threats to populations of *Adenium swazicum*

Anthropogenic threats such as habitat destruction, habitat degradation and illegal harvesting affected various *A. swazicum* populations during the survey period between 2009 and 2011. Some of the historic impacts such as habitat destruction were still occurring at the time of the study and is predicted to continue into the near future. The historic, current and future impacts affecting *A. swazicum* are listed in Table 2.5.

Habitat destruction and habitat degradation

Historically up to 50% of the suitable habitat for *Adenium, swazicum* was destroyed by habitat destruction, mostly for sugarcane cultivation (Raimondo et al. 2009). Most of the current populations, including those located in protected areas, were still threatened by habitat destruction in 2010. The main drivers of this habitat destruction were sugarcane farms and infrastructure development such as houses, roads and lodges. Historic infrastructure development included roads, lodges and houses within protected areas while recent developments included additional lodges, houses and roads. Numerous plants were destroyed by housing developments in population D, while a 200-bed hotel complex and associated road infrastructure have been proposed which will affect population C. However, the environmental impact studies and subsequent authorization for this 200-bed hotel had not yet been completed by the time this study was completed.

Habitat degradation includes a decline in habitat quality with the main drivers recorded during this study being trampling by cattle, especially in population O which was located on communal land where cattle are grazed year-round. Encroachment by alien plants is considered a severe threat for numerous plant species on a national scale (Von Staden 2009);

however, only population D was threatened by extensive invasions of pioneer alien plant species such as *Zinnia peruviana* and *Bidens pilosa*, which during summer formed dense stands often covering *A. swazicum* plants completely.

Harvesting: Medicinal/cultural

The biggest threat recorded during the survey period between 2009 and 2011, was the destruction of *A. swazicum* by medicinal plant harvesters or gatherers.

Population P decreased by 82% (from 115 plants to 20) between 2008, when plants were monitored as part of the Lowveld National Botanical Garden's Threatened Plant Project, and 2010 (pers. obs.). Interviews conducted at the Traditional Markets in Nelspruit in 2009 revealed that the tubers of *A. swazicum* were sold as an extract to cleanse the blood and stomach (Figure 2.7). Medicinal harvesters also targeted population D with at least ten adult plants (14%) removed in July 2010. Steel buckets and rope left in the holes where *A. swazicum* plants were removed from indicated that a ritual was performed during the removal (L. Nkuna, pers. comm.). In all instances, harvesting methods were destructive and killed the plant, with the tuber removed while the above ground stems, branches and leaves were discarded. Harvesting for traditional medicine was restricted to populations located outside protected areas.

Figure 2.7: Holes in the ground left behind following the harvesting of *Adenium swazicum* plants by medicinal plant gatherers in: (a) population P in April 2010, and (b) the muthi market in Nelspruit where *Adenium swazicum* is sold as an extract in 2 litre plastic bottles.

Harvesting: Horticultural

All *Adenium* species are popular ornamental plants in South Africa (pers. obs.). Despite this, the threat from the horticultural industry to the persistence of *A. swazicum* in the wild was perceived to be minimal, although it is not known how many *A. swazicum* plants have been removed from the wild historically. During the survey period, only one incidence was recorded where a branch was removed from a particularly large specimen with a sharp object such as a knife or secateurs, and it is presumed that this branch was used for grafting purposes. Although the threat from the horticultural industry was perceived to be low during this study, it should be noted that interest in *Adenium* is increasing in numerous countries and could therefore become a potential threat in the near future.

Propagating *Adenium* cultivars from seed and/or seedlings is not a popular practice since plants that have been grown from seed will take many months to flower, and the flowers on juvenile plants are smaller and of a lower quality compared to grafted plants. At the LNBG, *A. swazicum* was propagated exclusively from seeds that have been collected from wild populations as part of the threatened plants programme at the LNBG. It was found that seeds will germinate readily and did not require special treatment and seedlings had a high survival rate in *ex situ* collections.

Table 2.5: Historic, current and future threats to *Adenium swazicum* populations as observed and inferred during fieldwork conducted between January 2009 and January 2011 in South Africa.

Population	Locality	Land use	Threats		
			Historic	**Current**	**Future**
A	Southern Mpumalanga	Protected Area (Natural)	It is likely that some plants were destroyed during the construction of the roads and other infrastructure	One incidence of harvesting for horticultural purposes was recorded	None
B	Southern Mpumalanga	Protected Area (Natural)	It is likely that some plants were destroyed during the construction of the roads and lodges	None	None
C	Southern Mpumalanga	Sugarcane Farm (Cultivated)	Large areas were cleared of natural vegetation for sugarcane farms	The farmer is aware of the plants and will preserve the remaining natural areas	It is likely that medicinal plant gatherers will target this population once populations on communal land have been depleted.
D	Southern Mpumalanga	Urban/Residential (Degraded)	The construction of roads and houses impacted on plants within the area	Plants are harvested for medicinal purposes (14% decline). Continuous development of houses and roads also impacts on the remaining plants. Habitat degradation (overgrazing)	Current impacts are likely to continue in the future.
E	Southern Mpumalanga	Protected Area (Natural)	It is likely that some plants were destroyed during the	None	Proposed development of a hotel and associated road

			construction of the roads		infrastructure.
F	Southern Mpumalanga	Protected Area (Natural)	It is likely that some plants were destroyed during the construction of the road	None	None
G	Southern Mpumalanga	Protected Area (Natural)	It is possible that some plants might have been destroyed during the construction of the lodge	None	Off-road driving is permitted in private concessions and could impact on areas containing *Adenium swazicum*
H	Southern Mpumalanga	Protected Area (Natural)	It is possible that some plants might have been destroyed during the construction of the lodge	None	Off-road driving is permitted in private concessions and could impact on areas containing *Adenium swazicum*
I	Southern Mpumalanga	Protected Area (Natural)	It is likely that some plants were destroyed during the construction of the roads	None	Off-road driving is permitted in private concessions and could impact on areas containing *Adenium swazicum*
J	Southern Mpumalanga	Protected Area (Natural)	It is likely that some plants were destroyed during the construction of the roads	None	None
K	Southern Mpumalanga	Protected Area (Natural)	A road has been constructed through the middle of the population and it is therefore highly likely that plants were destroyed	Areas next to the entrance road were mechanically slashed during the survey period and plants were cut down to ground level but resprouted from underground tubers	None. The lodge was made aware of the plants within the concession and areas containing these plants were not cut
L	Central Mpumalanga	Protected Area (Natural)	It is likely that some plants were destroyed during the	None	None

			construction of the roads		
M	Limpopo	Protected Area (Natural)	None	None	None
N	Limpopo	Protected Area (Natural)	Only one plant was recorded and it is uncertain if this is a natural population.	None	None
O	Central Mpumalanga	Protected Area (Natural)	None	None	None
P	Southern Mpumalanga	Degraded (Communal land)	Large areas which were suitable habitat for *Adenium swazicum* were destroyed for sugarcane farms	Extensive harvesting by medicinal gatherers (82% population decline over survey period). Cattle and goats trample the plants, delirious fire regimes within the area are likely to impact on the reproduction.	Impacts are likely to continue in the future
Q	Southern Mpumalanga	Protected Area (Natural)	None	None	None
R	Swaziland	Unknown	Large areas surrounding this population have been transformed for sugarcane fields	Habitat destruction for sugarcane farms	Habitat destruction for sugarcane farms
S	Swaziland	Unknown	Large areas surrounding this population have been transformed for sugarcane fields	Habitat destruction for sugarcane farms	Habitat destruction for sugarcane farms
T	Swaziland	Unknown	Large areas surrounding this population have been transformed for sugarcane	Habitat destruction for sugarcane farms	Habitat destruction for sugarcane farms

			fields		
U	Swaziland	Unknown	None	None	None
V	Swaziland		Large areas surrounding this population have been transformed for sugarcane fields	Habitat destruction for sugarcane farms	Habitat destruction for sugarcane farms
W	Mozambique	Unknown	Unknown	Unknown	Unknown

2.5 DISCUSSION

The total population size of *A. swazicum* in South Africa, based on actual plants directly counted in the field in 2009 and 2010 was 1167 individuals spread over 17 populations in the Mpumalanga and Limpopo Provinces. The main threats currently affecting all populations are habitat destruction and harvesting for medicinal purposes. *A. swazicum* populations were found to be small (between 1 and 141 individuals), with many of these populations still declining.

Many rare and endangered species occur in small, local populations in a fragmented landscape (Franzen & Nilsson 2009), and this was also the case for *A. swazicum* with individual population sizes ranging from a single plant to 141. *A. swazicum* is restricted to the Savanna Biome where it occurs in six vegetation types namely Tshokwane-Hlane Basalt Lowveld, Delagoa Lowveld, Gabbro Grassy Bushveld, Malelane Mountain Bushveld, Granite Lowveld and Zululand Thornveld. The land uses associated with *A. swazicum* included state and privately owned game reserves (formal protected areas), nature reserves and hunting concessions, communal land, private sugarcane farms and municipal townships.

Historic distribution of *A. swazicum* prior to 2009 indicated that the species was recorded from 20 localities in 11 Quarter Degree Grid Cells (QDGC) in South Africa and Swaziland. One locality still contained suitable habitat but no plants were found during the survey period. Seven new localities for *A. swazicum* were confirmed during the present study, of which five were located in South Africa, one in Swaziland and one in Mozambique. Fieldwork was restricted to South Africa and it is therefore likely that there are other undiscovered populations in Swaziland and Mozambique. Based on this updated locality information, the Extent of Occurrence (EOO) for *A. swazicum* was estimated as 8 392km^2 while the Area of occupancy was estimated as 850ha.

Small populations have a greater risk of extinction from various causes (Shaffer 1981) however, small and restricted populations do not always go extinct (Da Silva et al. 2012). When a population decreases, the reduction in number of individuals threatens the genetic diversity of the population, making the population vulnerable to events that might lead to its extinction (Van Dyke 2003). It is therefore important to determine the minimum viable population (MVP) which is the minimum number of individuals needed for that population to persist during demographic stochasticity, environmental stochasticity, genetic stochasticity and natural catastrophes (Van Dyke 2003). Although large areas within the distribution range of *A. swazicum* have been destroyed by agriculture and infrastructure development, *A. swazicum* would have only occupied suitable patches within these areas. This type of distribution is linked to metapopulation theory, which states that “groups of small populations which are connected by occasional movements of individuals between them” (Husband & Barrett 1996). It is therefore likely that the population size of *A. swazicum* is limited by the availability of suitable microhabitat, especially for seedling establishment. Although this project did not study the genetic makeup of each population, by combining the historic and current distribution data, it is reasonable to assume that *A. swazicum* fits well within the metapopulation theory. However, due to the large scale destruction of habitat within the distribution of *A. swazicum,* the populations outside of Kruger National Park and other conservation areas, are greatly affected by habitat fragmentation and isolation, with little to no connection between these populations. With such isolation and small population sizes, coupled with high levels of exploitation and continued habitat destruction, it is likely that *A.*

swazicum will become locally extinct from localities on private and communal land. In contrast, populations within KNP are stable with distances between populations (approximately 2-10km) short enough that genetic exchange is likely.

There is a trend in South Africa towards increased commercialization of medicinal plants, which has already resulted in overharvesting, and even near-extinction of some valued indigenous species (Williams et al. 2000). Actual levels of exploitation of *A. swazicum* in the Lowveld of Mpumalanga might be higher than initially considered, with at least one population decreasing by 82% in less than three years. In addition to this, medicinal harvesters also affected population D, which was already under threat from housing development and habitat destruction. Echujine, the highly toxic glycoside has been isolated from the tubers, roots, stems and seed of *A. swazicum* (Newsinger 1996) but only the tuber is used for medicinal purposes (pers. obs.). According to Botha et al. (2004a), vendors interviewed in Mpumalanga indicated that the demand for species such as *A. multiflorum* or *A. swazicum* (ununakhulu) was higher when the plants were fresh. Cunningham (1992) indicated that species, which have high growth rates and/or resprouting/coppice responses are more easily able to withstand the impact of intensive harvesting. Since *A. swazicum* is able to resprout from its underground tuber (see Chapter 3), this species could potentially be harvested sustainably by utilizing the stems rather than the underground tuber.

3 CHAPTER 3: POPULATION BIOLOGY OF THE CRITICALLY ENDANGERED SUCCULENT, *ADENIUM SWAZICUM* STAPF

Abstract

No published information is available on the population size and structure or the impacts from herbivores on *Adenium swazicum.* Fifty plants in four representative populations were studied to determine plant size, herbivory, number of flowers, number of follicles and number of seed produced, between March 2009 and April 2011. Two of the studied populations were located inside a formally protected area, one on a private sugarcane farm while the fourth was located in a wildlife sanctuary with housing developments. The Area of Occupancy (AOO) of these four populations ranged from 0.37ha to 2.1ha, while the total number of individuals in the four populations ranged from 70 to 141. Size class distributions varied between populations, with population A having no individuals in size class 5 (1501-2000cm^2). Herbivores had a significant impact on the reproductive capability of *A. swazicum*, with intensive herbivory resulting in a threefold reduction in flower production. Plants that grew in full shade had a higher percentage of foliage impacted on by invertebrates, compared to those that grew in semi shade and full sun. *Ex situ* disturbance experiments, revealed that adult *A. swazicum* have a high disturbance tolerance and will resprout from the underground tuber even if all above ground parts have been destroyed. In 2010 insect species such as *Dacus frontalis* and *Leptocoris hexophtalma* destroyed all the follicles produced in population D as well as 91% of the follicles of population A. Although follicle predation was severe for certain *A. swazicum* populations during the survey period, this impact is likely to be less severe when one considers the entire population over a long term.

Keywords: Fire, follicles, herbivory, plant size, resprout, succulent, tuber.

3.1 INTRODUCTION

After rainfall and soils, fire and herbivory are two of the main determinants of savanna ecosystems with fire considered the major role player in distinguishing between vegetation types such as grassland, woodland and forests (Adams 2013; Masunga et al. 2013). Herbivory is however complex to study since it may be different between habitats, populations and vary along environmental gradients (Maron & Crone 2006). Due to these challenges, insect herbivory research usually only focuses on the interaction between insects and adult plants, and seldom considers the impact on juveniles (Crawley 1983, Ehrlen 1995; Stanforth et al. 1997). Among insect feeding guilds seed predation is generally considered to have strong effects on plant populations (Crawley 1990), while plants tolerate considerable damage to non-productive tissue such as leaves (Ancheta & Heard 2011). Despite this, herbivory might hinder the production and/or maturation of flowers into viable fruit (Stephenson 1981; Pfab & Witkowski 1999).

The effect of fire on savannas has been well researched (e.g. Govender et al. 2006; Staver et al. 2009; Smith et al. 2010) although most of these studies focussed on woody vegetation. The impact of fire on succulent species growing in fire prone biomes are not clearly understood, however it is generally considered that these species are vulnerable to fire and are therefore often found growing in refugia such as rocky areas or open patches devoid of fire fuel (Thomas 1991; Witkowski et al. 1997; Witkowski & Liston 1997; Pfab & Witkowski 1999b). Furthermore, some succulent species might resprout from lignotubers or protected meristems after fire (Moreno & Oechel 1990; Thomas & Goodson 1992). Despite the fact that fire management could be detrimental to the survival of *A. swazicum*, no studies have been conducted to determine the effect of fire on this Critically Endangered succulent.

The aim of this study was to understand aspects of the population biology of *A. swazicum* through the following objectives:

- Determine the plant size and population structure of representative populations;
- Determine the extent and effect of herbivory on adults and seedlings;
- Determine the follicle and seed production per adult (flowering plant) as well as per population;
- Determine the extent of seed and follicle predation; and
- Determine the effect of fire on the survival and reproduction of *A. swazicum.*

3.2 MATERIALS AND METHODS

3.2.1 Description of populations studied

In order to determine population structure of *A. swazicum*, studies were conducted in four populations over a three-year period between March 2009 and April 2011. Fifty individual plants in each population, randomly selected by walking through the population were marked with aluminium tags (Figure 3.1). The four populations were selected based on the following:

- Land use: Since three different land uses (a state owned protected area, private sugarcane farm and Municipal Township in a wildlife sanctuary), were associated with the four populations, the effect of these land uses on plant size, herbivory/threats and reproduction could be assessed;

- Proximity of populations: Since the researcher was based at the Lowveld National Botanical Garden (LNBG) in Nelspruit, it was important that the populations could be studied by conducting day trips; and
- Size of the population: The studied populations had to each have more than 50 individuals.

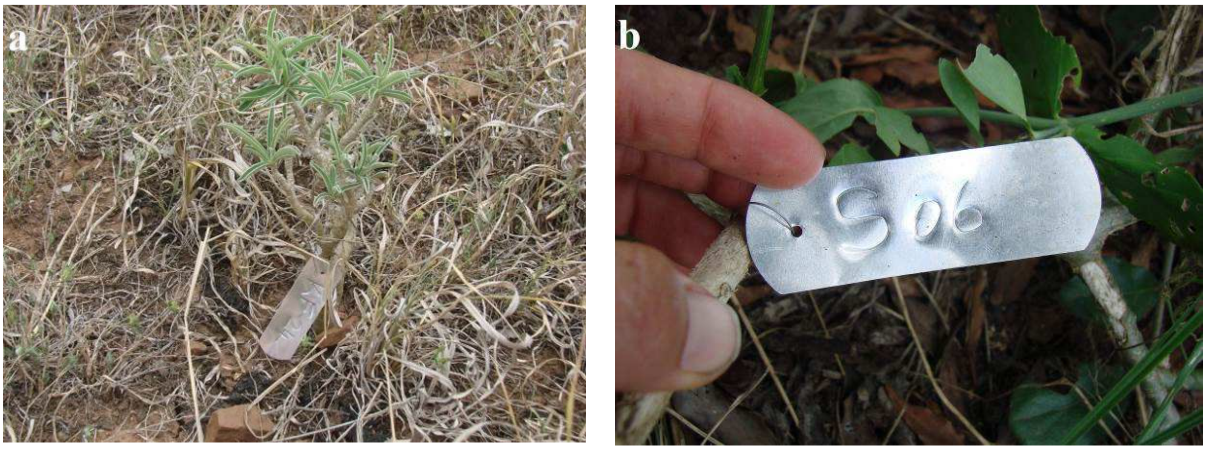


Figure 3.1: Fifty *Adenium swazicum* plants in each of the four populations were marked with numbered aluminium tags.

3.2.2 Plant size and population Structure

The AOO of each population was determined using a Garmin Quest 2 Global Positioning System (GPS) by walking in a circle around the population and recording all the individuals on the boundary of the population. GPS readings of these plants on the edges were connected to determine the AOO. Plant size was determined for fifty plants in each of the four populations. Plant size was expressed as canopy area and the following data were collected in January 2010:

- Height of plant from soil level to top of canopy (closest cm);
- Maximum canopy diameter (cm); and
- Diameter at right angle to the maximum canopy diameter (cm);

Canopy area was calculated (see Knowles & Witkowski 2000):

$$\text{Canopy Area} = \frac{\pi(D1)}{2} x \ \frac{(D2)}{2}$$

where (D1) is the maximum canopy diameter, (D2) is the diameter at right angles to the maximum. Plants were grouped based on canopy size into the following classes: class 1 = $\leq$ 100cm^2; class 2 = 101-500cm^2; class 3 = 501=1000cm^2; class 4 = 1001-1500cm^2, class 5 = 1501-2000cm^2 and class 6 = >2000cm^2.

The number of stems/plant as well as three classes of shading (full sun, semi-shade or shade) from other plants, including grass, shrubs and trees (Figure 3.2) were also recorded for the fifty plants in the four populations. Since herbivores often eat above ground parts such as stems and branches down to the ground, aboveground size is not necessarily a good indication of age or overall size (including belowground size/mass). It is likely that underground size/mass provides a good indication of age, but since *A. swazicum* is a threatened species and possible negative effects of exposing underground structures is unknown, this was considered an unsuitable method to assess age. Adult *A. swazicum* plants do not flower every year; therefore, the presence or absence of flowers was not a reliable indication of age. Based on this, only two stage categories namely, seedling/juvenile and adult were applicable to *A. swazicum*. A plant was considered to be a seedling/juvenile when the stems were green in colour while adult plants had grey to dark brown, woody stems (Figure 3.3).

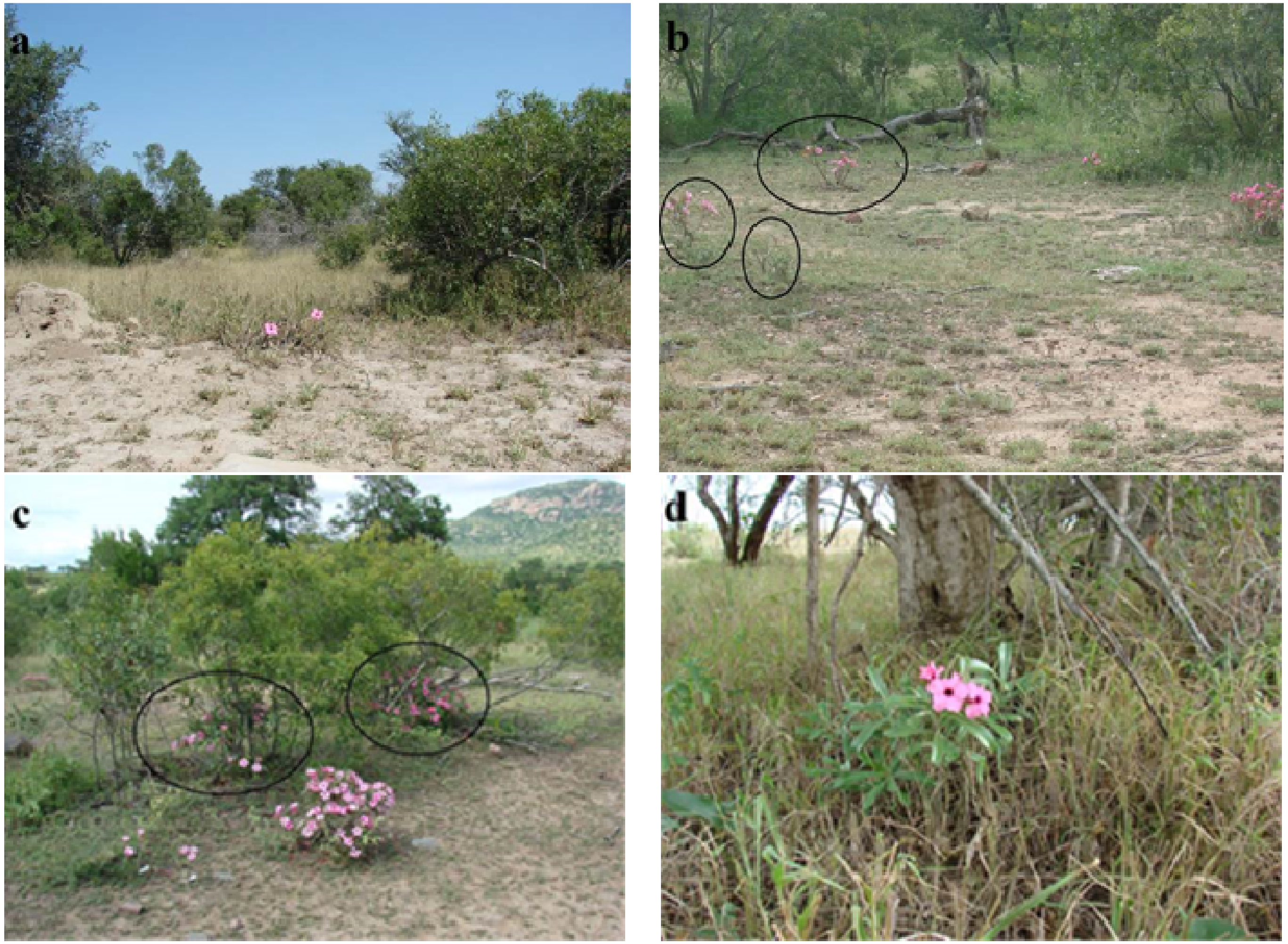


Figure 3.2: Three classes of shading for *Adenium swazicum* included: (a & b) full sun where plants were not shaded by trees, grass or shrubs, (c) semi-shade which included some shade during the day and (d) full shade when plants were in deep shade and did not receive full sun.

Figure 3.3: Age classes for *Adenium swazicum* included (e) adults with grey to brown, woody stems and (f) seedlings/juveniles with green, soft stems.

3.2.3 Herbivory on leaves, flowers, branches and stems

In situ *herbivory observations*

Damage to aboveground parts (i.e. leaves, flowers and branches/stems) of the monitored plants by vertebrates and invertebrates was recorded and expressed as total percentage of plant damaged. In addition to this, any invertebrates observed feeding on *A. swazicum* were collected and identified by the author, Kruger National Park's scientific services or the Agricultural Research Council (ARC) in Nelspruit, Mpumalanga. Lepidopteran (moths and butterflies) larvae found feeding on *A. swazicum* were captured and taken to the LNBG where each was placed in a 30cm x 30cm box filled with soil. The box was covered with a nylon stocking to prevent larvae and adults from escaping. Fresh flowers and leaves collected from the *ex situ* collection at LNBG were provided to the larvae on a daily basis. The emerged adults were identified to determine if they are potential pollinators and/or insect herbivores of *A. swazicum.*

Ex situ *experiments to simulate herbivory*

Three prominent herbivores, observed that affecting *A. swazicum* in the wild, included *Diceros bicornis* subspecies *bicornis* L (Black Rhinoceros), *Phymateus morbillosus* (Linnaeus) & *P. viridipes* (Stal) (Orthoptera: Pyrgomorphidae) (Common Milkweed Locust) and *Chrysolina (Naluhia) confluens* (Gerstaecker) (Coleoptera: Chrysomelidae) (Leaf Beetle). Black Rhinoceros are browsers feeding on a wide range of woody and succulent species, which often include unpalatable or toxic plant species such as *Datura stramonium*, *Euphorbia* spp. and *Euclea divinorum* (Oloo et al. 1994; Braselton 1995). Since it is difficult to quantify the effect of herbivory on *A. swazicum*, herbivory of vegetative tissues observed in wild populations was simulated in *ex situ* collections at the LNBG in Nelspruit, Mpumalanga. The *ex situ* collection for *A. swazicum* at LNBG was established in 2003 by Johan Hurter (Chief Horticulturist at the time) from seed collected from population P. The plants were grown in 20cm plastic pots until December 2007 when they were transplanted into full sun within a flowerbed containing local soil. Twelve of these plants were used to simulate herbivory.

Black Rhinoceros has a distinct feeding manner in which twigs and shoots are clipped cleanly often at a 90° angle (Oloo et al. 1994). These feeding signs were observed in population G, K and M located in the formally protected areas, which Black Rhinoceros frequented. Young branches were neatly clipped at a 90° angle, and this was simulated in *ex situ* experiments by removing branches with sharp secateurs (Figure 3.4). In two plants, all above ground parts were removed to determine if *A. swazicum* could resprout from the underground tuber. Insects such as *Phymateus morbillosus* (Linnaeus) and *P. viridipes* (Stal) (Orthoptera:

Pyrgomorphidae) (Common Milkweed Locust) were observed feeding on the bark of branches and stems, this was simulated in experiments on *ex situ* plants by removing bark with a sharp knife (Figure 3.5). Recovery was monitored on the 14th of September (approximately two weeks after the experiments were conducted), 8th of October 2009 (approximately 5 weeks after the experiments) and concluded on the 15th of January 2010 (20 weeks). Between the 27th of September and 7th of October, 10.5 mm of rain was measured at the LNBG.

Figure 3.4: Browsing impact by *Diceros bicornis* L. (Black Rhinoceros) observed in; (a) wild populations of *Adenium swazicum,* and (b) simulated in *ex situ* disturbance experiments.

Figure 3.5: *Phymateus viridipes* (Stal.) (Milkweed Locust) (a) was frequently observed feeding on the bark, stems and flowers of *Adenium swazicum,* (b) in the wild. These impacts were simulated (c & d) in *ex situ* plants by scraping the bark from branches using a sharp knife.

3.2.4 The impact of herbivores on seedlings

The impact of herbivores on seedling emergence and survival in the wild was determined by establishing three small plots *in situ.* The plots were 1m x 1m in size and placed next to *Euclea divinorum* trees to replicate *in situ* seedling observations. Due to low seed production in September/October 2009, only one hundred seeds were collected from population A, while two hundred seeds were collected from population B. Both populations occur in the same geographical area approximately 11km apart with isolated individuals confirmed between the populations. Plot one was established in population A (with seed collected from the same population) while plots two and three were established in the Nkhuhlu full exclosures in the KNP. Exclosures were first established in the KNP in 1954 with the aim of measuring and monitoring the long-term effects of fire and herbivory (Van der Schijff 1958) with the Nkhuhlu exclosures built in 2002 to determine the effect of elephants and fire by excluding them from the ecosystem (Siebert & Eckhardt 2008). The Nkhuhlu exclosures consist of a full exclosure section which is fenced and electrified and large vertebrates have been removed. At the time of this study, the original exclosures had been protected from herbivory for 34 years and from fire for seven years (Smith et al. 2010).

However, the Nkhuhlu exclosures were only protected for seven years. Regular patrols are conducted in the exclosure as well as along the boundaries to prevent large vertebrates from re-entering the exclosures. Scientific services in KNP approved the establishment of the experimental plots provided that plot two and three which consisted of steel and wire cages (Figure 3.6), were placed inside the Nkhuhlu full exclosures while the open plot was established in population A. This would limit the chances of these cages being destroyed by animals such as *Crocuta crocuta* Erxleben 1777 (Spotted Hyena), *Loxodonta africana* Blumenbach 1797 (African Elephant) and *Ceratotherium simum* Burchell 1817 (White Rhinoceros). A small existing population of 20 adult *A. swazicum* plants was recorded within the Nkhuhlu full exclosure (Population O). The three plots (Figure 3.6), their location, a description of the plots as well as number of seed and date of sowing are summarized in Table 3.1. In all three plots, the seeds were covered with approximately 5mm of soil to prevent wind and rain from displacing the seeds. Seedling emergence and survival were scored in January, February and May 2010.

Table 3.1: Three experimental plots to test the effect of herbivory on seedlings including the location of the plots, description of the plots, and number of seed in each plot and date the seeds were planted in the Nkhuhlu exclosures, South Africa.

Plot name	Location	Plot Description	Number of Seed	Date planted
Plot one	Population A	Open	100	16 October 2009
Plot two	Nkhuhlu full exclosure	Steel frame covered with 50mm poultry mesh	100	20 October 2009
Plot three	Nkhuhlu full exclosure	Steel frame covered with 50mm poultry mesh and 1 mm pore size mosquito net	100	20 October 2009

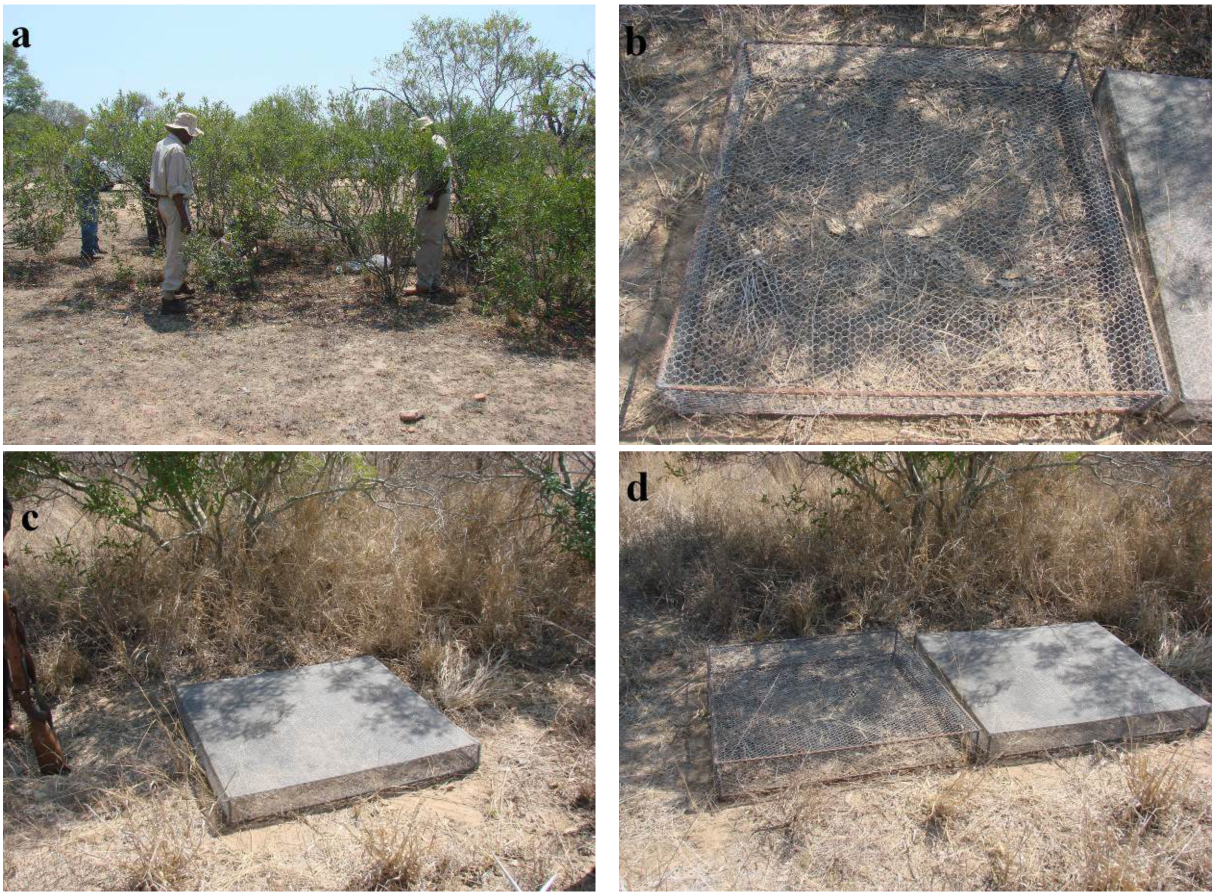


Figure 3.6: The three plots established to determine seedling emergence and survival of *Adenium swazicum* in the wild; (a) plot one was located in population A and was not covered; (b) plot two was located in the Nkhuhlu exclosures and was covered with 50mm poultry mesh to exclude vertebrates, and (c) plot three was also located in the Nkhuhlu exclosures and covered with 50mm poultry mesh as well as 1 mm mosquito net to prevent any access to seed or seedlings.

3.2.5 Seed and follicle predation

Follicle formation and predation were recorded in July and September 2009 as well as August, September and October 2010. Healthy follicles were firm and approximately 15-20cm in length while predated/infected follicles were soft with curled tips (Figure 3.7). Seeds were collected from follicles and inspected for holes or any other deformities which would indicate predation and/or seed abortion. Average number of seed per follicle was determined from nineteen intact follicles, which were collected in October 2009 from population A (n = 3), population B (n = 14) and population C (n = 2). Potential number of seed (based on all follicles produced) produced by sampled plants was calculated by multiplying the total number of follicles (including predated follicles) in each population by the average number of seed per follicle. The number of seed released by sampled plants in each population was calculated by multiplying only intact follicles with the average number of seeds per follicle. Viable seed production/sampled plant was estimated for each sampled reproductive plant as follow: Viable seeds/sampled plant = seeds released from intact follicles * mean number of seeds per follicle * (% seed viability for population/100). Seed viability was tested using tetrazolium testing (see Chapter 5). Due to a lack of seed from population B when seed viability was tested, viability results for population A that was located approximately 11km away from population B were used. Fruit (follicle) set was determined as the percentage of flowers that produced follicles.

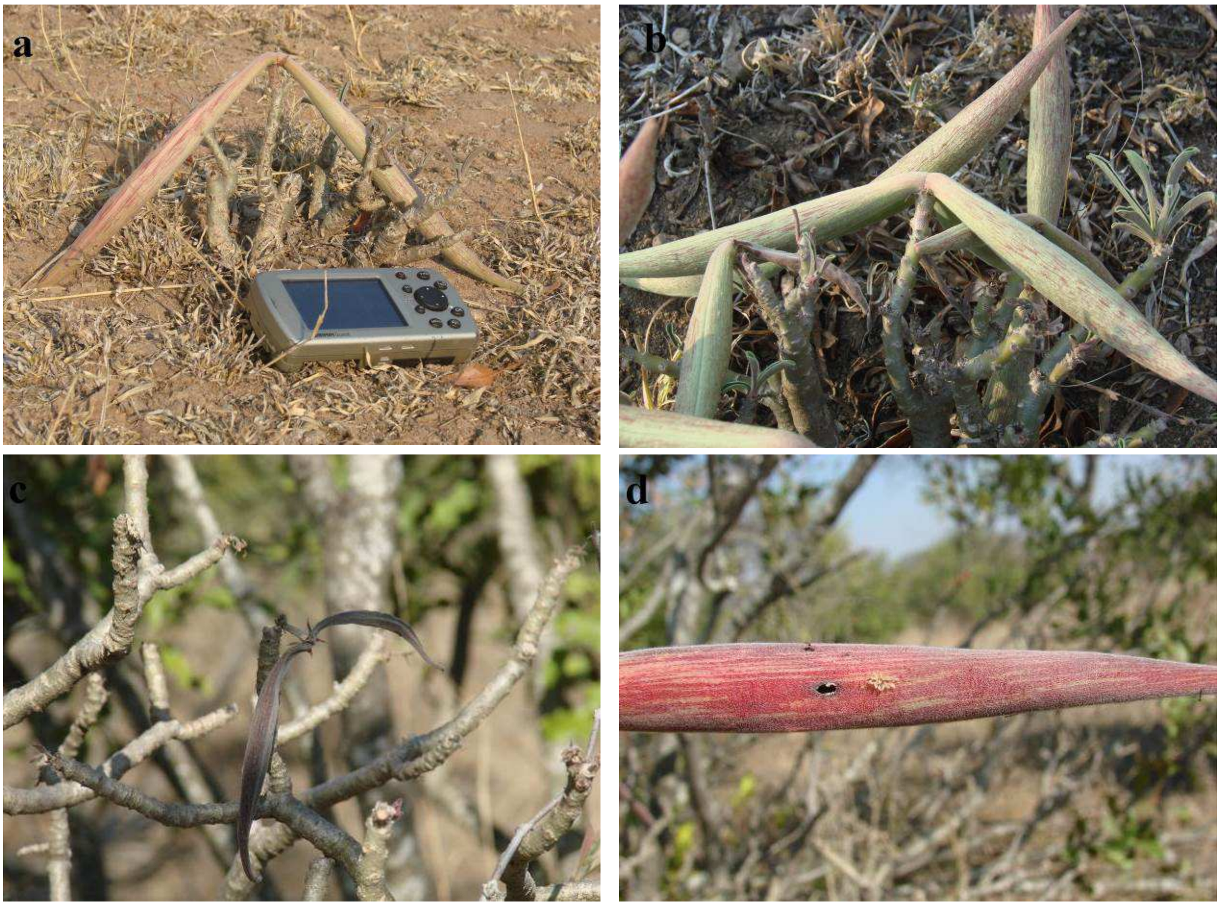


Figure 3.7: Healthy, mature *Adenium swazicum* follicles are (a) approximately 15-20cm in length and firm, ranging from reddish pink to (b) green in colour. Aborted/predated follicles were (c) soft, desiccated with curled tips, with (d) holes where invertebrates hatched sometimes visible.

3.2.6 The impact of fire on *Adenium swazicum*

Six adult, single stemmed *A. swazicum* plants of the same age at the LNBG, were covered with approximately 1 kg of dry flammable stems and leaves from grass species such as *Hyparrhenia hirta* (L) Stapf. on the 3rd of September 2009 and set alight between 11:00 and 12:00 in the morning (Figure 3.8). The temperature at the time of the fire was 28°C with 0% cloud cover, wind speed was 10km/hour and the humidity was 27% (worldweatheronline.com). The recovery of plants was recorded on a weekly basis after each treatment for eight months. The development of underground tubers was investigated by excavating four plants (three months, 12 months, 24 months and 84 months old), at the LNBG. A plant that was 84 months (6 years) old was one of the plants used for the fire experiment described above.

Figure 3.8: To determine the effect of fire on *Adenium swazicum*, (a) six adult plants at the Lowveld National Botanical Garden with approximately 1 kg of dry flammable stems and leaves of grass species such as *Hyparrhenia hirta*, and (b) set alight.

3.2.7 Statistical analysis

Where necessary, data were tested for normality using the Kolmogorov-Smirnov one sample test and when not normal were transformed using Log_{10}. One-way ANOVAs were used to compare differences in canopy area, plant height, and follicle and flower production between the four populations, while Fisher's Least Significant Difference (LSD) was applied to statistically significant results. Regression analyses were used to explore the relationship between canopy area (cm^2) and inflorescence production. Spearman ranked correlation was used to determine the relationship between herbivory and flower production. The intensity of herbivore damage was compared between plants growing in shade, semi-shade and sun using a one-way ANOVA followed by Fisher's LSD test. *In situ* seedling emergence and seedling survival were compared between three plots (i.e. open to all herbivores, excluding vertebrates only, and excluding all herbivores) using a chi-square contingency table. The results were generally presented as means $\pm$ SE. Analyses were conducted using the software Analyse-it, version 2.30 as well as XLStat, version 2014.6.04.

3.3 RESULTS

3.3.1 Population structure of monitored plants

Population B had the largest aerial extent at more than 2.1ha while population C was confined to the smallest area of 0.37ha (Table 3.2). Overall, size class five (1501-2000cm^2) was underrepresented in all populations with population A having no plants in this size class. Less than 8% of the measured plants in population B and population C fell in size class one (<100cm^2) (Figure 3.9). The average canopy area (cm^2) of sampled plants were significantly different amongst the four populations with the largest plants (canopy area) recorded in population A (1231$\pm$221cm^2) and population D (1474$\pm$289cm^2; ANOVA; $F_{3,196} = 2.39$; $P = 0.0698$; n = 200). However, population B was not significantly different from populations A and D. Population C had the most stems/plant (5.7$\pm$0.69), although this was not significantly different to the number of stems/plant for population B (4.5$\pm$0.55). Plants growing in population D were significantly taller (48.8$\pm$2.25cm) than those growing in population B (40.7$\pm$2.52cm) and population C (34.6$\pm$2.30cm; ANOVA, $F_{3,196} = 6.71$, $P = 0.0002$; n = 200).

There was a weak positive significant relationship between canopy area and the height of the plants for all the populations combined ($r^2 = 0.235$; n = 200; P < 0.001).

Plants that grew in full sun were significantly shorter (35.4$\pm$1.70cm) than those that grew in full shade (44.6$\pm$2.43cm; ANOVA, $F_{2,197} = 8.46$; P = 0.0003; n = 200), although there was no difference between the height of plants that grew in full shade and semi shade (46.7$\pm$2.10cm). Plants that grew in full sun also produced significantly more stems (5.4$\pm$0.55) compared to those that grew in semi-shade (3.5$\pm$0.43) and full shade (3.0$\pm$0.40; ANOVA, $F_{2,197} = 8.56$; P < 0.0003; n = 200). The canopy area (cm^2) of plants that grew in full shade were significantly larger (1601$\pm$207cm^2) compared to those that grew in semi shade (1033$\pm$180 cm^2) and full sun (763$\pm$150 cm^2; ANOVA, $F_{2,197} = 4.22$; P = 0.0161; n = 200).
The percentage of sampled plants that grew in sun, semi shade and full shade was significantly different between the four populations ($\chi^2_6 = 16.89$; P = 0.0097; Figure 3.10).

Table 3.2: Aerial extent, population size, density of the population (number of plants/ha), height, number of stems and mean (±SE) canopy area per plant for the four *Adenium swazicum* populations, in South Africa. Different subscript letters indicate significant differences (P<0.05, LSD).

Population No	**A**	**B**	**C**	**D**
Land Use	Protected Area	Protected Area	Commercial Sugarcane Farm	Residential Area
Aerial extent of population (ha)	0.95	2.1	0.37	1.57
Size of population (no. of plants)	121	137	141	70
Density (number of plants/ha)	127	65	381	44
Canopy area (cm^2 per plant)	$1231\pm221_{ab}$	$896\pm142_{ab}$	$776\pm130_{b}$	$1474\pm289_{a}$
Number of stems/plant	$3.7\pm0.57_{b}$	$4.5\pm0.55_{ab}$	$5.7\pm0.69_{a}$	$3.0\pm0.35_{b}$
Plant height (cm)	$43.3\pm2.53_{ac}$	$34.6\pm1.87_{b}$	$40.7\pm1.73_{bc}$	$48.8\pm2.73_{a}$

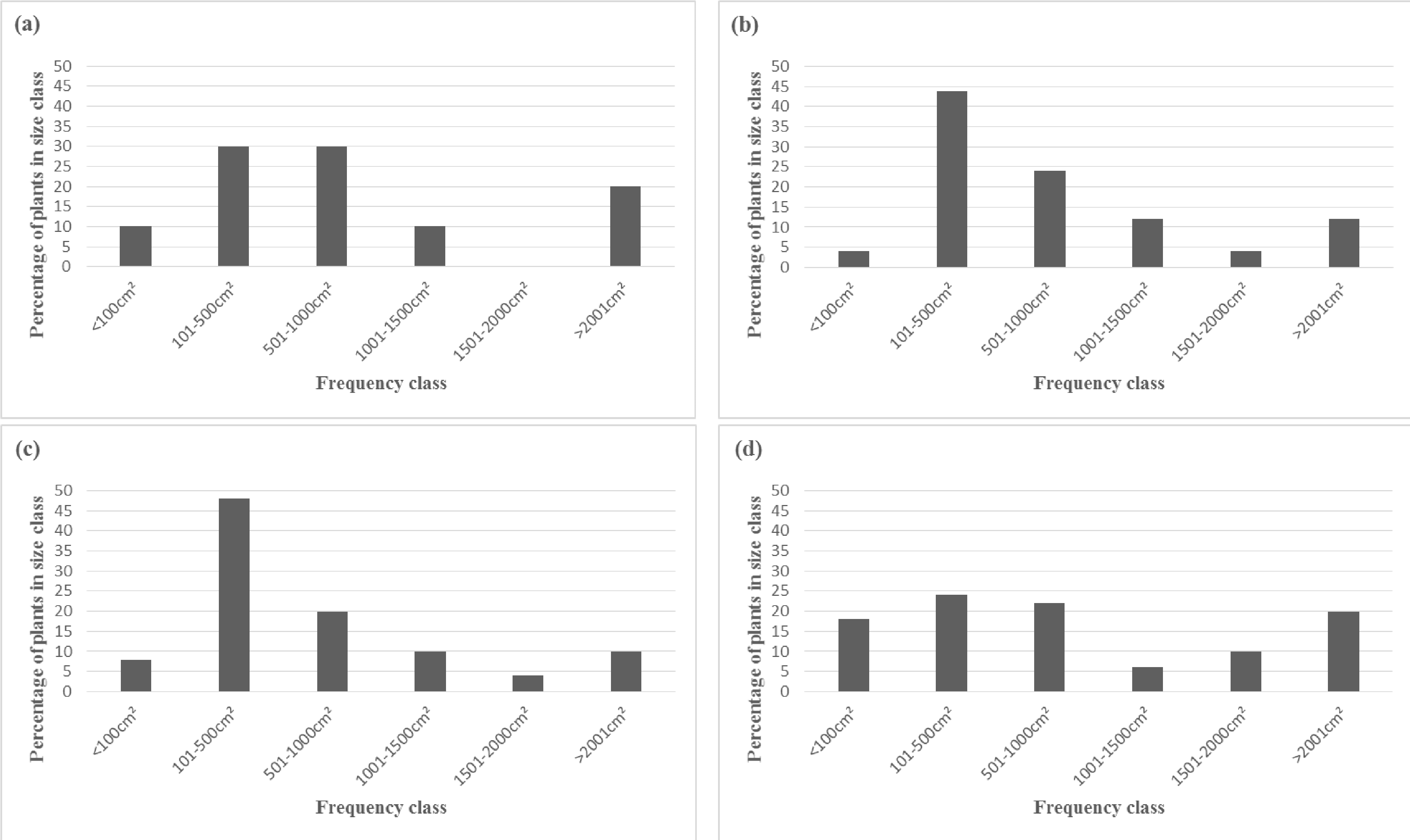


Figure 3.9: Frequency distribution of canopy areas (cm²) of *Adenium swazicum* for population A, B, C and D (n = 50 for each population). Population A (a) had no plants in frequency size class 1501-2000cm², while (b & c) most of the studied plants in population B and population C had canopy areas of 105-500cm². There was slightly more plants (d) in frequency size 101-500cm² than in the other frequency classes in population D.

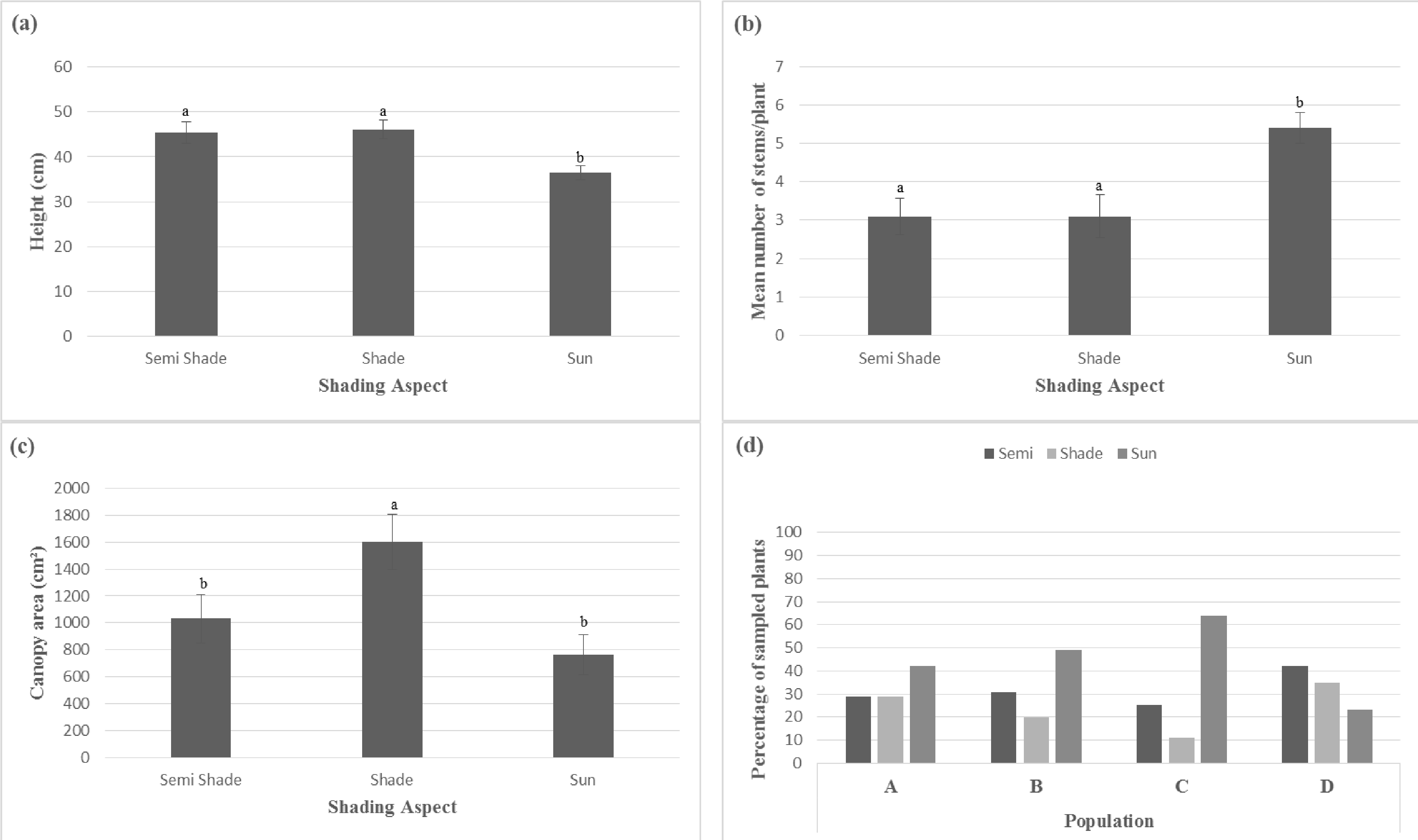


Figure 3.10: ***Adenium swazicum*** **plants (a) that grew in full sun were significantly shorter (35.1±1.74) compared to those in shade (44.7±2.08) and semi-shade (46.9±2.08), however (b) the plants that grew in full sun also produced significantly more stems (5.5±0.55) compared to those in semi shade (3.5±0.43) and shade (3.1±0.40). Plants (c) that grew in full shade had a larger canopy area (1601±207) compared to plants in semi shade (1033±180) and full sun (763±150) (c). The percentage (d) of sampled plants growing in each shading condition was different between the four populations. Different letters indicate significant differences (LSD, P < 0.0001).**

3.3.2 Vertebrate and invertebrate impact on leaves, flowers, branches and stems

Only one vertebrate species, Black Rhinoceros was confirmed to feed on *A. swazicum* plants in populations A, G, K and M in protected areas, as observed by Section Rangers D. English & R. Sowry in 2010, (pers. comm.). Three insect species were recorded feeding on the leaves, flowers, branches and/or stems of *A. swazicum* during the survey period:

1) <u>*Chrysolina (Naluhia) confluens* (Gerstaecker) (Coleoptera: Chrysomelidae) (Leaf Beetle).</u>

This species was recorded throughout the survey period and within all four populations. *Chrysolina* is a large genus of leaf beetles in the subfamily Chrysomelinae and all the species are phytophagous, feeding on specific food plants (Picker 2004). According to B. Grobbelaar (pers. comm. 2009), these beetles have been recorded feeding on *A. multiflorum* in Skukuza, KNP as well as *A. somalense* in Somalia, and no other host plant information had been published to date. During fieldwork conducted between 2009 and 2011 for this project, it was observed that *Chrysolina confluens* laid their eggs on or very close to *A. swazicum*, and the various instars as well as adults were observed feeding on the leaves and flowers of *A. swazicum* throughout summer in all four populations (Figure 3.11). *A. swazicum* continuously produces leaves and flowers throughout the growing season to replace damaged leaves (October to April) and it is therefore difficult to quantify herbivory.

Figure 3.11: *Chrysolina (Naluhia) confluens* (a) eggs recorded on *Adenium swazicum* leaves, (b) the first instar feeding on the flowers, (c) second instar feeding on the leaves, and (d) an adult feeding on flowers.

2) <u>*Phymateus morbillosus* (Linnaeus) and *Phymateus viridipes* (Stal) (Orthoptera: Pyrgomorphidae) (Milkweed Locust).</u>

Phymateus species (Milkweed Locusts) have bright warning colours and are capable of producing a foamy defensive secretion when threatened (Picker 2004). Most species in this genus feed on toxic plant species such as *Gomphocarpus fruticosus* (L), but will also feed on other plants such as *Solanum panduriforme* and various garden plants (Gade 2002; Picker 2004). *Phymateus morbillosus* is a reduced or flightless species in which the males are capable of short flights of less than 1 minute, while females are sedentary (Kutsch et al. 2002). These locusts usually congregate in large numbers while feeding (Gade 2002; Picker 2004). *Phymateus viridipes* is capable of strong flight, and have the ability to form swarms of many thousands to migrate long distances (Skaife 1979; Picker 2004). Both *Phymateus* species were recorded feeding on the leaves, bark and stems of *A. swazicum* during the summer months (October to April) between 2009 and 2011.

3) *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) (Cotton Bollworm).

Helicoverpa armigera eggs were collected in population O on the 6th of May 2010 and taken to the LNBG. Two larvae hatched on the 11th of May 2010 and on the 2nd of June 2010, the larvae buried into the soil and two adults emerged one month later on the 5th of July 2010 (Figure 3.12). *Helicoverpa armigera* is very common and widespread in Europe, Africa, Asia and Australia, and it is considered a generalist feeder using a wide range of plant species as host plants, namely *Datura stramonium*, *Pelargonium* spp. and *Solanum panduriforme* as well as crop species including cotton, chickpeas, maize and tobacco (Razmjou et al. 2014). Adult *Helicoverpa armigera* was however not observed on the flowers of *A. swazicum* during any of the pollinator observations and it is therefore not likely that this species plays an important role in the pollination of *A. swazicum* and it appears that *H. armigera* only uses *A. swazicum* as a host plant for its larvae.

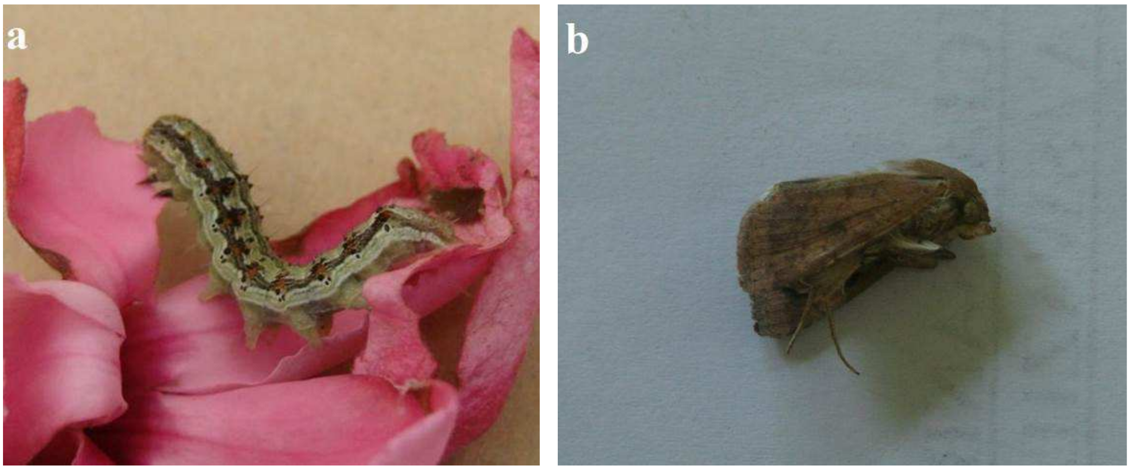


Figure 3.12: The moth *Helicoverpa armigera* (a) found feeding on wild populations of *Adenium swazicum*, (b) was raised to maturity for identification.

Population A had the lowest percentage herbivore damage in 2010 with only 26% of the sampled plants affected by herbivores. Populations B, C and D all had a high incidence of herbivory with 68%, 66% and 70% respectively (n = 50 for each population; Figure 3.13a). When plants without damage were excluded, the severity of herbivore damage (i.e. percentage of leaves, flowers and bark consumed) was significantly different between the sampled plants in population A (21.1$\pm$8.26%) and population B (40.4$\pm$5.03; ANOVA; $F_{3,110} = 1.74$; $P < 0.0001$; n = 114; Figure 3.13b).

Insects accounted for 99.7% (n = 114) of the recorded herbivory (leaves, flowers and bark) in the representative populations, with vertebrate damage limited to population B. Furthermore, of all the plants that were impacted on by herbivores, 40% (n = 14) had damage to the branches/stems only, while 27% had herbivory to their leaves only with a further 9% having damage to flowers or flower buds but not to the leaves or branches/stems (Figure 3.13c). The percentage of sampled plants with leaf damage was the highest in population D (62%) while 52% of the sampled plants in population B had herbivore damage to the stems and branches (Figure 3.13d). There was a negative relationship between percentage herbivore damage and the number of flowers produced per plant ($r^2 = -0.365$; $p < 0.0001$; $n = 114$). Plants that had no insect damage produced significantly more flowers/plant (45.6 ± 5.4) compared to plants that had insect damage (13.5 ± 4.63; ANOVA; $F_{1,198} = 20.81$; $p < 0.0001$; $n = 200$), in fact over three times more flowers. The percentage herbivore damage per plant was significantly higher for plants that grew in shade ($42.5 \pm 5.9\%$) compared to those that grew in semi shade ($27 \pm 4.6\%$) and sun ($29 \pm 4.6\%$; ANOVA; $F_{2,111} = 2.313$; $P < 0.0001$; $n = 114$).

Ex situ experiments to simulate herbivory

The removal of bark from the main stem and branches did not have an effect on *A. swazicum* as damaged parts were sealed with a clear latex within 24 hours. Even in experiments in which stems were ring-barked, *A. swazicum* continued to grow (Figure 3.14). The destruction of branches and/or the main stem caused significant coppice regrowth with up to seven new stems produced by January 2010. In both experiments where the stems were cut down, locusts ate the new growth that was recorded in October 2009. Despite this, both plants resprouted for a second time, with plant Ad14 producing seven stems by January 2014 (Figure 3.15). However, in cases where the stem was cut down, and /or all of the branches were removed; the plants recovered but did not produce any flowers during the same season (Table 3.3). These results were also confirmed by *in situ* observations where plants were often heavily impacted by various insect species, although it seems like these impacts did prevent the production of leaves. This indicates that *A. swazicum* has a high tolerance to disturbance by herbivores and will resprout from the underground tuber.

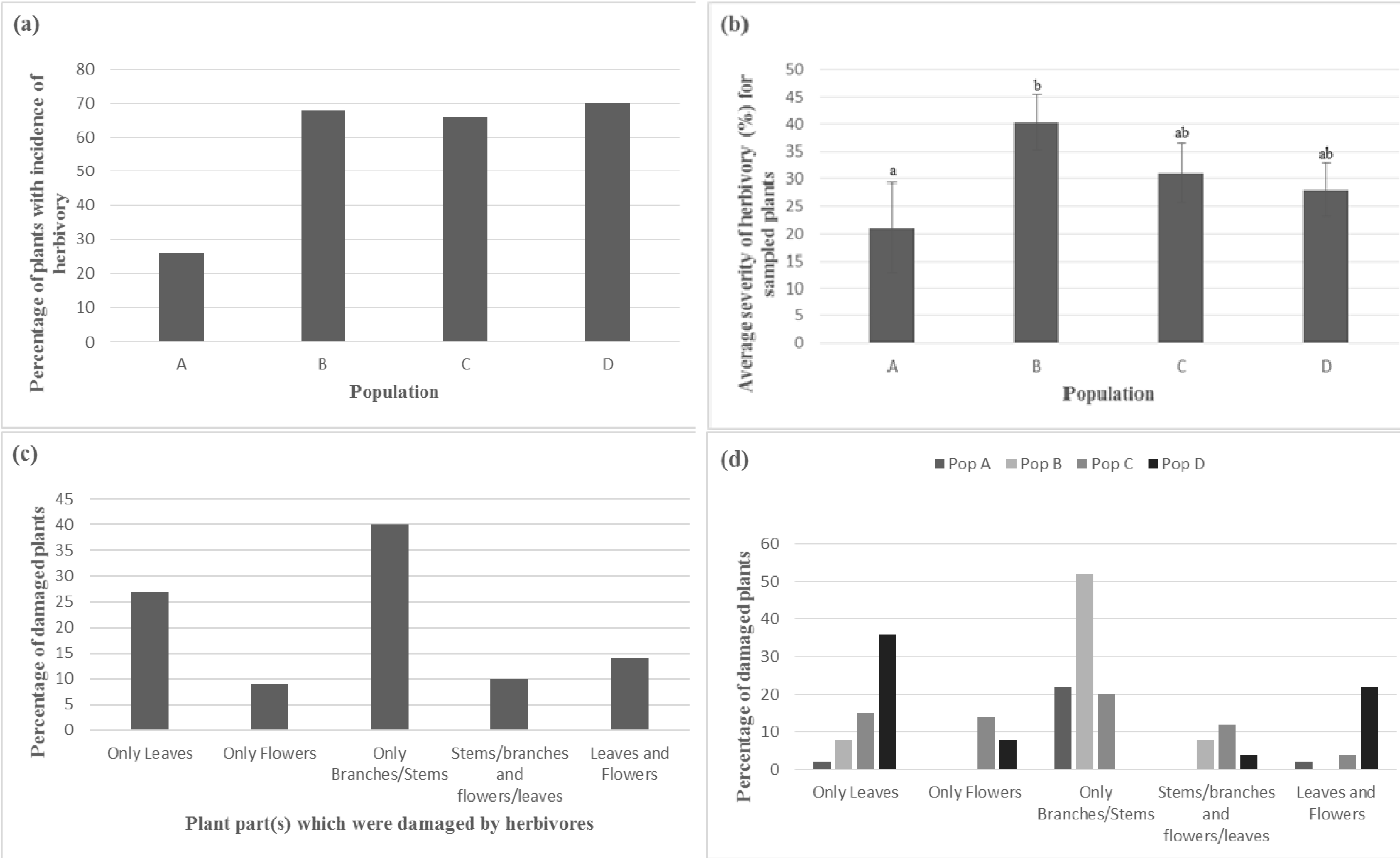


Figure 3.13: Bar charts showing (a) the percentage of sampled plants of *Adenium swazicum*, which had incidence of herbivory in 2010, as well as (b) the mean (+ S.E.) severity (percentage of foliage consumed) of herbivory of the monitored plants. Of the monitored plants (c) which were damaged by herbivores in 2010, most had damage to their branches/stems only, while (d) population B had the highest percentage of sampled plants with damage to the stems and branches (d). Values with different letters are significantly different between populations (LSD, P<0.05).

Table 3.3: A summary of *ex situ* experiments conducted in September 2009 to determine the effect that herbivores are likely to have on *Adenium swazicum*.

Plant id.	Experiment description	Two weeks after experiment (14 September 2009)	Five weeks post experiment (8 October 2009)	Conclusion of experiment (January 2010)
Ad07 & Ad08	Bark removed from less than 50% of the stem	Damaged areas sealed	Damage appears not to have an effect on the plant	Plant fully recovered and flowering
Ad09	Stem ring barked (all the bark removed around the stem)	Damaged areas sealed	Leaves produced but smaller when compared to new leaves from other plants	Plant produced mature leaves but no flowers
Ad10 & Ad11	A third of the branches were debarked	Damaged areas sealed	Damage appears not to have an effect on the plant	Plant fully recovered and flowering
Ad12	90% of the bark from one branch removed while the second branch was not damaged	Damaged areas sealed	Damage appears not to have an effect on the plant	Plant fully recovered and flowering
Ad13	The stem was cut off at ground level	Damaged stem sealed and firm, but no growth visible	Four small stems resprout on the cut surface. Locusts destroyed all the new growth again	A single stem was produced on the original cut area, no flowers
Ad14	The stem was cut off at ground level	Damaged stem sealed and firm, but no growth visible	Four small stems resprout on the cut surface. Locusts destroyed all the new growth again	Seven stems produced around the original stem, no flowers
Ad15	All the branches were cut off against the main stem	Cut surfaces sealed	New growth visible around the each of the cut areas	Plant produced ten new stems from areas where original branches were removed. No flowers.
Ad16, Ad17 & Ad18	All the branches were cut off against the main stem	Cut surfaces sealed	New growth visible around the each of the cut areas	Four new stems produced from cut surfaces. Flowers

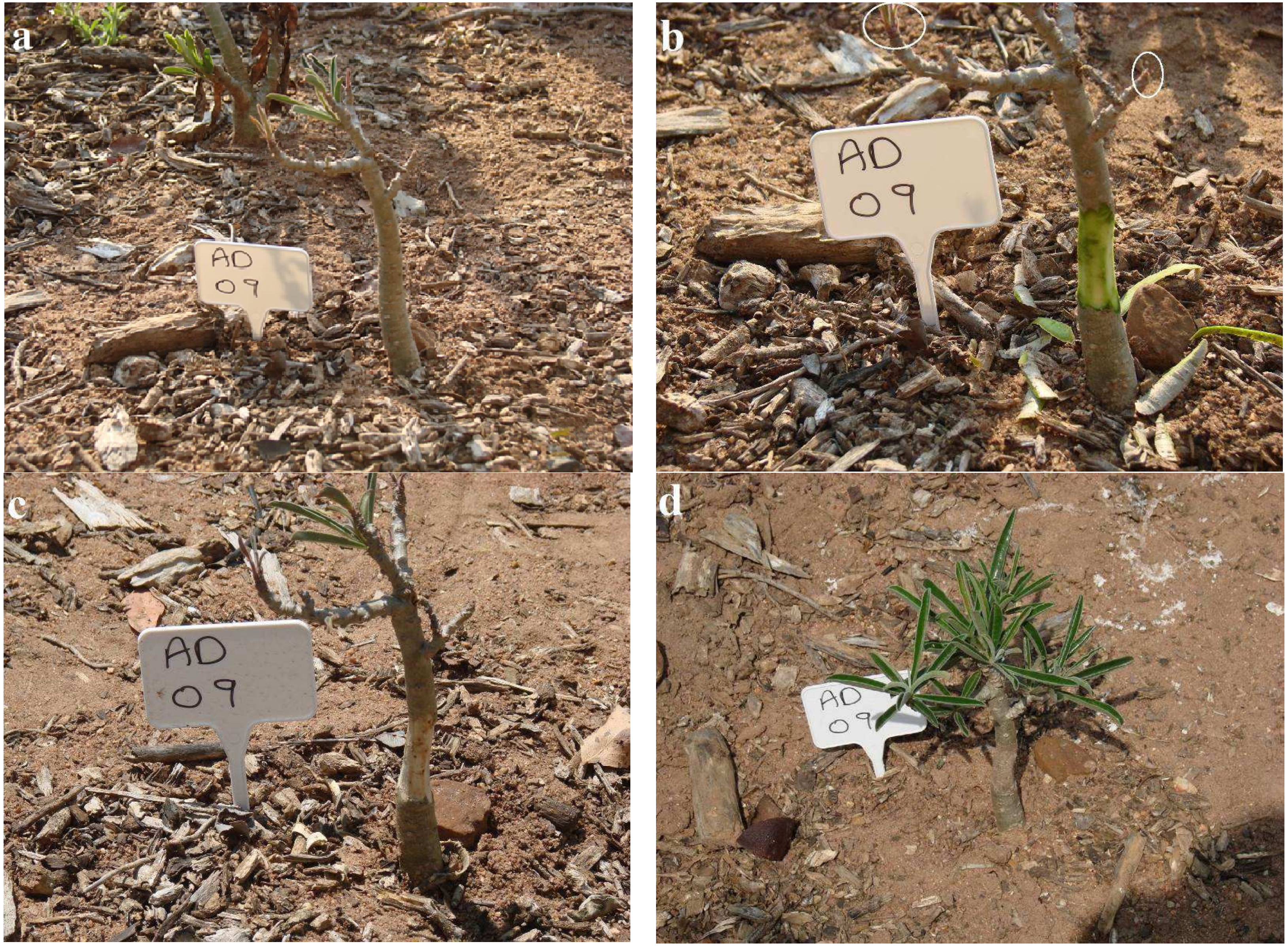


Figure 3.14: The figure illustrates that (a & b) if bark is removed from adult single stemmed plants to simulate herbivory, (c) damaged areas sealed within 24 hours and (d) the plants produced leaves in the same season.

Figure 3.15: The figure illustrates the ability of adult *Adenium swazicum* plants with (a) all the aboveground parts removed to (b) regrow within the same season.

3.3.3 The impact of herbivores on seedling emergence and establishment

Observed germination percentage, or at least emergence of seedlings above the soil surface, in the open plot, which was not protected from herbivores was the lowest (6%) with only 2% of the emerged seedlings surviving the first six months. Seedlings in the plot which was protected from all herbivores had the highest germination percentage (58%) with all the emerged seedlings surviving the initial six months. The germination percentage of seedlings in the plot which excluded vertebrates but not invertebrates, was 12% with 9% surviving the first six months (Figure 3.16). A contingency table χ^2 test revealed that there was no association between seedling emergence and survival in the three plots (open access, excluding vertebrates and excluding invertebrates and vertebrates) (χ^2_2= 2.10; p = 0.351) while there was a strong association between the percentage of seedlings that emerged and those that did not emerge (χ^2_2= 77.77; p <0.001) in the three plots. Since seedling survival was very low (2% and 9%) in plots which were open to invertebrates, it is likely that invertebrates have a significant impact on the establishment of *A. swazicum* seedlings in the wild. Germination might have been underestimated since newly germinated seedlings might have dried up or died prior to a monitoring visit. However, the germination observations corresponds with herbivory records of mature plants in which invertebrate species such as *Chrysolina (Naluhia) confluens* (Leaf Beetle), *Phymateus morbillosus* and *P. viridipes* (Common Milkweed Locust) were widely recorded. However, the exclusion of herbivores also resulted in other herbaceous plants around the seedlings growing more vigorously which are likely to provide better microclimates (moisture, temperature and litter) and the higher germination and seedling survival rate could thus be attributed to the effect of nurse plants rather than the effect of herbivores.

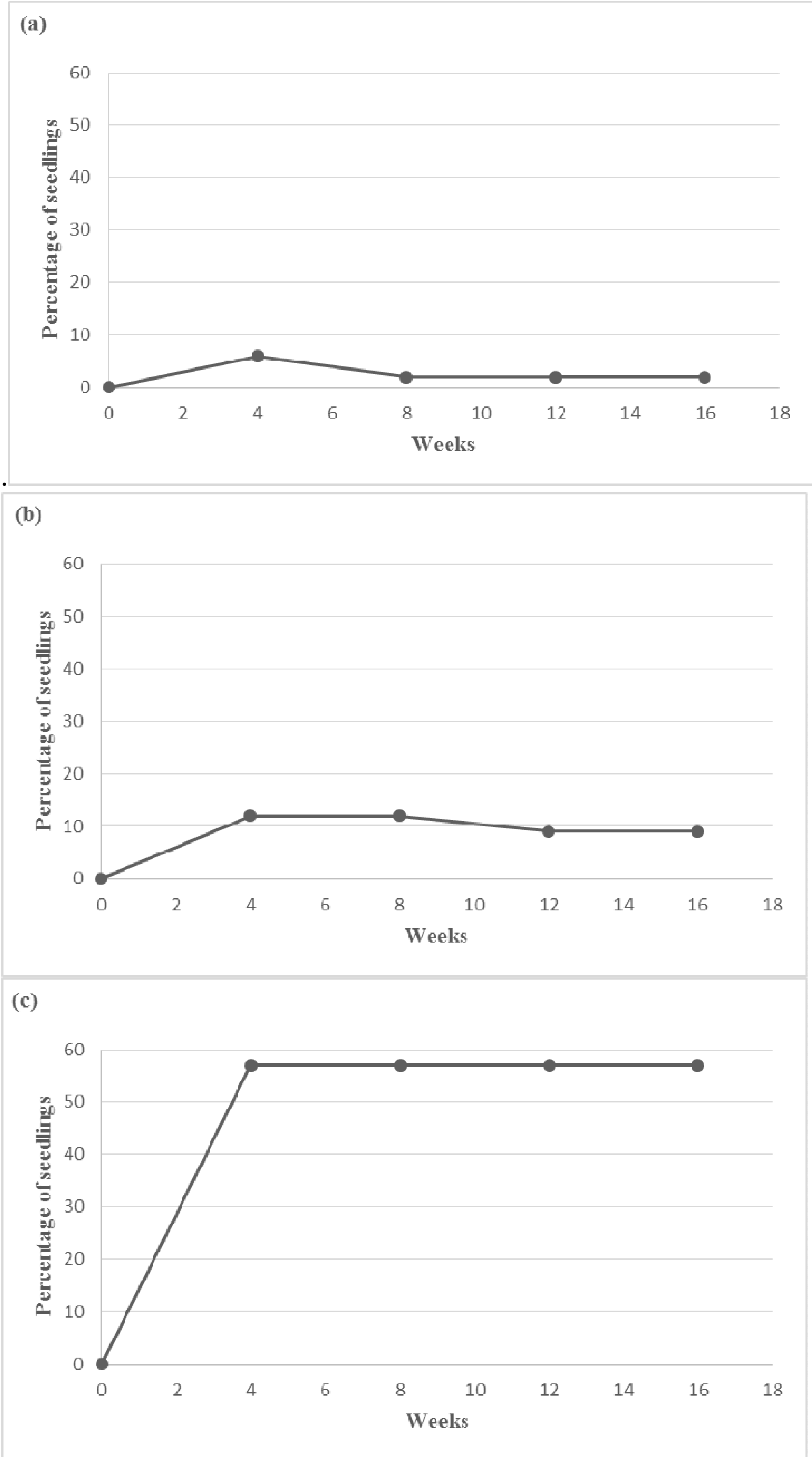


Figure 3.16: Number of *Adenium swazicum* seedlings emerging and surviving over time in the (a) open plot, (b) the plot which excluded only vertebrates as well as (c) the plot which excluded vertebrates as well as invertebrates.

3.3.4 Seed and follicle predation

Two invertebrate species were recorded on the follicles of *A. swazicum* during the fruiting period (between 2009 and 2010). *Leptocoris hexophthalma* (Thunberg) (Rhopalidae: Hemiptera) was recorded in population A in 2009 and 2010. *Leptocoris hexaphtalma* was observed on mature follicles of *A. swazicum* where the adults as well as various instars were feeding on the seed, often causing the follicles to rot before the seed was released (Figure 3.17). In October 2010, 91% of the follicles produced in population A (n = 221), 50% of follicles in population B (n = 28) and 100% of follicles in population D (n = 6) were impacted on by follicle and/or seed predators (Figure 3.18).

Figure 3.17: *Leptocoris hexaphtalma* (a) eggs and larvae, and (b) adult on *Adenium swazicum* follicles in population A in October 2010.

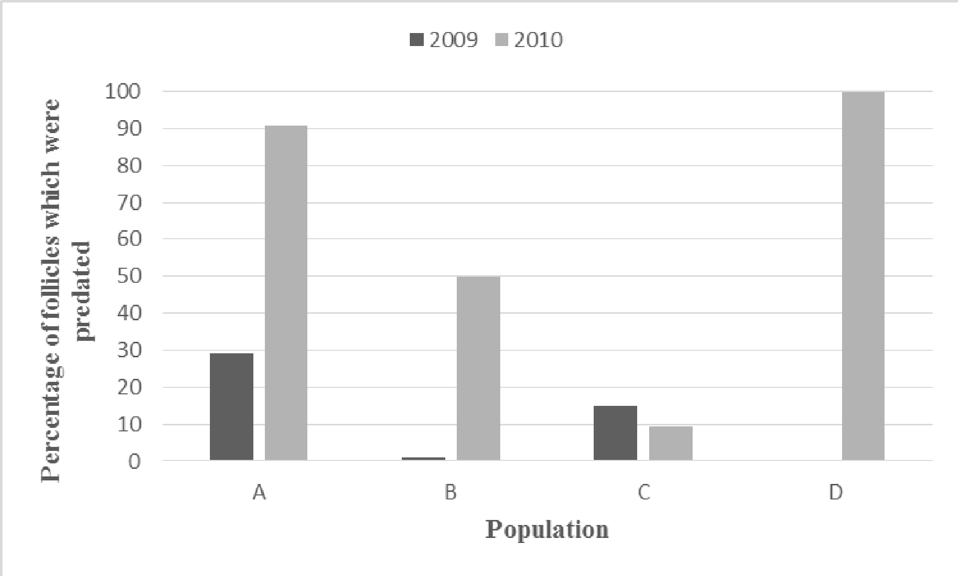


Figure 3.18: Percentage of *Adenium swazicum* follicles predated in all the four populations (i.e. A, B, C & D) in October 2009 and October 2010.

The genus *Dacus* consists of fruit flies, which mimic wasps in appearance and this often leads to the misidentification of species in this genus. The larvae have fluid-feeding mouthparts and often inhabit the bacterial soup in the decaying areas of infested fruit while *Dacus* species pupate within the fruiting body of *Asclepiadaceae* (Drew et al. 2004). These records were consistent with observations in *A. swazicum* with pupae found in the decayed follicles (Figure 3.19).

Figure 3.19: The figure shows, (a) newly emerged adults on follicles, and (b) an infected follicle of *Adenium swazicum* with *Dacus frontalis* in Population A in October 2010, in South Africa.

Extensive infestations by *Dacus frontalis* were recorded in 2010 in population A, and together with *Leptocoris hexaphtalma*, they destroyed 91% of the follicles and seed in population A. Although seed predation could be considered severe for *A. swazicum*, it did not affect all the populations at the same time. Significantly less viable seed/sampled plant was released in 2009 in population A (5.28$\pm$3.2) compared to population B (70.2$\pm$20.1) and population C (45.3$\pm$12.25; ANOVA; $F_{2,147} = 5.69$; $P = 0.0042$; $n = 150$). Despite lower seed viability for population C (69.9%) compared to population A and B (85.8%) (see Chapter 5), population C still released significantly more viable seed in 2010 (41.2$\pm$11.0) compared to population A (14.3$\pm$5.4) and population B (10.6$\pm$7.8; ANOVA; $F_{2,147} = 3.99$, $P = 0.0204$; $n = 150$; Table 3.4). Population D was not studied in 2009 and since all the follicles were predated before it reached maturity, no seed was produced in 2010 and it was therefore excluded from further analysis.

Table 3.4: One-way ANOVA results of potential seed production (including predated follicles), actual seed production (excluding predated follicles) and number of viable seed released from sampled plants (n=50 for each population) 2009 & 2010. Due to lack of seed production, population D was excluded from the analyses. Different subscript letters indicate significant differences (LSD, P<0.05).

Attribute	Population A	Population B	Population C	F	P	Df
2009						
Potential seed production	$29.29 \pm 10.0_{a}$	$82.72 \pm 23.6_{b}$	$29.92 \pm 9.7_{a}$	3.717	= 0.027	2, 147
Number of seed released	$6.2 \pm 26.7_{a}$	$81.8 \pm 165.8_{b}$	$65.1 \pm 124.5_{b}$	5.42	< 0.005	2,147
Number of viable seed released	$5.28 \pm 3.2_{a}$	$70.2 \pm 20.1_{b}$	$45.3 \pm 12.3_{b}$	5.69	=0.0042	2, 147
2010						
Potential seed production	$194 \pm 41.6_{a}$	$24.6 \pm 13.8_{b}$	$65.1 \pm 17.6_{b}$	10.52	< 0.0001	2,147
Number of seed released	$22.0 \pm 5.01_{a}$	$12.32 \pm 9.1_{a}$	$58.9 \pm 15.7_{b}$	7.15	< 0.0001	2,147
Number of viable seed released	$14.3 \pm 5.4_{a}$	$10.6 \pm 7.8_{a}$	$41.2 \pm 11.0_{b}$	3.99	= 0.0204	2,147

3.3.5 The impact of fire on *Adenium swazicum*

The plants used in experiments to determine the effect of fire on *Adenium swazicum*, had single stems and were firm, however after the fire, the stems were soft and appeared to have been destroyed by topkill (i.e. complete destruction of above ground parts). Rain experienced in October 2009 (30 days after the fire), stimulated all six plants to resprout from the underground tuber. By January 2010, the plants had all produced more than 10 stems/plant and they all flowered (Figure 3.20).

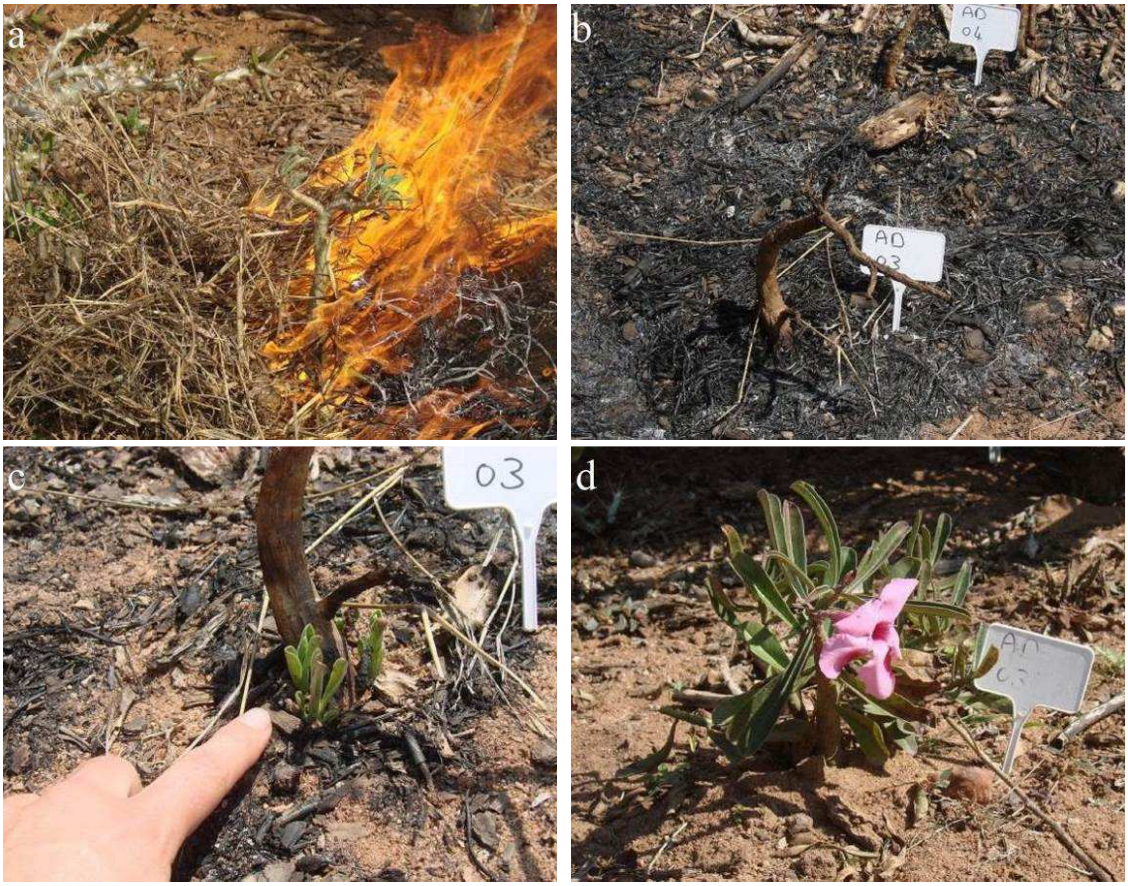


Figure 3.20: The figure illustrates (a) *ex situ* fire experiments with (b) the main stem soft and apparently dead, with (c) regrowth in October to (d) burnt plants flowering in January 2010.

Experiments to determine the effect of disturbances such as herbivory and fire on *A. swazicum* revealed that the species has a high tolerance to disturbance, which includes the destruction of all above ground parts and they will regrow from the underground tuber. Underground tuber formation in the four excavated plants indicated that the above ground parts (stem and leaves) develop during the first 12 months, after which there is significant growth in the underground tuber while the above ground parts remain generally the same (Figure 3.21).

Figure 3.21: The figure illustrates stem and tuber size in *ex situ* plants at (a) three and twelve months, (b) twenty four and thirty-six moths, and (c) all four age categories.

3.4 DISCUSSION

Plants that grew in full sun were significantly shorter, produced more stems and were the least affected by herbivores compared to plants that grew in semi shade and full shade. While vertebrates had very little impact on *A. swazicum*, invertebrates were recorded feeding on the leaves, flowers and branches/stems in all four populations and had a negative impact on flower production. Fire had no detectable effect on the survival of adult plants in *ex situ* experiments and although the effect of fire on seedlings was not determined, it is unlikely that seedlings will be able to survive fires. Follicle and/or seed predation had a considerable impact on *A. swazicum*.

Studies into the effect of herbivory on the reproduction capability of endangered plants had contrasting results which included no effect on reproductive output of *Liatris ohlingerae* plants damaged by invertebrates (Kettingrang et al. 2009) to a significant reduction in the number of flowers recorded on plants which have sustained herbivore damage (Ehrlen 1995; Pfab & Witkowski 1999a). Maron & Crone (2006) found that insect herbivory had a strong impact on population dynamics of some plant species. For *A. swazicum* it was found that insect herbivory had a significant impact on the production of flowers, with an increase in herbivory resulting in a threefold reduction in flower production. In addition to this, plants that grew in full shade had the highest percentage of herbivore damage compared to plants that grew in full sun.

Resprouting is a mechanism that allows plants to regenerate from taproots or lignotubers after the destruction of aboveground biomass and thereby allowing these

species to persist in ecosystems with recurrent disturbances (Pate et al. 1990; Morena & Oechel 1991; Bond & Midgley 2001). Vesk and Westoby (2004) proposed the use of a semi-quantitative classification of no, weak and strong resprouters. *Ex situ* disturbance experiments revealed that adult *A. swazicum* plants are strong resprouters, even if new resprouts are destroyed by herbivores in the same season. Although these experiments determined the effect of fire and/or herbivores on adult *A. swazicum* plants, it is likely that seedlings and/or juveniles younger than 24 months will be destroyed by fire and/or herbivory since the underground tuber is not yet well developed at this stage and plants would therefore be unlikely to have sufficient resources to resprout. Although plants with underground storage organs/lignotubers usually provide these species with an ability to withstand intense herbivory and fire, it has been found that an increase in fire intensity decreased the survivorship or at least, delayed resprouting (Moreno & Oechel 1990; Lesica 1999). It is possible that *A. swazicum* is restricted to sodic sites due to the higher intensity fires occurring in the upland and riparian zones surrounding sodic sites and that these higher intensity fires result in the mortality of seedlings, juveniles or even adult plants. In other words, the sparsely vegetated sodic sites provide a fire refuge similar to rocky hillslopes.
When disturbed *Leptocoris* species release a strong odour and this coupled with them being unpalatable ensures that they are not predated on and therefore often congregate in large numbers (Picker et al. 2004). Dense infestations of *L. hexaphtalma* were also recorded in *Mangifera indica* (Mango) and *Litchi chinensis* (Lychee) orchards in the Malelane region during 2010 (S. Schoeman 2010, pers. comm.). *Dacus* species exhibit characteristic behaviour where the male utilizes the larval host plants as

species-specific waiting places and territories for courtship and mating (Drew et al. 2004).

Since *A. swazicum* is a strong resprouter, the presence of multiple stems on nearly all the plants in populations A-D indicate extensive disturbance and/or destruction of aboveground parts due to herbivores, fire and/or human activities. Plants that grew in full sun produced significantly more stems compared to those that grew in semi-shade and full shade. In addition to this, plants in population C had the highest mean number of stems/plant and since this population is surrounded by sugarcane fields which are burned before harvesting, it is likely that the higher number of stems recorded in this population is a result of more frequent fires than experienced by the other populations.

Seedling emergence and survival in the wild was generally considered to be very low with less than 10% of the seedlings surviving in the plot which was open as well as the plot which allowed access to invertebrates but excluded vertebrates. However, 58% of the seedlings emerged and established in the plot which excluded all herbivores indicating that seedling mortality is likely due to invertebrate species such as *Phymateus morbillosus* and *P. viridipes* which were frequently recorded feeding on the bark and stems of adult *A. swazicum* plants. However, since the open plot was located at a different site (in population A) than the other two plots (Nkhuhlu full exclosure), the possibility that the low seedling emergence and establishment associated with the open plot could be due to a site effect cannot be ruled out, although it is considered unlikely since seedlings were very seldom observed in the wild. Based on these results, it is likely that the dispersal of *A. swazicum* is limited by invertebrate herbivory, availability of suitable microsites for germination and the effect of fire on seedlings and/or juvenile plants.

Studies into plant reproduction have revealed that seed production can be controlled throughout the reproduction period by abortion of inflorescences and immature seeds (Witkowski 1990). Although some plant species are likely to tolerate considerable damage by herbivores, it is important to determine the effects of insects on the entire population, especially for rare/threatened species (White & Robertson 2009; Ancheta & Heard 2011). This study found that herbivores had a considerable impact on *A. swazicum* between 2009 and 2011, with plants damaged by herbivores producing significantly less flowers. In addition to this, seed predators destroyed 91% of the follicles produced in population A in 2010 while the combined seed production/sampled plant in populations A and B, which were located in a protected area, were significantly less than seed production/plant in population C which was not within a protected area. Common pest species that affect sugarcane in South Africa include insect orders such as Hemiptera (Joshi & Viraktamath 2004; Thompson 2004), Homoptera (Singh et al. 2004) and Lepidoptera (Conlong 1990; Keeping & Meyer 2002) and these insects are commonly controlled with an aerial application of contact pesticides such as creoline and glufosinate ammonium (Leibbrant & Snyman 2003). It is possible that the application of these pesticides have impacted on *A. swazicum* seed predators (Hemiptera) resulting in lower infestations in population C which was located in a sugarcane matrix land where the application of non-specific pesticides is common and therefore might have affected pest species associated with *A. swazicum*. Seed predation might vary greatly between populations (Menges et al. 1986; Louda 2001) and this was found in *A. swazicum* populations during the study period. Although seed predation was severe for certain populations, this impact is likely to be less when one considers the entire population over a longer term.

4 CHAPTER 4: REPRODUCTIVE ECOLOGY OF THE CRITICALLY ENDANGERED SUCCULENT, *ADENIUM SWAZICUM* STAPF

Abstract

The regeneration of *Adenium swazicum* has not been studied with the number of flowers and seed produced not known. Pollination studies have not been conducted for any *Adenium* species, although it has been suggested that it can only be pollinated by an insect with a proboscis length of at least 15mm due to the anatomy of the flower. The aspects of the reproductive biology of *A. swazicum* were studied by comparing the number of flowers and follicles produced as well as fruit and seed set and seed production/plant in four representative populations (i.e. A, B, C & D). The colour, shape and size of *A. swazicum* flowers were described based on flowers which were produced on *ex sit* and *in situ* populations. Observations of potential pollinators were also made. Despite large floral displays, *A. swazicum* attracted very few insects with the only potential pollinator observed being a *Sphingidae* Linnaeus 1758 (Hawk Moth) which was observed after dusk. Plants that grew in full sun produced significantly more flowers (24$\pm$2.81) compared to those that grew in semi shade (9.3$\pm$3.36) and full shade (10.4$\pm$3.88; $P < 0.001$). In 2010, sampled plants in population B, which was located in a formally protected area, had the lowest percentage of flowering plants (32%) but produced significantly more follicles/flowering plant ($X^2_3 = 25.69$; $P < 0.001$) than the other three populations, in which 72-92% of plants flowered. In all four populations, less than 12% of the flowering plants produced follicles, indicating that *A. swazicum* has a low rate of seed production. The average number of seed per follicles was 44.1$\pm$13.2 (mean $\pm$ S.E.).

At a population level, population C produced 2363 viable seeds while the other populations produced 257 (A), 242 (B) and 0 (D) in 2010. The low (and highly variable) number of viable seed produced, as well as low seedling emergence and survival in the wild indicates that *A. swazicum* has a low reproduction success, which could increase its vulnerability to local extinctions.

Keywords: Apocynaceae, *Dacus frontalis*, flowers, follicles, hawk moth, pollinators, seed abortion, seed predation, seed production.

4.1 INTRODUCTION

In order to understand community processes such as regeneration, establishment, succession, species survival strategies and causes of rarity it is important to have an understanding of a plant's reproductive ecology (Kaye 1999; Cousins et al. 2013b). Critical determinants of a plant's reproductive success, which influences its distribution and populations size, are the species ability to produce seed and effectively disperse viable seed (Steffan-Dewenter et al. 2012). For a species to persist in the long term, effective regeneration through seed or vegetative reproduction is essential (Weiersbye & Witkowski 1998; Kaye 1999; Helm et al. 2011). Although it is known that insects are the main floral visitors of Apocynaceae (Rowley 1980; Fallen 1986, Endress et al. 2007), information on plant-pollinator relationships and field observations of flower visitors are scarce. Large Apocynaceae flowers may have evolved a pollination mechanism that attracts insects with long and strong mouthparts to penetrate the flowers and obtain the nectar which is located at the base of the flower (Rowley 1999; Araujo et al. 2014).

Self-incompatible species might experience difficulty in setting seed, especially in small populations (Lamont et al. 1993; Byers 1995). Although no studies have been done on the reproduction of any species in the genus *Adenium,* low fruit production coupled with high fruit abortion has been reported for species in the family Apocynaceae, which includes the genus *Adenium* (Willson & Rathke 1974; Willson & Price 1977; Darrault & Schlindwein 2005).

The aspects of the reproductive biology of *Adenium swazicum* were studied through undertaking the following objectives:

- Description of the flower colour, shape and size;
- Determine the number of flowers produced in four representative populations;
- Observations of potential pollinators;
- Determine follicle and seed production/plant and per population in four representative populations;
- Determine fruit and seed set of *A. swazicum;*
- Determine the viable seed set for *A. swazicum;* and
- Determine total viable seed production at a population level for 2010.

4.2 MATERIALS AND METHODS

4.2.1 Flower Characteristics

Flower descriptions (i.e. colour, size and shape) were based on flowers which were photographed *in situ* as well as the *ex situ* collection at the LNBG. Flower shape, size and colour were recorded during the flowering period (October to April) between 2009 and 2011. Floral structure descriptions were based on Rowley (1980) and supplemented by personal observations which included approximately 90 photographs. Individual flower development was recorded on a daily basis in *ex situ* collections at LNBG in February 2011.

4.2.2 Flower production

In order to determine flower production of *A. swazicum*, data were collected from four populations (i.e. A, B, C & D) in January 2010 (this study was only initiated in March 2009 which was at the end of the flowering season for *A. swazicum*). Fifty individual plants in each population were randomly selected and marked with aluminium tags during initial fieldwork conducted in March 2009. The number of plants that flowered, the number of flowers per plant, plant size as well as the degree of shading experienced by each flowering plant were recorded (January 2010). Plant size, specifically canopy area, of each flowering plant was determined and calculated as follow (see Knowles & Witkowski 2000) in January 2010:

$$\text{Canopy area} = \prod \left(\frac{D1}{2}\right) \times \left(\frac{D2}{2}\right)$$

where (D1) is the maximum canopy diameter, (D2) is the diameter at right angle to the maximum. Shading from other plants, including grass, shrubs and trees were divided into three classes, namely full sun, semi-shade or shade.

4.2.3 Observation of potential pollinators

Pollinator observations were conducted in all four populations during the flowering period (October to April) for *A. swazicum* between 2009 and 2011. Although only populations A, B, C and D were studied, population Q provided a safe environment in which dusk studies could be conducted, so it was also included. Pollination observations were conducted at 30-minute intervals during early morning (07:00-08:00), mid-morning (10:00-11:00), midday (12:00-13:00), late afternoon (16:00-

17:00) and dusk (18:00-19:30). Observations conducted in the late afternoon and dusk were conducted by six people in population A (February 2010 & March 2011) and population Q (November 2010), for 90 minutes at a time (total observation time = 870min or 14 hours). Pollinator observations in populations B, C and D were limited to diurnal studies (i.e. early morning, mid-morning, midday and late afternoon). Where possible insects landing on *A. swazicum* flowers were caught with a standard butterfly net and placed in glass bottles for identification. To determine the presence of floral guides, ten potted plants from the *ex situ* collection at LNBG were placed in a dark garage with no windows and inspected with a 21 LED Ultra-violet (UV) torch in March 2011.

4.2.4 Follicle and Seed production

Follicle and seed production were assessed during the fruiting period of *A. swazicum* (July-October) in 2009 and 2010 for population A, B and C. Population D was only discovered in January 2010, therefore follicle and seed production for this population was only assessed for July-October 2010. *Adenium swazicum* seeds are wind dispersed. To ensure that all the seeds in the follicles were collected; nylon stockings were placed over each follicle with the ends secured with cable ties. Nylon stockings allow sunlight and air to reach the follicle and therefore are unlikely to have a significant impact on the development of the follicle (Figure 4.1). To determine the average number of seeds per follicle, nineteen follicles in total were collected in October 2009 from population A (n = 3), population B (n = 14) and population C (n = 2). Since limited follicles were produced in each population, the number of follicles collected from each population varied. Potential number of seed produced by sampled plants in each population was calculated by multiplying the total number of follicles (including predated follicles) on sampled plants by the average number of seed per follicle. Follicle set was determined as the percentage of flowers that produced follicles, while seed set was the average number of seeds per follicle. Viable seed production (sampled plants and total population) was calculated by multiplying the average number of seeds produced (intact follicles only), by the percentage seed viability for each population determined through Tetrazolium staining protocol tests (see Chapter 5). Since seeds from population B were not available for tetrazolium testing, seed viability results from population A, which was located 11km west population B, were applied to population B.   A flow diagram to indicate the steps followed to determine follicle and seed production are given in Figure 4.2.

Figure 4.1: Developing *Adenium swazicum* follicles covered with nylon stockings, (a) to allow follicles development to maturity, and (b) to prevent loss of seeds on release.

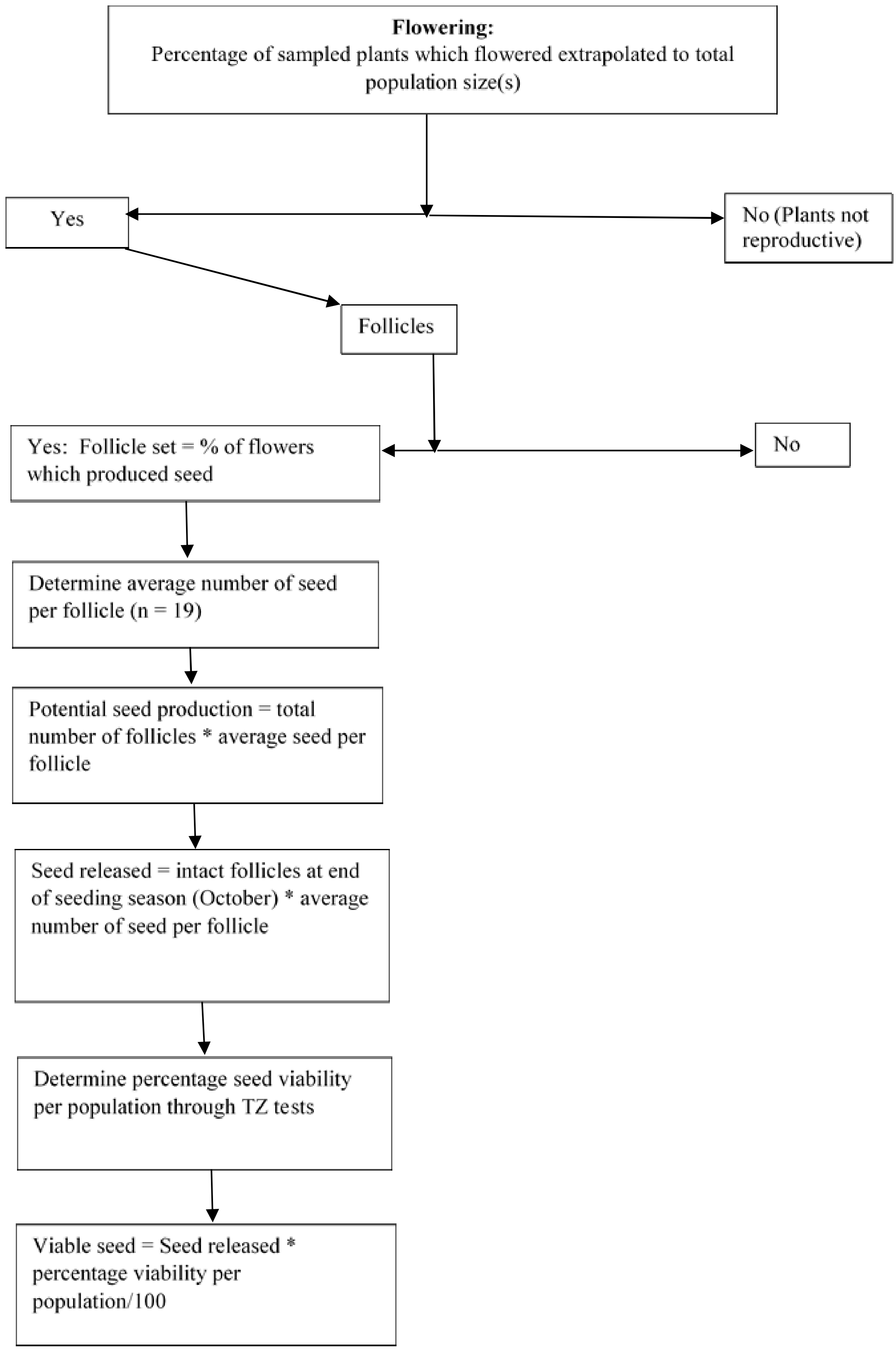


Figure 4.2: Flow diagram of methods used to determine follicle production and seed set of sampled plants and total population(s) for *Adenium swazicum* in South Africa.

4.2.5 Statistical analysis

Statistical analyses were conducted using Analyse-it for Microsoft Excel (version 2.30) as well as XLStat (version 2014.6.04). Regression analysis was used to explore the relationship between canopy area/height of plants and number of flowers produced. One-way ANOVA followed by Fisher's LSD was used to compare the number of flowers produced on plants that grew in full sun, semi shade and full shade. A Chi square test was used to compare the percentage of flowering plants in the four populations that produced intact follicles. The number of seeds per follicle was counted for nineteen follicles to determine the average (±S.E., and range) number of seeds per follicle. An estimate of the potential number of seeds produced by the sampled plants was calculated by multiplying the total number of follicles (including predated follicles) with the average number of seeds per follicle, while the number of seed produced was estimated by using intact follicles only. Percentage fruit (follicle) set was calculated as the percentage of flowers that produced fruit. One-way ANOVA followed by Fisher's LSD was used to compare the number of seeds produced per plant between the three populations in 2009 and the four populations in 2010, as well as fruit production/plant, seed production/plant, and fruit and seed set between populations. The results were generally presented as means ± SE.

4.3 RESULTS

4.3.1 Floral Structure

Adenium swazicum flowers were mostly light pink or mauve in colour, although darker pink/red, while only one pure white flower was observed in the wild populations (Figure 4.3). Flower colour in the *ex situ* collection at Lowveld National Botanical Garden did not show a great variety, which is most likely due to all the plants originating from seeds collected population P (seeds were collected for *ex situ* collections at the LNBG in 2003). Individual flower size ranged from 2-6cm in width, corolla lobe shape varied from round to star shaped with smooth or wavy margins (Figure 4.4). *Adenium swazicum* flowers are borne in clusters at the tips of the branches and open sequentially from the top to the bottom. Up to 15 flowers in different states of development can be seen simultaneously on one individual flowering branch. Flower development from bud to open flower takes less than 48 hours and mature flowers remain on the plant for four to five days before they wilt and drop to the ground (Figure 4.5). Flowers are scentless and remain open during the day and night. Flowers are produced throughout the flowering season between October and April with isolated flowers still visible in May.

Figure 4.3: Flower colour of *Adenium swazicum* in the wild ranged from (a) mauve, (b & c) light pink to (d) pure white (colour frequency: 300-400nm).

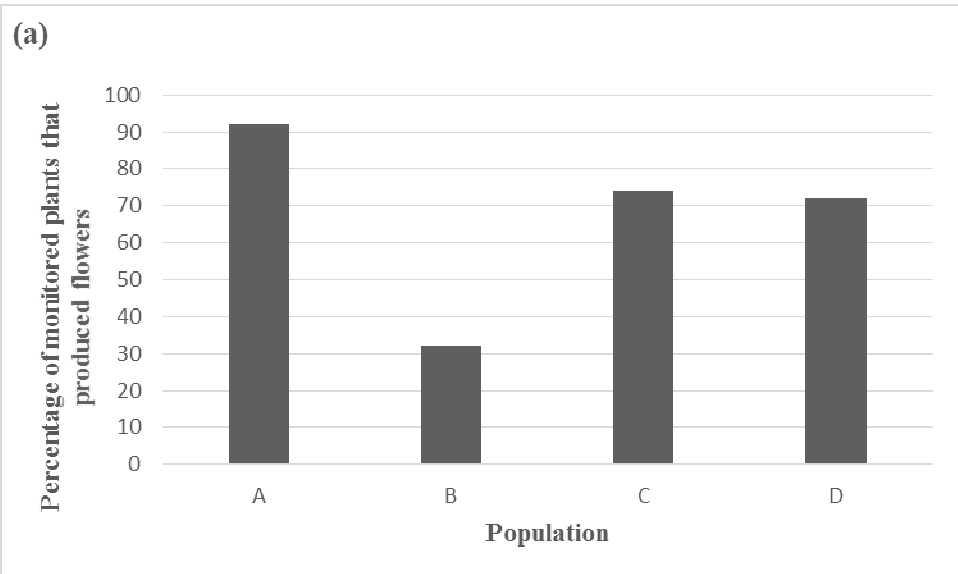
Figure 4.4: Flower shape observed in *Adenium swazicum* between 2009 and 2011 included (a) round to (b) star shaped while the corolla margins were (c) wavy to (d) smooth.

Figure 4.5: Flowers (a) in different states of development can be seen simultaneously on one flowering branch of *Adenium swazicum* with (b) mature flowers remaining open for 4-5 days before they wilt.

4.3.2 Flower production

In January 2010, 92% of the sampled plants in population A produced flowers (Figure 4.6). When non-flowering plants were excluded, significantly more flowers/plant was produced in population A (68.8±13.27 flowers/plant) compared to populations B (5.2±2.21), C (23.8 ±4.51) and D (11.7 ± 2.21; ANOVA; $F_{3,196} = 15.90$; $P < 0.0001$; $n = 200$). There was however, no significant difference between the number of flowers produced per plant in population B, C and D in January 2010 (Figure 4.6).

For all populations combined, there was no relationship between canopy size (cm²) and number of flowers ($r^2 = 0.012$, $P < 0.001$; $n = 200$) or height of plants and the number of flowers ($r^2 = 0.04$, $P < 0.001$; $n = 200$). When non-flowering plants were excluded, there was still no relationship between canopy size (cm²) and number of flowers ($r^2 = 0.0008$, $P < 0.001$; $n = 136$) or height and number of flowers ($r^2 = 0.0399$; $P < 0.001$; $n = 136$; Figure 4.7). There was no difference between the number of flowers produced on sampled plants in full sun (29.3±5.83), semi shade (30.2±7.20) and full shade (19.6±8.29; ANOVA: $F_{2,197} = 0.57$; $P = 0.5650$; $n = 200$; Table 4.21). This remained the same when non-flowering plants were excluded.



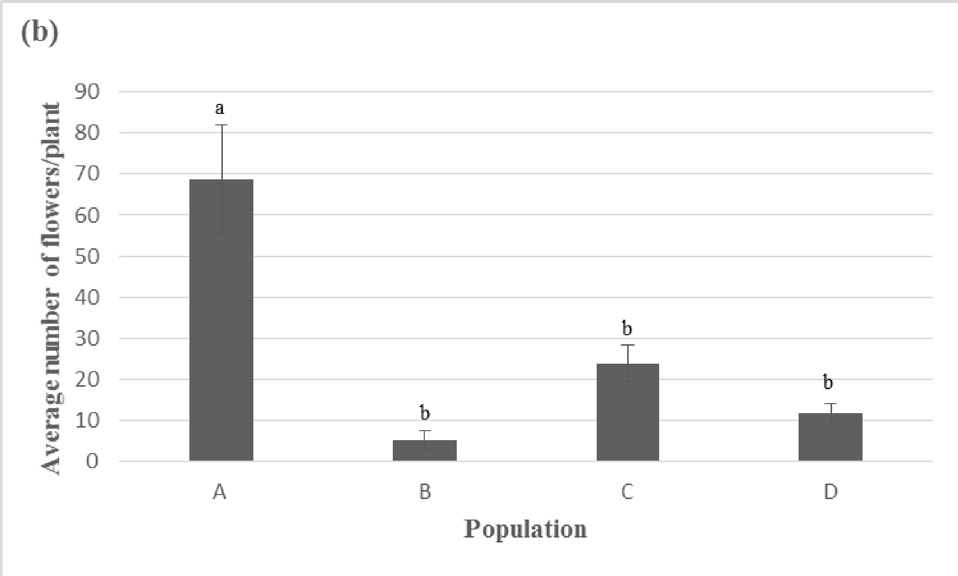


Figure 4.6: The percentage of sampled plants in each population (n = 50 for each population) of *Adenium swazicum* (a) which produced flowers in January 2010 as well as (b) the average number of flowers/plant produced by sampled plants in each population (only flowering plants). Values with different letters are significantly different between populations (LSD, P < 0.05; error bars represent standard error of the mean).

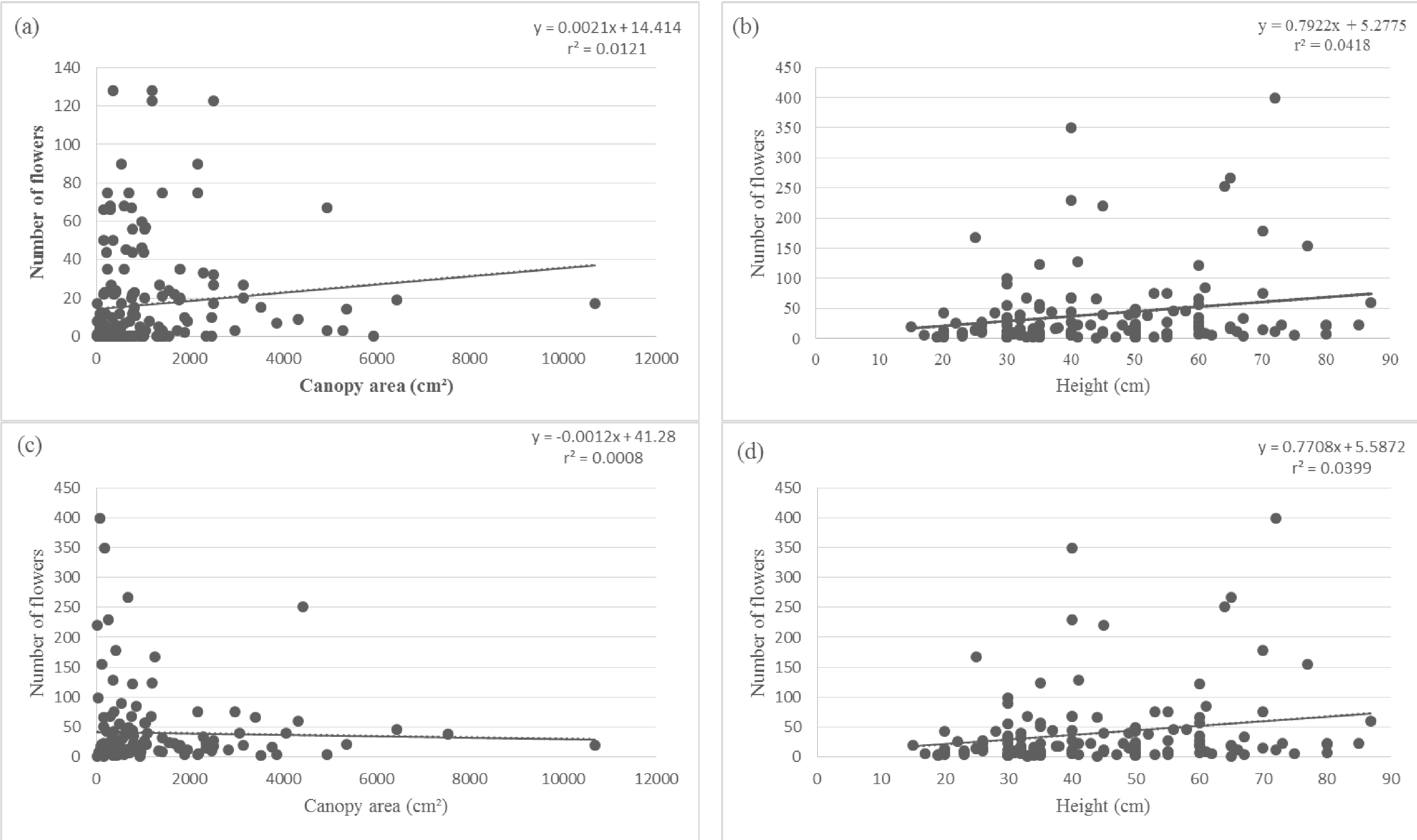


Figure 4.7: For all populations (n = 200) there was (a) no relationship between canopy area (cm^2) and number of flowers, (b) or height of plants and the number of flowers. For only flowering plants (n = 136) there was (c) still no relationship between canopy area (cm^2) and number of flowers, or (d) height and number of flowers.

4.3.3 Pollinator Observations

Pollinator observations revealed that very few insects visited *A. swazicum* flowers even during good flight times which were windless and hot. Two bee species, *Amegilla calens* and *Amegilla terminate* Friese 1897 (Hymenoptera: Apidae) were collected during the day on *A. swazicum* flowers. In both species, the proboscis length was less than 10mm and therefore not considered a possible pollinator of *A. swazicum* which requires a proboscis length of at least 18mm (Rowley 1980). *Xylocopa caffra* Linnaceus 1767 (Hymentoptera: Apidae) (Carpenter Bees) were observed hovering in front of *A. swazicum* flowers but did not land or insert its proboscis. A fast flying *Sphingidae* Linnaeus 1758 (Lepidoptera: Sphingidae) (Hawk Moth) was observed at flowering *A. swazicum* plants in population A and population Q. The moth always appeared after sunset and hovered in front of open flowers, briefly inserting its proboscis. Due to the darkness and quick flight pattern of the moth, attempts to capture a specimen were unsuccessful but, the fast flying and hovering behaviour is characteristic of *Sphingidae*, which have an apparent lack of reaction to scent and good vision at very low intensities (Proctor & Lack 1996). No floral guides, nectar guide or any other patterns were observed under UV light on mature *A. swazicum* flowers.

Table 4.1: The number of plants, percentage of flowering plants, average number of flowers, percentage of sampled plants which produced follicles as well as the percentage of flowering plants that produced follicles for plants growing in full sun, semi shade and full shade for the four populations (i.e. A, B, C & D) in South Africa.

Population A	**Full Sun**	**Semi Shade**	**Full Shade**
Number of plants (n = 50)	23	13	14
Percentage of sampled plants that produced flowers	91%	100%	86%
Average number of flowers per plant (mean $\pm$ S.E)	57.2$\pm$17.6	111.3$\pm$33.3	48.3$\pm$19.7
Percentage of sampled plants with follicles	52%	69%	64%
Percentage of flowering plants that	57%	69%	75%

produced follicles			
Population B			
Number of plants (n = 50)	24	17	9
Percentage of sampled plants that produced flowers	42%	35%	0%
Average number of flowers per plant (mean $\pm$ S.E)	7.0$\pm$3.4	5.1$\pm$2.8	0
Percentage of sampled plants with follicles	12.5%	11.7%	0%
Percentage of flowering plants that produced follicles	30%	33%	0%
Population C			
Number of plants (n = 50)	33	12	5
Percentage of plants that produced flowers	76%	66%	80%
Average number of flowers per plant (mean $\pm$ S.E)	34.7$\pm$6.76	7.8$\pm$2.82	2.8$\pm$0.86
Percentage of sampled plants with follicles	40%	35%	0%
Percentage of flowering plants that produced follicles	44%	50%	0%
Population D			
Number of plants (n = 43)	10	18	15
Percentage of plants that produced flowers	60%	89%	60%
Average number of flowers per plant (mean $\pm$ S.E)	9.4$\pm$4.24	12.3$\pm$1.95	5.1$\pm$1.60
Percentage of plants with follicles	0%	0%	0%
Percentage of flowering plants that produced follicles	0%	0%	0%

4.3.4 Follicle and seed production

Adenium swazicum follicles are between 15 and 20cm in length with two follicles produced per flower. The seed has a woody outer layer which protects the soft embryo, the individual seeds weigh 0.0022$\pm$0.00029 g (n = 10; range: 0.001-0.004) and are 9-13 mm in length with hairy tufts on both ends (Figure 4.8). Average number of seed produced per follicle was 44.1$\pm$ 3.04 (mean $\pm$ S.E; n = 19; range: 25-69). Population B produced the highest number of follicles (94) in 2009 from the 50 sampled plants. Population A produced 221 follicles in 2010, but 202 (91%) of these follicles were predated before they reached maturity in October (Figure 4.9). For sampled plants in all populations combined, there was no relationship between canopy size (cm^2) and number of follicles ($r^2 = 0.063$, $P < 0.001$; n = 200), this remained the same when non-reproductive plants were excluded from the analysis ($r^2 = 0.0338$, $P < 0.001$; $n = 136$). However, there was a positive relationship between the number of flowers and the number of follicles ($r^2 = 0.441$; $P < 0.001$; $n = 136$). There was no difference between the number of follicles produced on plants that grew in full sun (1.6$\pm$0.34), semi shade (1.3$\pm$0.51) and full shade (0.6$\pm$0.32; ANOVA: $F_{2,197} = 1.32$; $P = 0.2702$; $n = 200$).

In 2010, population B had the highest fruit (follicle) set (% of flowers which produced follicles) at 11% compared to populations A (6.4%), C (6.2%) and D (1%).
In 2009, the average number of seed/plant produced in population B was significantly more (82.7$\pm$23.6) compared to populations A (29.3$\pm$26.7) and C (29.9$\pm$9.7; ANOVA, $F_{2,147} = 5.42$; $P < 0.005$; $n = 150$). Population D was not studied in 2009 and was therefore excluded from the analyses. However, in 2010 the average number of seed produced/plant in population C was significantly more (58.9$\pm$15.7) compared to populations A (22.0$\pm$5.01), B (12.32$\pm$9.1) and D (0; ANOVA, $F_{3,196} = 7.15$; $P < 0.0001$; $n = 200$).

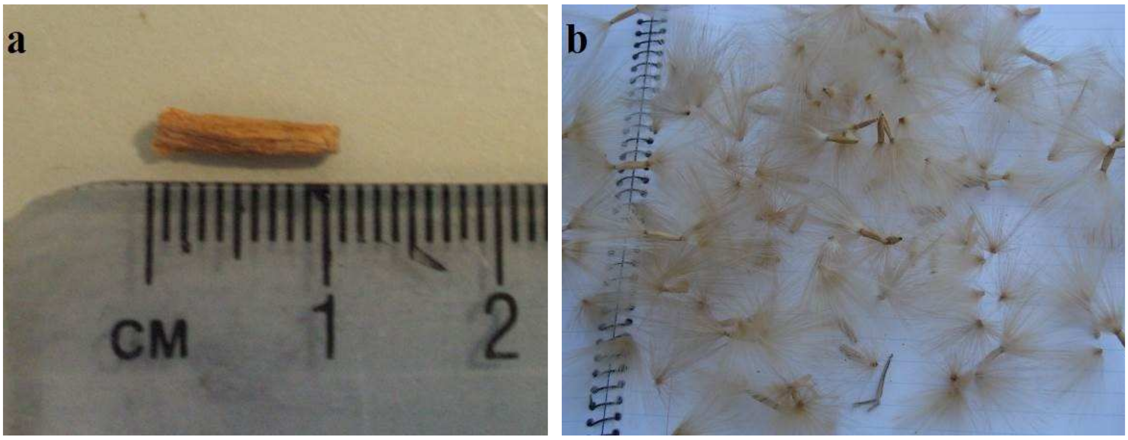
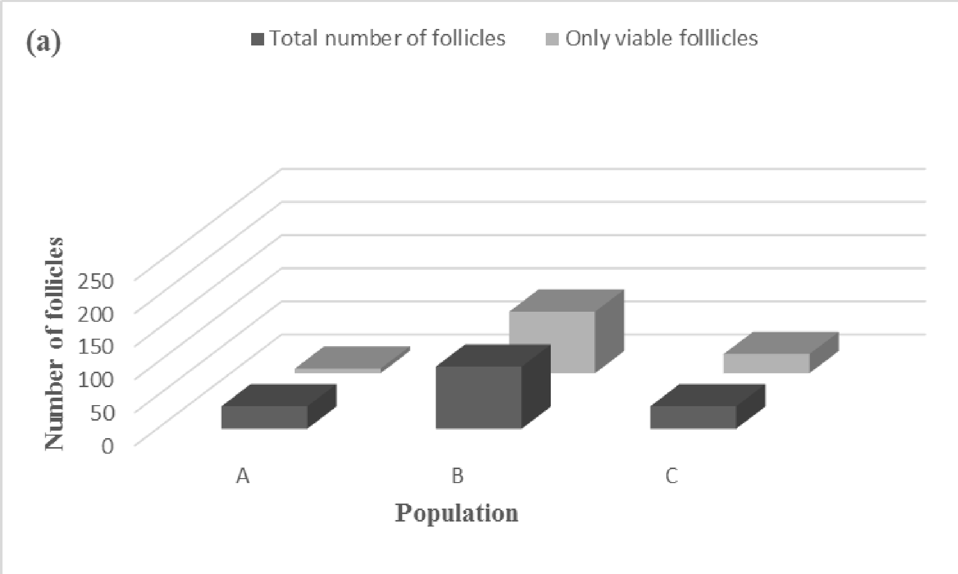


Figure 4.8: *Adenium swazicum* seeds have (a) a woody outer layer, are 9 to 13mm in length (a), weigh 0.0020 $\pm$ 0.0029 g and (b) have hairy tufts on both ends to aid in wind dispersal.



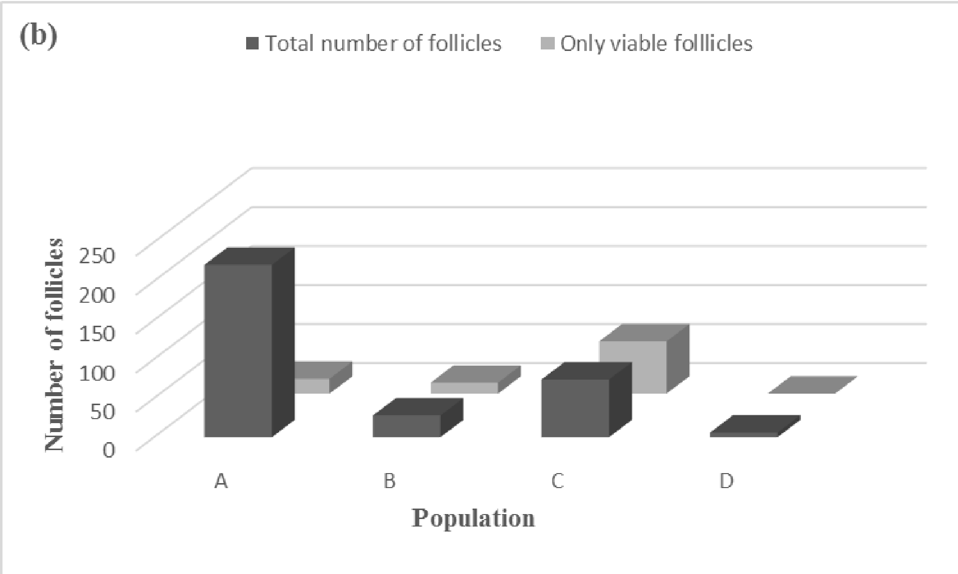


Figure 4.9: Follicles produced by sampled *Adenium swazicum* plants, (a) in 2009 and (b) 2010.

The percentage of flowering plants that produced intact follicles compared with those that produced none, differed significantly between the four populations ($X^2_3 = 25.69$; $P < 0.001$) with only two (5.6%) of the flowering plants in population D producing follicles in 2010, and none of these were intact by the end of the fruiting period in October (due to predation) (Figure 4.10). There was a significant positive linear relationship between the number of flowers and follicles produced for all four populations combined (excluding non-flowering plants) ($r^2 = 0.44$; $p < 0.001$; $n = 136$) (Figure 4.11). At population level, population C produced the highest number of viable seed (2363) in 2010 compared to populations A (257), B (242), and D (0) (Table 4.2).

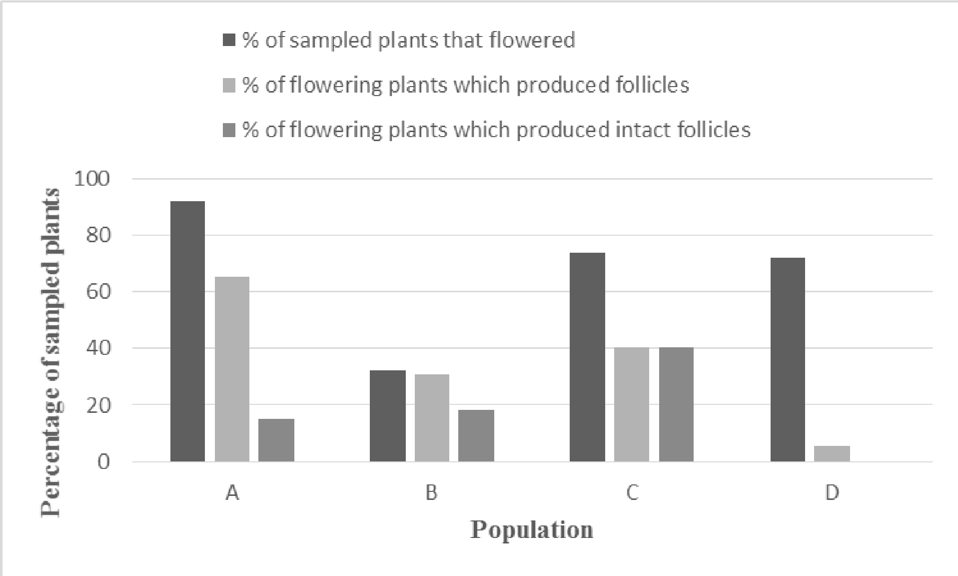


Figure 4.10: The percentage of sampled plants in each population (n=50/population) of *Adenium swazicum* which produced flowers, the percentage of the flowering plants that produced follicles as well as the percentage of flowering plants which produced intact follicles.

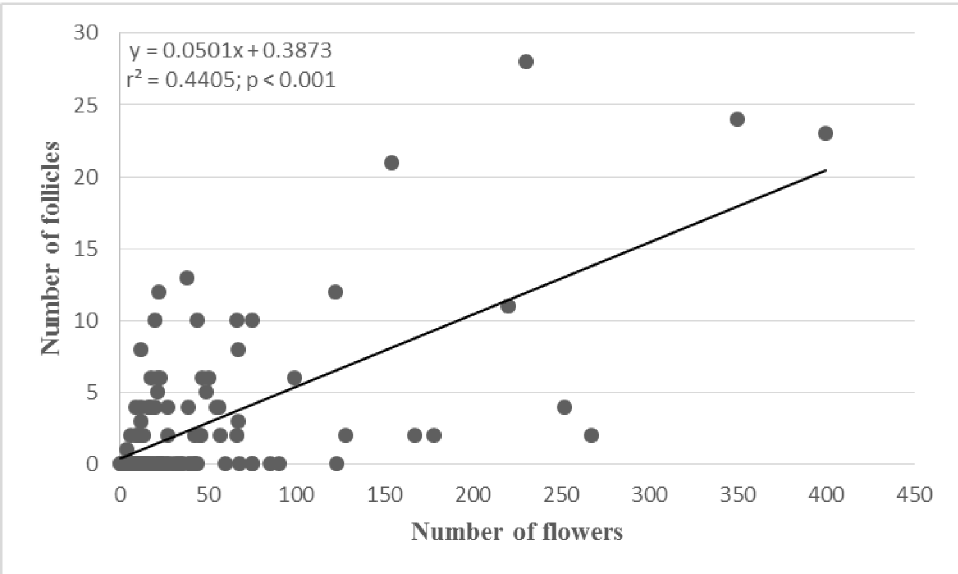


Figure 4.11: Relationship between the number of flowers and the number of follicles produced in 2010 for all populations combined (n = 136).

Table 4.2: The number of plants that produced flowers, average number of follicles/plant, number of seed released from intact follicles and number of viable seeds released for 50 plants sampled/population and extrapolated to the whole population (data in Table 3.4).

Attribute	Population A		Population B		Population C		Population D	
	Sampled Plants	**Total population**	**Sampled Plants**	**Total population**	**Sampled Plants**	**Total population**	**Sampled Plants**	**Total population**
Sample/Population Size	n = 50	n = 121	n = 50	n = 137	n = 50	n = 141	n = 50	n = 70
Number of plants which produced flowers (2010)	46 (92%)	111	17 (34%)	47	37 (74%)	104	36 (72%)	50
Number of flowering plants which produced intact follicles [A]	7 (15%)	17	3 (17%)	8	15 (41%)	43	0	0
Average number of seeds/follicle[i] [B]	44.1$\pm$13.2	44.1$\pm$13.2	44.1$\pm$13.2	44.1$\pm$13.2	44.1$\pm$13.2	44.1$\pm$13.2	44.1$\pm$13.2	44.1$\pm$13.2
Number of follicles/plant (mean$\pm$S.E) [C]	0.4$\pm$0.25	0.4$\pm$0.25	0.8$\pm$0.41	0.8$\pm$0.41	1.8$\pm$0.28	1.8$\pm$0.28	0	0
Average number of intact follicles produced/plant [D] = [A*C]	2.8	6.8	2.4	6.4	27	77	0	0
Number of seeds released from intact follicles [E] = [B*D]	123	299	105	282	1190	3396	0	0
Seed viability for population [F]	85.8%	85.8%	85.8%[ii]	85.8%[ii]	69.6%	69.6%	n/a	n/a
Number of viable seeds released = [E*F]	105	257	90	242	828	2363	0	0

[i]Seeds/follicle were only determined from a sample of 19 intact follicles gathered from populations A, B and C in October 2009.
[ii]Due to a lack of available seed in October 2009, seed viability could not be determined for population B therefore, seed viability which was determined for population A, which was located 11km from population B was applied.

4.4 DISCUSSION

Population A produced significantly more flowers/plant in 2010 compared to the other three sampled populations and although population A produced significantly more follicles in 2010 compared the other three populations, 91% of the follicles were predated before seed was released in October. At the same time, population C produced the highest number of intact follicles and subsequently the highest number of seed in 2010. Hence there is considerable variation in seed production between populations and between the years.

Plant size is often closely related to flower production and larger floral displays should result in more fruit production because they attract more pollinators and represent larger energy investments in reproduction (Udovic 1981; Sih & Baltus 1987; Ollerton & Lack 1998). Furthermore, many individual plants of a species flowering at the same time will be more attractive to pollinators compared to fewer plants producing less flowers (Udovic 1981; Echart 1991; Ollerton & Lack 1998). No relationship was found between plant size (canopy area) and the number of flowers, which could be partially due to disturbance factors (see Chapter 3) which reduced the actual levels of flower production as the plant, recovers. A positive relationship between the number of flowers/plant and number of follicles/plant for all populations combined, indicated that plants with large floral displays attract more pollinators. One unlikely possibility is that the species might self-pollinate. This would require an exclusion experiment to confirm this, which would be very difficult to undertake in the KNP as animals such as baboons would destroy the exclusion bags. Witkowski & Wilson (2001) found that a significant higher percentage of flowering plants of the alien invasive *Chromolaena odorata* (L) (Asteraceae) were growing in full sun compared to plants that grew in semi shade and shade. However, for *A. swazicum* there was no difference between the number of flowers/plant for plants that grew in full sun, semi-shade and full shade possibly indicating that sunlight was not a prerequisite for flowering.

Despite large, pink floral displays, low insect activity was observed around *A. swazicum* and although surprising, similar observations have been made for *Nerium oleander*, another member of the Apocynaceae family (Herrera 1991). Diurnal and nocturnal Sphingids (Hawkmoths) use colour vision to find and recognize flowers and feed while hovering in front of flowers (Kelber et al. 2003). Although a specimen *Sphingidae* Linnaeus 1758 (Hawk Moth) could not be captured, its feeding behaviour was consistent with studies on hawkmoth pollination (White et al. 1994; Proctor & Lack 1996; Sugiura & Yamazaki 2005; Araujo et al. 2014), and it is considered likely that this moth is the pollinator for *A. swazicum.*

Limited fruit set can be a result of poor pollination or lack of resources (Copland & Whelan 1989) although studies on the same plant species have sometimes yielded opposite results with pollination setting the limit to fruit set in some seasons but not others (Whelan & Goldingay 1989). However, it has been argued (Willson & Rathke 1974; Willson & Price 1977) that pollinators do not limit fruit production in *Asclepias* species even though the proportion of flowers producing mature fruits is low and fruit abortion is common. Asclepiadaceae is treated as a subfamily in Apocynaceae (Endress & Bruyns 2000), which includes the genus *Adenium.* Fruit and subsequent seed set was highly variable in *Adenium swazicum* in 2009 and 2010, with population B producing 94 follicles in 2009, but only 28 in 2010, while in population A 12 plants produced follicles in 2009 while 28 plants produced follicles in 2010. Janzen et al. (1980) suggested that annual alternation of good and poor reproduction may result from resource limitations in individual plants, and this was considered possible for *A. swazicum.* Reproductive failure can include failure of fruit initiation and failure of fruit to mature (Willson & Price 1977). Failure of fruit to mature can be due to abortion, which is usually caused by competition amongst the fruits for nutrients supplied by the parent plant and/or abortion due to anomalies, or due to predation of the fruit (Willson & Price 1977). Failure of fruit to mature (mostly due to predation) had a significant impact on population A in which 91% of the follicles did not reach maturity in 2010. Although this study did not distinguish between follicle abortion and predation, large concentrations of follicle and seed predators such as *Leptocoris hexophthalma* (Thunberg 1784) and *Dacus frontalis* (Becker 1922) were observed on the follicles in population A. During the same time, dense infestations of *L. hexaphtalma* were confirmed by the Agricultural Research Centre (ARC) in *Mangifera indica* (Mango) and *Litchi chinensis* (Lychee) orchards in the Malelane region during 2010 (S. Schoeman 2010, pers. comm.). It is therefore highly likely that failure of follicles to mature in *A. swazicum* was largely due to predation and not follicle abortion due to limited resources.

Mutualism between plants and pollinators is likely to be disrupted in small, isolated populations, and this may reduce plant reproductive success (Sih & Baltus 1987; Olensen & Jain 1994; Argen 1996).

The Allee effect is the relationship between population size or density and overall population growth rate (Le Cadre et al. 2008). Van Kleunen & Johnson (2005), describe two types of Allee effects; ecological Allee effect due to a reduction in pollen deposition in small populations and genetic Allee effect which relates to the quality of the deposited pollen. Therefore, plant fitness in small populations may be reduced because of both ecological and genetic factors (Berec et al. 2007). This Allee effect might have been present in population D which had a small number of plants, low pollinator activity and was isolated from other *A. swazicum* populations resulting in reproduction failure in 2010. Reproductive failure in small populations have been recorded in various studies for example Lamont et al. (1993) found that total seed production of *Banksia goodii* populations tended to increase with the number of plants in the population, while a significant positive correlation has been found between population size and seed production in the self-incompatible herb, *Lythrum salvicaria* (Argen 1996). Due to an insufficient number of founders, fragmented populations within deteriorating patches may go extinct over time while colonization of new sites becomes increasingly unlikely (Ovaskainen & Hanski 2001). Although population D was only studied for one year, it is possible that this population suffers from both ecological and genetic Allee effects and continued reproduction failure. Destruction of the remaining plants by the housing development as well as collection of the species for medicinal purposes, could lead to the local extinction of this population. The above indicates that there is a need for reproductive studies to be conducted over several consecutive years in order to better understand the reproductive ecology of *A. swazicum*.

5 CHAPTER 5: SEED VIABILITY, GERMINATION AND SEEDLING ESTABLISHMENT OF THE CRITICALLY ENDANGERED SUCCULENT, *ADENIUM SWAZICUM* STAPF IN SOUTH AFRICA.

Abstract

Temperature is considered the most important environmental factor governing seed germination. Maximum, minimum and optimal germination temperatures for *Adenium swazicum* were determined under controlled conditions. Tetrazolium staining protocol tests were used to determine seed viability. Seedling emergence and establishment experiments were carried out in 25 x 25cm seedling trays under 40% shade netting in ambient nursery conditions at the Lowveld National Botanical Garden (LNBG) in Nelspruit and at the Skukuza indigenous nursery in Kruger National Park (KNP), Mpumalanga, to compare germination results between the two localities. Germination medium, moisture requirements, depth of sowing and the effect of shading on seedling emergence and establishment were determined under ambient nursery conditions in the LNBG. Seed viability was significantly different between seed samples, with population A in 2009 having the highest percentage of viable seed (85.8$\pm$2.11%) compared to seeds collected from population C in 2009 (69.6$\pm$2.19%) and 2010 (49.9$\pm$2.37%; ANOVA: $F_{2,15} = 58.29$; $P < 0.0001$; $n = 18$). The highest percentage seed germination was obtained under 25°C and 20/30°C alternating temperature (both 90 $\pm$ 3.33%). No germination was recorded at 5°C and 10°C, with limited germination recorded at 40°C (26 $\pm$ 4.05%). The optimal germination temperature for *A. swazicum* is 25°C and 20/30°C alternating temperature, although high germination percentages were also reached at 20°C (86 $\pm$ 3.36%), 30°C (86 $\pm$ 3.36%) and 35°C (82$\pm$3.8%). The germination and seedling

emergence / establishment had three main characteristics: high germination percentage between 20°C and 30°C; a rapid germination rate; and seedling establishment is highly dependent on shade. Seedlings readily emerged irrespective of the soil media tested or depth of planting, although seedling establishment was highly dependent on the presence of shade. *Adenium swazicum* has a transient soil seed bank and seeds do not appear to display any dormancy mechanism.

Keywords: Apocynaceae, germination medium, germination temperature, mean germination time, shading, soil seed bank, time to minimum germination.

5.1 INTRODUCTION

Two basic alternatives that limit plant recruitment include the availability of viable seed and the availability of suitable microsites at which seedling establishment is possible (Eriksson & Ehrlen 1992). Temperature is considered the most important environmental factor governing the maximum germination percentage and rate of germination and germination is usually only possible within well-defined temperature limits (Heydecker 1977 cited in Mattana et al. 2010; Garcia-Huidobro et al. 1982). This leads to a consideration of the cardinal temperatures, which include the maximum, minimum and optimum temperatures. The optimum germination temperature for a species is characterised by maximum germination in the shortest time, while no germination will occur beyond maximum and minimum temperatures (Probert 2000). Once a species' cardinal temperatures are reached, some species have a fast germination response to rain, with these fast germination responses favoured by rainfall patterns that lack long intermittent drought periods (Fennel & Thompson 2005). Choinski & Tuohy (1991) found that 90-100% of *Colophospermum mopane,* *Acacia tortillis, A. karoo* and *Combretum apiculatum* seeds germinate within 4-5 days at 30°C.

Soil seed banks can be classified as transient in which seed stocks disappear within a year of dispersal, or as persistent seed banks in which seeds remain in the soil for more than a year (Thompson & Grime 1979). The presence of seed in soil seed banks can play an important role in the persistence of a species, since seed will be available for regeneration even in years with no seed yield (Baskin & Baskin 1998).

No research has been conducted on the seed germination, seed dormancy and seedling establishment of *A. swazicum* in Southern Africa. The aim of this study was to understand seed germination, seed dormancy and seedling establishment through the following objectives:

- Determine seed viability of *A. swazicum* and compare seed viability between *A. swazicum* populations;
- Determine optimum, minimum and maximum germination temperatures for *A. swazicum*;
- Determine Mean Germination Time (MGT), minimum time to onset of germination (T_{min}), and time to maximum germination (T_{max}) for set temperature intervals;
- Determine the importance of soil medium, depth of planting, moisture stress and shading on seedling emergence and establishment; and
- Compare germination response and seedling establishment under nursery conditions within and outside the natural distribution range of *A. swazicum.*

5.2 MATERIALS AND METHODS

5.2.1 Seed collection and storage

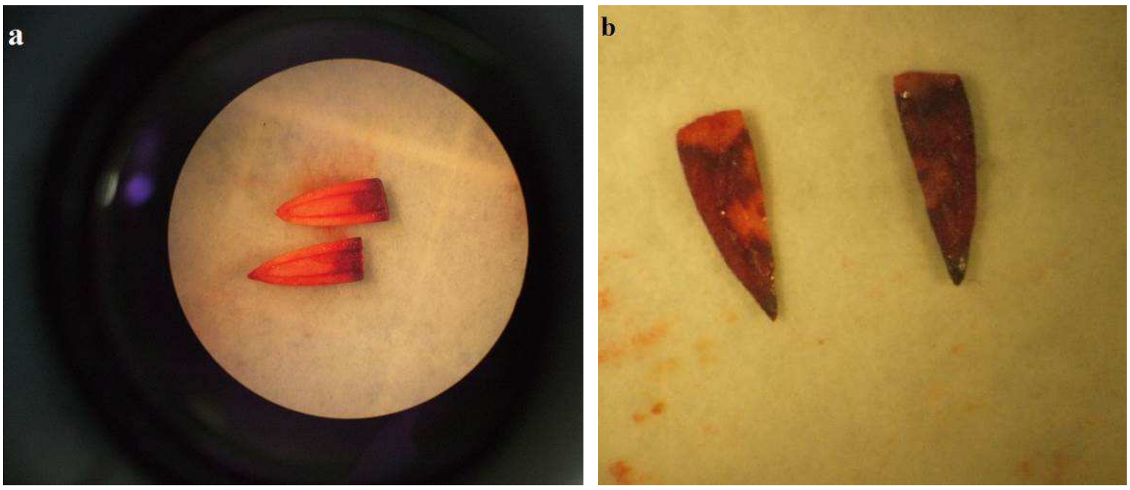
In total 411 seeds were collected in total, 147 and 87 seeds from population A in 2009 and 2010 respectively, and 177 seeds from population C in 2009. The seeds were stored under ambient conditions, in brown paper bags at the Lowveld National Botanical Garden in Nelspruit, Mpumalanga. A permit was obtained from Mpumalanga Tourism and Parks Agency to export the seeds to the Kew Millennium Seed Bank Project, Wakehurst, England.

Seeds used for seedling emergence and establishment experiments conducted under ambient nursery conditions were collected in October 2010 from population B and C. All seeds were collected at the point of natural dispersal by securing nylon stockings over developing follicles, securing both ends lightly with a cable tie to avoid seed dispersal by wind. The seeds were stored under ambient conditions, in brown paper bags at the LNBG.

5.2.2 Seed viability

Standard tetrazolium staining protocol was used to assess the percentage of viable and non-viable seeds. The initial tetrazolium tests were conducted with 27 seeds from each of the three different seed batches, this allowed for 25 seeds for the test as well as an additional two seed per locality for cutting experiments and error. Seeds were prepared for the TZ test by placing them in 50mm diameter petri dishes, which were suspended over distilled water inside an airtight container for 24 hours and at 20°C. After 24 hours, the seeds were transferred to 90mm diameter petri dishes, which contained 1% agar solution and stored at 20°C for an additional three days. In preparation for staining the seeds were placed in plastic vials containing 2, 3, 5-triphenyltetrazolium chloride solution and each vial was wrapped in heavy-duty aluminium foil to prevent reaction to light. The vials were incubated at 30°C in an 8/16 hour light/dark cycle for two days. At the end of day two, the seeds were removed from the vials, washed with distilled water and evaluated immediately using recommendations by Peters (2000). Seed viability was based on tissue characteristics and divided into one of the following categories (Patil & Dadlani 2009):

- Sound/Viable: Seed was firm and could be removed from the woody seed coat without damaging the seed inside. Tissue was stained gradually and uniformly without distinct boundaries (Figure 5.1a);
- Weak viable tissue: Seeds were firm and could be removed from the woody seed coat but stained tissue had a mottled appearance (Figure 5.1b);
- Non-viable tissue: Seeds were soft, mush, and could not be removed from the woody seed coat (Figure 5.1c). Tissue was an off white to chalky white colour (Figure 5.1d).



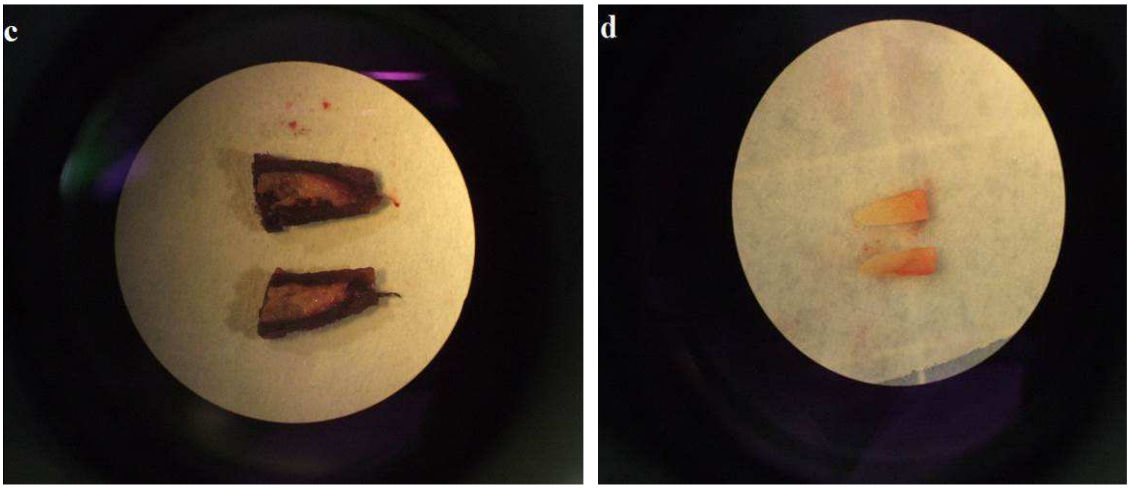


Figure 5.1: Seed viability of *Adenium swazicum* based on a standard tetrazolium staining test with (a) sound/viable tissue indicating gradual staining, (b) mottled appearance of weakly viable tissue. Non-viable tissue was (c) soft and could not be removed from the woody seed cover, or (d) tissue did not stain and had an off-white appearance.

5.2.3 Seed germination

Seed germination experiments were conducted in June 2010 at the Kew Millennium Seed Bank Project laboratory. Seeds were not treated or rinsed before germination experiments. Seeds were placed in 90mm petri dishes containing agar solution within nine different environmental control incubators set at an 8/16 dark/light cycle at 5°C temperature intervals between 5°C and 40°C as well as one alternating temperature of 20/30°C. A limited number of seeds were available for germination experiments, especially from population A (2009), and therefore the more extreme temperature intervals of 5°C, 10°C, 15°C and 40°C were not tested for population A (2009) but only used seeds from population C collected in 2009 and 2010 (Table 5.1). Seeds from population B and D were not available at the time of these experiments. Temperatures selected for the experiments were based on temperatures found across the natural distribution of *A. swazicum.*

Table 5.1: Number of petri dishes and seed used per replicate for a particular population to determine germination response of *Adenium swazicum* at various temperature intervals.

Temperature	Population C 2009	Population A 2010	Population A 2009

40°C	3 (petri dishes) x 5 (seeds)	2 x 5	0
35°C	3 x 10	2 x 5	3 x 10
30°C	3 x 10	2 x 5	3 x 10
25°C	3 x 10	2 x 5	3 x 10
20°C	3 x 10	2 x 5	3 x 10
15°C	3 x 5	2 x 5	0
10°C	3 x 5	2 x 5	0
5°C	3 x 5	2 x 5	0
30/20°C	3 x 10	2 x 5	3 x 10

Germination scoring was done three times a day for three weeks or until 100%, germination success was achieved. Germination was defined as emergence of the radicle. Final germination scores were based on percentage of viable seed that germinated.

5.2.4 Seedling emergence

Seeds for emergence experiments were collected from population A, B and C during 2009 and 2010 and stored under ambient conditions, in brown paper bags at the LNBG. Germination tests were carried out in 25 x 25cm seedling trays under 40% shade netting in ambient conditions outside at the LNBG and at the Skukuza indigenous nursery in Kruger National Park (KNP), Mpumalanga. Germination and seedling emergence for all experiments was scored once a week on Fridays, for eleven weeks between the 29th January 2010 and 16th of April 2010. Seedlings were scored as emerged once the cotyledons were visible above the ground. The following seedling emergence experiments were conducted:

Germination medium:

To test the effect of germination medium on seedling emergence and establishment, three germination media were used: (a) 100% seedling mix (Braaks, South Africa), (b) mixture of 50% seedling mix (Braaks, South Africa) and 50% river sand, and (c) 50% river sand mixed with 50% red, loamy soil obtained from the grounds of the

LNBG. All three trays were watered once a day and seeds were planted 5mm below the surface.

Depth of planting:

To assess the influence of planting depth on seedling emergence and establishment, three identical trays were prepared with the 100% seedling mix (Braaks, South Africa) and seeds were planted (a) on the surface, (b) 5mm below the surface, and (c) 10mm below the surface. The trays were watered once a day.

Available moisture:

The effect of available moisture/watering regime on seedling emergence and establishment was determined by preparing four identical trays with 100% seedling mix and seeds planted 5mm below the surface. The trays were watered (a) once every 14 days, (b) every seven days (once a week), (c) every three days and (d) kept moist in the mist house having automated sprayers producing a mist spray for 20seconds every 2 hours, 24 hours a day.

Shading:

The effect of shading on the emergence and establishment of seedlings was determined by planting 50 *A. swazicum* seeds in full sun in the LNBG and preparing a 25cm x 25cm tray covered with 40% shade netting with the same soil medium (100% seedling mix - Braaks, South Africa) and placing it next to the open area experiment. Trays only received natural rainfall (no artificial watering).

To determine if there was a difference between the germination results for studies conducted within the distribution range (Skukuza) and outside the distribution range

(LNBG), three identical germination experiments in three trays were prepared on the 12th of October 2010. Two trays contained seed collected from population A, while the third tray contained seed collected from population C. Two trays, one containing seed from population A, while the other contained the seed from population C, were kept in the nursery at LNBG while the third tray, which contained seed from population A was taken to Skukuza nursery in KNP. All three trays were prepared as follow:

- Soil medium: 100% seedling mix;
- Depth of planting: 5mm below the surface;
- Watering: Daily at 14:00;
- Shading: In nurseries, under 40% shade net.

Seedling emergence was scored on a daily basis at 14:00 between 12th of October until the 10th of November 2010, Monday to Friday. Total seedling emergence was expressed as a percentage of the total number of seeds sown per treatment. The mean maximum temperature recorded in Nelspruit and Skukuza during the experimental period was 27.2 $\pm$ 0.86°C and 32.6 $\pm$ 0.90°C, while the mean minimum temperature was 15.0°C $\pm$ 0.38 °C and 17.7 $\pm$ 0.59°C respectively (KNP Scientific Services; Lowveld NBG weather records; TuTiempo weather; World Weather Online).

5.2.5 Statistical analysis

Mean germination time (MGT) was calculated as follow:

$$MGT = \sum(G't')/G_m$$

where G' is the number of seed which germinate at hour t'. G_m is the maximum number of seed that germinated at the temperature interval (Heydecker 1977 in Garcia-Huidobro et al. 1966). Germination percentages were arcsine transformed before analysis. Minimum time to germination (T_{min}) represented the time (hours) to first germination per temperature interval, while time to maximum germination (T_{max}) was time (hours) to maximum germination (only viable seed) per temperature interval). One-way ANOVAs were used to compare the MGT between temperature treatments followed by Fisher's LSD. A series of χ^2 tests compared germination under (a) different soil media, (b) depth of planting and (c) watering regime under nursery conditions. Results were generally represented as mean $\pm$ S.E. Statistical analyses were conducted using Analyse-it for Microsoft Excel (version 2.30).

5.3 RESULTS

5.3.1 Seed viability

The tetrazolium staining test revealed that there was a significant difference between the percentages of viable seed from all three seed batches. Seeds collected from population A in 2009 had the highest percentage of viable seed (85.8$\pm$2.11%) compared to seeds collected from population C in 2009 (69.6$\pm$2.19%) and seeds collected from population C in 2010 (49.9$\pm$2.37%; ANOVA: $F_{2,15} = 58.29$; $P < 0.0001$; n = 18; Figure 5.2).

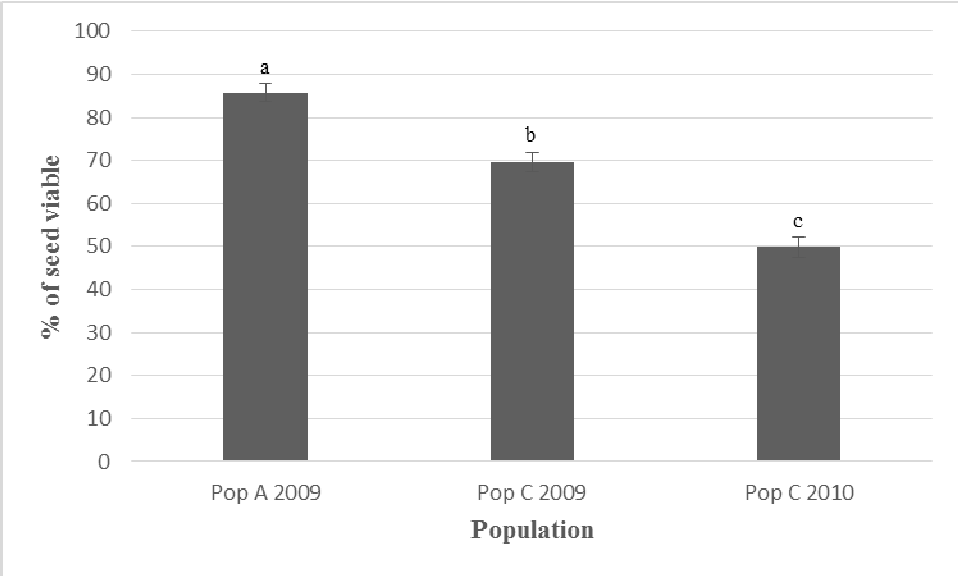


Figure 5.2: The percentage of viable seed differed significantly between the three seed batches with population A having the highest percentage of viable seed (85.8 ± 2.11; ANOVA; $F_{2,15}$ = 58.29; P < 0.0001; n = 18). Values with different letters are significantly different between seed batches (LSD, P<0.05; error bars represent standard error of the mean).

5.3.2 Seed germination

There was a significant difference in maximum seed germination (G_m) among the temperature treatments (ANOVA: $F_{8,13}$ = 144.03, P < 0.0001; n = 22; Figure 5.3). The highest percentage seed germination was (90 ± 0%) obtained under 25°C and 20/30°C alternating temperature. No germination was recorded at 5°C and 10°C during the experiment, with low germination recorded at 15°C (40 ± 4.5%) and 40°C (26 ± 4.05%). Although it appears that the optimal germination temperature for *A. swazicum* is around 25°C and 20/30°C, high germination percentages were also reached at 20°C (86 ± 3.8%), 30°C (86 ± 3.3%) and 35°C (82 ± 3.8%). Hence *A. swazicum* has a relatively broad germination temperature optimum, which may reflect its distribution range from Mpumalanga, Swaziland and Mozambique where summers are hot. Minimum time (hours) to the onset of germination (T_{min}) was significantly different among temperature treatments (ANOVA: $F_{6,11}$ = 17.80; P < 0.0001; n = 18) with the shortest and longest-time to germination recorded at 35°C (30.0 ± 3.5 hours) and 15°C (199 ± 7.0 hours), respectively. T_{max} was also significantly different amongst temperature treatments (ANOVA: $F_{6,11} = 3.39$; $P = 0.034$; $n = 18$) with shortest and longest time to maximum germination recorded at 35°C (80 $\pm$ 24.58 hours) and 15°C (236 $\pm$ 30.10 hours), respectively (Figure 5.4). Temperature treatments 5°C and 10°C were excluded due to 0% germination. Mean Germination Time (MGT) was significantly different amongst temperature treatments (ANOVA; $F_{6,11} = 4.76$; $P < 0.0104$; $n = 18$) with the fastest MGT recorded at 35°C (50.1$\pm$2.55 hours) while the longest MGT was recorded at 15°C (149$\pm$56.8 hours). These results indicate that *A. swazicum* will germinate rapidly once temperatures are between 20°C and 35°C are experienced although the highest germination percentages are achieved at 25°C, 30°C and 30/20°C alternating temperatures.

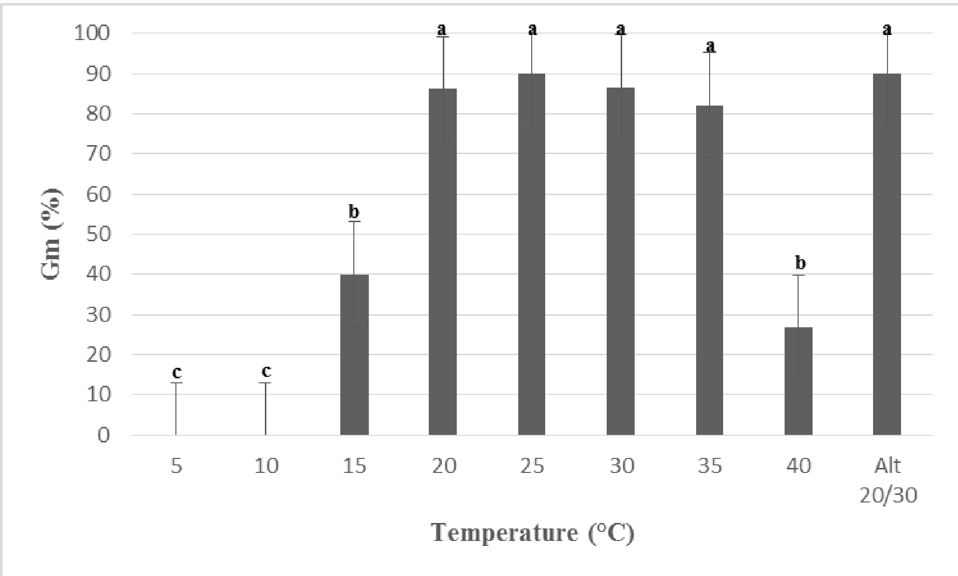


Figure 5.3: Maximum germination percentage (G_m) for *Adenium swazicum* under nine different temperature treatments. No germination occurred at 5°C and 10°C with low germination success recorded at 15°C (40$\pm$5%) and 40°C (26.7$\pm$8.3%). Values with different letters are significantly different between temperatures (LSD, $P < 0.05$; error bars represent standard error of the mean).

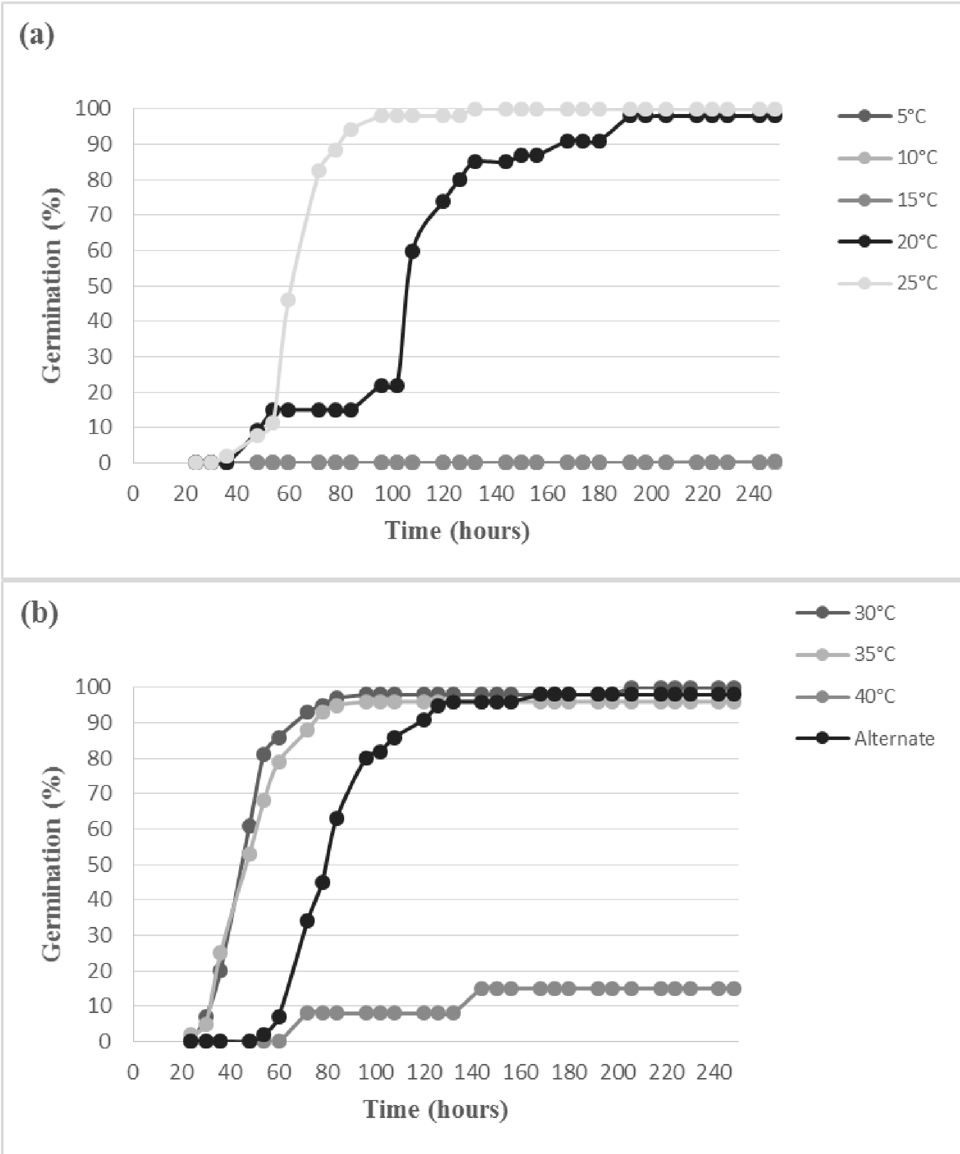


Figure 5.4: The course of cumulative germination for *Adenium swazicum* at (a) 5°C, 10°C, 15°C, 20°C and 25°C, as well as (b) 30°C, 35°C, 40°C and alternating temperature 20/30°C.

5.3.3 Seedling emergence and seedling establishment

It is acknowledged that seedling emergence will be less than or equal to actual germination percentages, as some germinated seeds might not emerge above the soil surface. Seedling emergence percentages were similar for the three soil media, 100% seedlings mix (38%), 50% seedlings mix: 50% sand (38%) and 50% sand: 50% local soil (30.6%) (χ^2_2 = 1.83; P = 0.4005; Figure 5.5a) as well as depth of planting, surface (26.5%), 5mm below surface (35%) and 10mm below surface (26.5%) (χ^2_2 = 2.32; P

= 0.3135; Figure 5.5c). However, seed planted at 10mm below the soil surface emerged two weeks after seed that were planted on the soil surface and those planted 5mm below the soil surface (Figure 5.5c). Water regime had a significant effect on seedling emergence, with the highest emergence recorded for the tray which was kept moist (46%), followed by the tray which was watered once every three days (24%). For the tray which was watered once every seven days, 16.4% of the seedlings emerged, while no emergence was recorded for the tray which was watered once every 14 days (χ^2_3 = 64.75; $p < 0.0001$; Figure 5.5b). In most treatments, seedling establishment (over an 11 weeks duration) was similar to seedling emergence, except for the shading experiment in which 100% of the emerged seedlings died by week 2 (Figure 5.6) when in full sun (open).

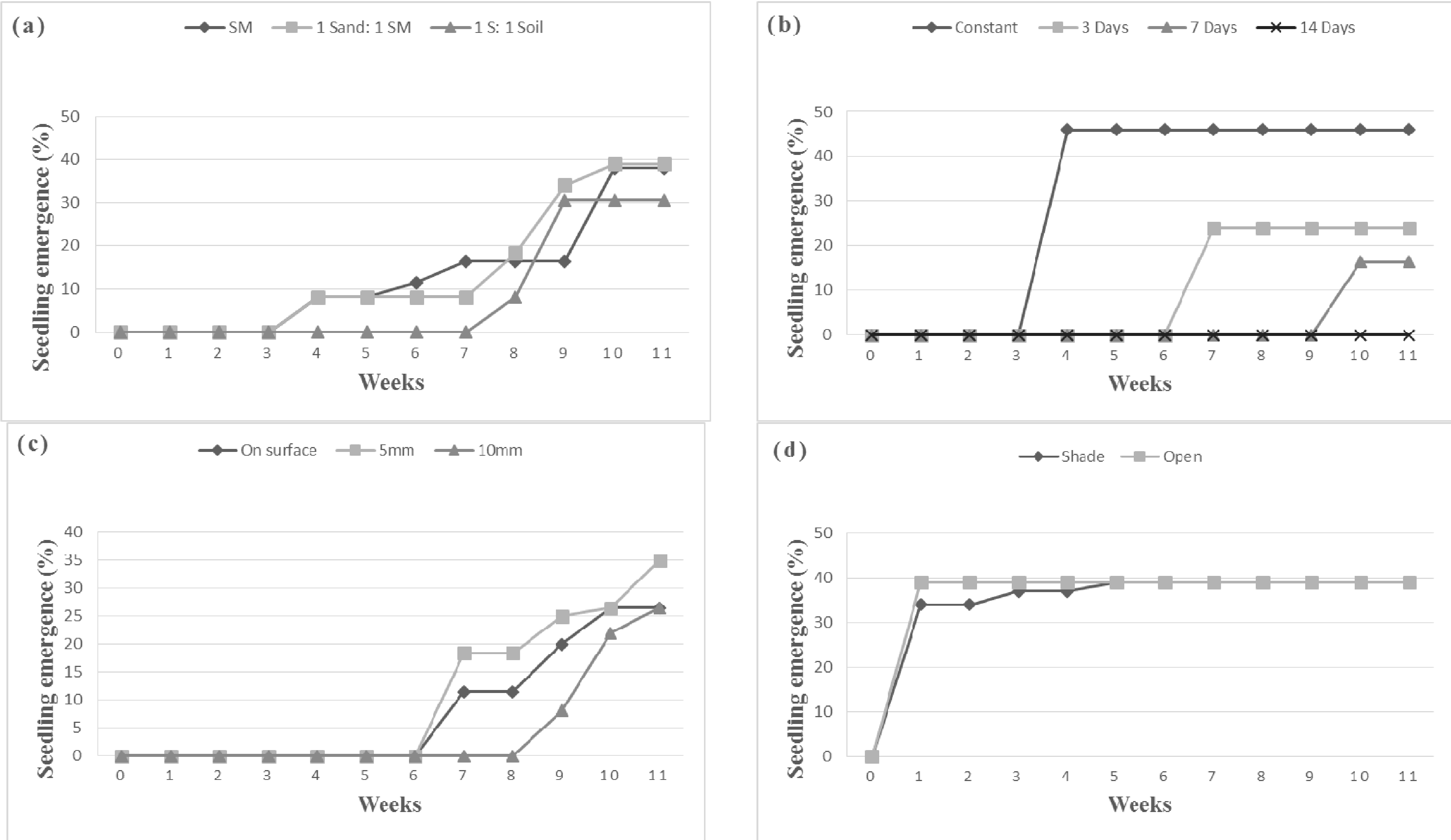


Figure 5.5: Seedling emergence (%) over time for (a) soil media, (b) watering regime, (c) depth of planting and (d) shading aspect.

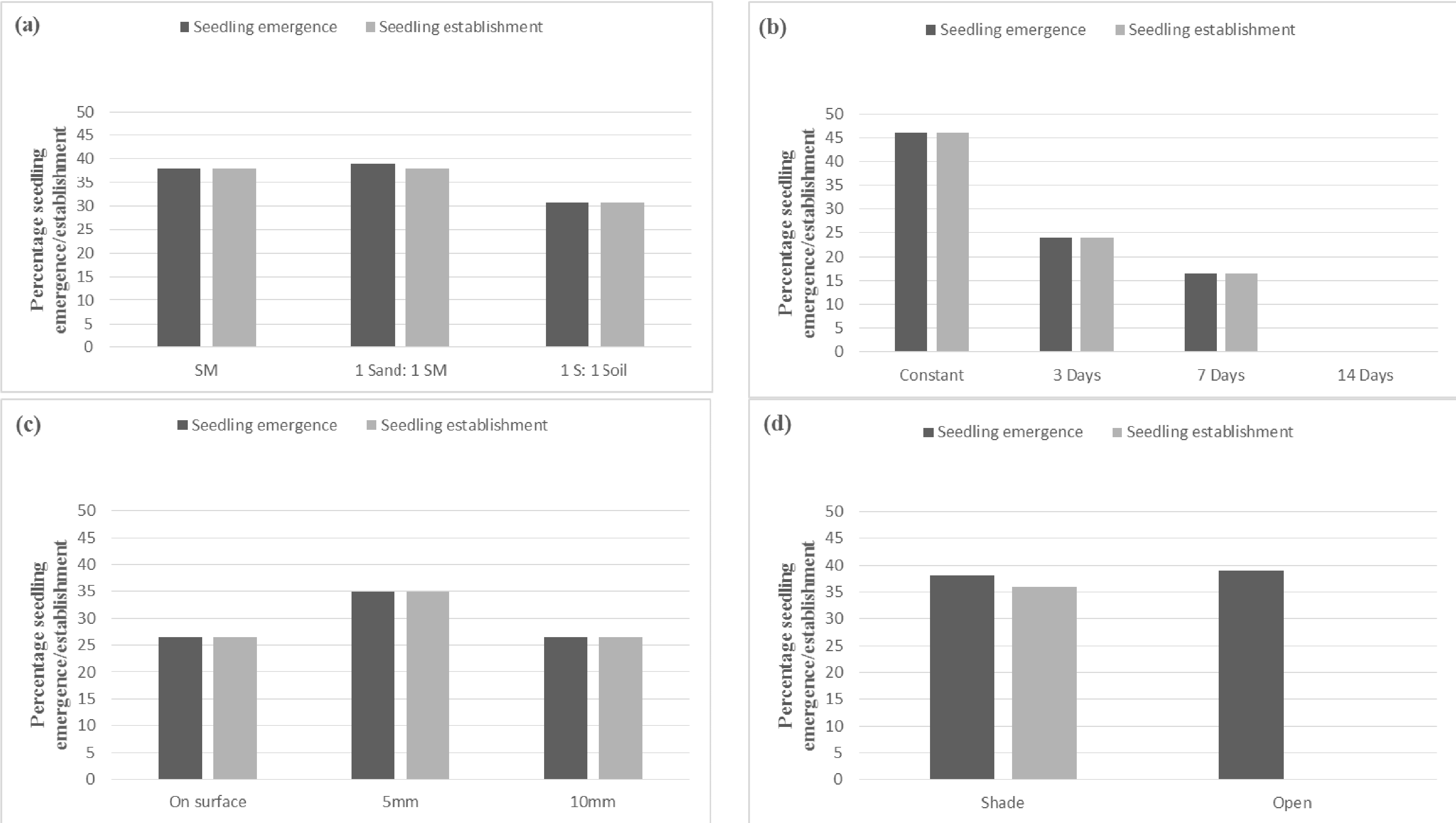


Figure 5.6: The percentage seedling emergence and final seedling establishment after 11 weeks for (a) soil media, (b) watering regime, (c) depth of planting and (d) shading for *Adenium swazicum* as determined under ambient nursery conditions at the Lowveld National Botanical Garden, Nelspruit, South Africa.

Shading had an important effect on seedling survival though 40% seedling emergence was recorded in full sun as well as those under 40% shade netting, the seedlings in full sun all died by the second week, while the seedlings under the 40% shade cloth survived and became established. These seedlings were not watered and received rain only (36mm). This indicates that once minimum temperatures for germination (above 20°C) have been reached, (a) moisture is essential to initiate germination, (b) emerged seedlings do not require constant moisture to survive, and (c) shading is not essential for emergence, but it is essential for seedling establishment. Seedlings observed in the wild were located under the mother plant or other vegetation which provided a microclimate for the seedlings to become established. Since adult *A. swazicum* plants were often recorded in full sun, it is possible that the seedlings which germinate under nurse plants (Figure 5.7) outcompete these plants over time. It is however also possible that the sodic soils with higher clay content could retain moisture for a longer period compared to the Braaks seedlings mix which was used during the *ex situ* experiments.

Figure 5.7: *Adenium swazicum* seedlings in the wild were only recorded under nurse plants and not in full sun.

Despite lower temperatures recorded at LNBG (27.2 ± 0.86°C) when compared to KNP (Skukuza nursery) (32.6 ± 0.90°C), MGT was shortest for seed from population

A (11.5 days) and population C (9.9 days) at LNBG, while the tray at Skukuza had the longest MGT at 17.4 days (Figure 5.9). Final germination and seedling emergence were high for both trays containing seed collected from population A (LNBG-A = 82%; SKUK-A = 94%). However, low final percentage germination (42%) and seedling establishment were recorded for seed from population C (LNBG-C) (Figure 5.9). Germination experiments conducted under controlled conditions and subsequent tetrazolium tests (see section 5.3.1 and 5.3.2) revealed low seed viability for population C. It is likely that the low seedling emergence from population C was due to low seed viability.

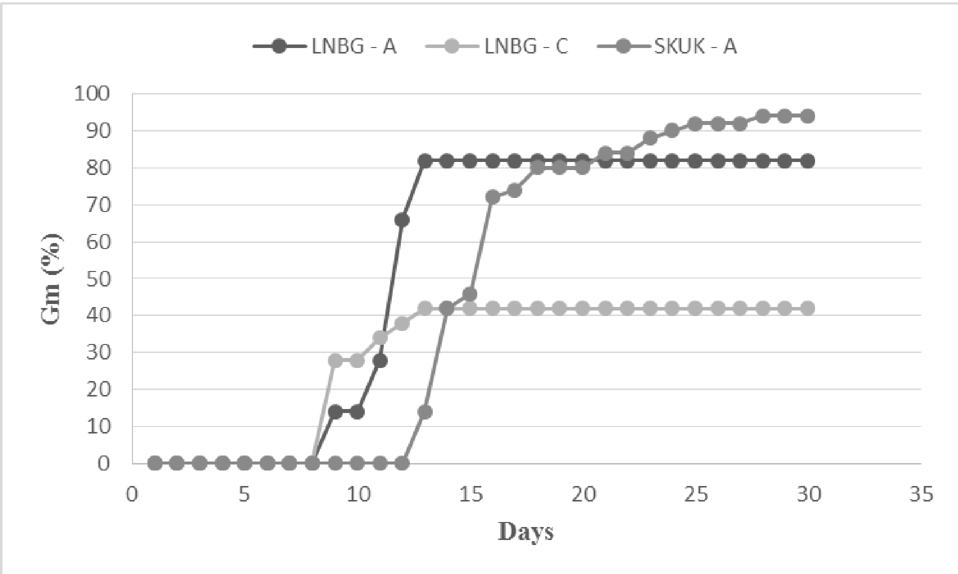


Figure 5.8: The course of cumulative germination time (days) for *Adenium swazicum*.

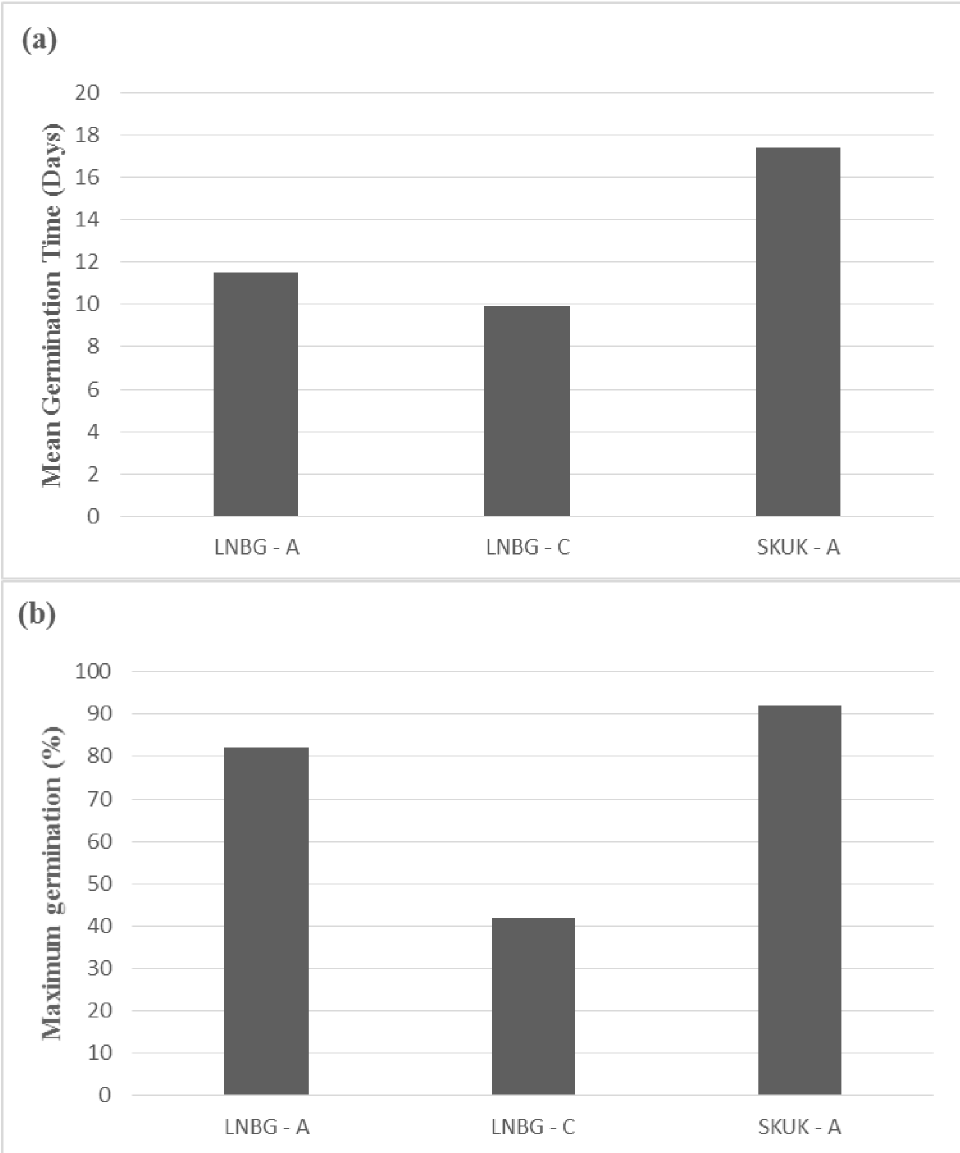


Figure 5.9: The (a) mean germination time (MGT), and (b) final germination percentage for two trays at Lowveld National Botanical Garden and one tray at Skukuza.

5.4 DISCUSSION

Tetrazolium tests revealed that seed viability was significantly lower in population C when compared to seed from population A. This low seed viability of population C was also confirmed in seedling emergence experiments with final germination percentages of less than 42% recorded for seed collected from population C compared to more than 80% germination for seeds from population A.

Greater production of non-viable seeds in populations could be related to inbreeding depression and reduced fitness resulting from homozygosity which can be severe enough to affect the viability of small and isolated wild populations (Keller & Waller 2002 in Mattana et al. 2010). Population C consisted of approximately 140 individuals, which was the largest of the four studied populations, however this population was also located more than 25km away from the nearest known population. Inbreeding depression and/or reduced fitness could result in low seed set (Ronce 2007; Cadre et al. 2008) which could explain the low seed set in Population C in 2009 and 2010. However, long term reproduction monitoring is required to determine if environmental conditions resulted in low seed viability in 2009 and 2010.

Studies from savannas confirm that seed germination of savanna species seldom occurs below 10°C, while the optimal temperatures are between 20°C and 35°C (Hoffman et al. 1989; Baskin & Baskin 1998; Stevens et al. 2014). This was also confirmed to be the case for *A. swazicum* where high germination success was achieved at temperatures between 25°C and 35°C, with no germination recorded at 10°C or 5°C and low germination recorded at 15°C and 40°C. Once suitable germination temperatures were reached, seed germination of *A. swazicum* was initiated within 48 hours. Rapid germination is a common occurrence for arid-adapted plants, which allows the seed to take advantage of short window periods when water is available (Wilson & Witkowski 1998; Daws et al. 2002; Kos & Poschlod 2010). In the presence of moisture, *A. swazicum* reached maximum germination in less than 90 hours at 25°C, 30°C and 35°C and took slightly longer to reach maximum germination (120 hours) at the alternating temperature of 20/30°C indicating that this species also has rapid germination to allow for germination in short window periods of suitable conditions.

In situ seedling observations for *Nerium oleander*, a member of the Apocynaceae family revealed that soil media did not affect germination, although seedlings in open areas died soon after germination (Herrera 1991). The cultivation of *A. swazicum* from seed was highly successful, with the different soil media tested having no apparent influence on seedling emergence and establishment. The depth, at which seeds were planted, did not influence final seedling emergence, although seeds that were planted 10mm below the soil surface took longer to emerge. This indicates that *A. swazicum* seeds have the ability to germinate even if it is covered with soil and/or litter. Bradstock & Auld (1995) found that soil temperatures in areas which have been blackened and vegetation removed due to fire, could be as high as 70°C. Although soil temperature was not measured during this study, it is likely that the barren areas associated with sodic sites will also reach very high temperatures. Shade was not essential for germination and seedling emergence, although it appears to be essential for seedling establishment in the wild. This was confirmed by *in situ* observations, in which all seedlings were located underneath nurse plants with no seedlings observed in full sun during the survey period.

There are four potential limiting factors in plant recruitment, which include limited seed production, competition, microsites and herbivory (Crawley 1990). Seed, germination and seedling emergence experiments for *A. swazicum* revealed that the factors which are likely to limit recruitment for this species are the availability of microsites which provide suitable germination temperatures and herbivory. It has been suggested that the availability of microsites is predominant in plant populations (Fowler 1988; Crawley 1990) although Eriksson and Ehrlen (1992) argued that the dichotomy of seed versus microsite limitation is of minor importance in recruitment limitation for certain species. Although germination in *A. swazicum* was triggered by moisture and temperature, *ex situ* seedling emergence and seedling establishment experiments revealed that seedlings would only survive at suitable microsites that provide sufficient shade. It should be noted that microsite availability is episodic in most habitats, and in many cases, the carrying capacity of an area might fluctuate from generation to generation with seedling establishment in woody plants likely to be episodic (E.T.F. Witkowski unpublished; Crawley 1990). There were a limited number of seedlings present in the four studied populations of *A. swazicum* between 2009 and 2011, but all the seedlings recorded during this period were located under nurse plants or mother plants. However, it is possible higher seed germination and seedling establishment could be recorded in subsequent years when microsite conditions are more favourable. In addition to this, suitable habitat patches close to current populations, which were devoid of any *A. swazicum* during the survey period, could be colonized in years when there is an abundance of seed and suitable microsites.

Soil seed banks play an important role in plant population dynamics through the maintenance and restoration of plant communities in response to environmental disturbance (Ferrandis & Herranz 2001). Seed dormancy and the formation of seed banks within the soil are important factors which prevent seed from germinating under short favourable conditions, especially towards the end of a growing season (Degreef et al. 2002). Plant species that form seed banks usually have hard, seed coats, which are impermeable to water enabling these species to spread their germination over a long period of time (Degreef et al. 2002; Venter & Witkowski 2013). The soft, woody outer layer of their seeds, as well as rapid germination under suitable conditions (moisture and temperature), indicate that *A. swazicum* does not form soil seed banks. It was furthermore found that *A. swazicum* seeds do not display any dormancy mechanism although seed will not germinate at temperatures below 10°C.

6 OVERALL CONCLUSION

This concluding chapter aims to:

- Synthesize the results of this study indicating how the different parts of the study link to each other;
- Discuss the ecology of *A. swazicum* based on the findings of this study;
- Recommend management actions for *in situ* and *ex situ* conservation of *A. swazicum*; and
- Recommend future research to aid in the conservation of this species.

6.1 GENERAL DISCUSSION

The risk of extinction is considered the greatest for species which have small populations and occur in restricted areas (Shaffer 1981; Gaston 2011), with many rare and endangered species occurring in small, local populations in a fragmented landscape (Franzen & Nilsson 2009). This was also the case for *A. swazicum* with the total population in South Africa comprising only1167 known individuals, spread over 17 populations in Limpopo and Mpumalanga Provinces. Large-scale habitat destruction within the historic distribution range of *A. swazicum* has resulted in isolation of most populations occurring outside of large formally protected areas. When a population decreases, the reduction in individuals threatens the genetic diversity of the population, making the population vulnerable to events that might lead to its extinction (Van Dyke 2003). Small populations might also be more vulnerable to the impacts from insect herbivory with some studies revealing that plants which sustained extensive herbivory had a significant reduction in number of flowers produced (Ehrlen 1995; Pfab & Witkowski 1999a). Insects such as *Chrysolina*

(Naluhia) confluens (Gerstaecker) (Coleoptera: Chrysomelidae) (Leaf Beetle), *Phymateus morbillosus* (Linnaeus) and *Phymateus viridipes* (Stal) (Orthoptera: Pyrgomorphidae) (Milkweed Locust) had a significant impact on the production of flowers in the studied *A. swazicum* plants with an increase in herbivory resulting in far fewer flowers produced per plant. The ability of a plant to regenerate from taproots or lignotubers after destruction of the aboveground biomass enables a species to persist in ecosystems with recurrent disturbances (Pate et al. 1990; Morena & Oechel 1991; Bond & Midgley 2001). *Ex situ* disturbance experiments revealed that adult *A. swazicum* plants are strong resprouters even if aboveground parts are destroyed more than once in the same season. Although this strong resprouting ability of *A. swazicum* enables it to withstand extensive herbivory, it has been found elsewhere that an increase in fire intensity decreases survivorship (Moneno & Oechel 1990; Lesica 1999). It is therefore possible that *A. swazicum* is restricted to sodic sites due to the higher intensity fires occurring in the upland and riparian zones surrounding sodic sites and that the higher intensity fires result in the mortality of seedlings, juveniles and even adult plants.

The reproduction (biology and ecology) of *A. swazicum* was largely unknown with no previous studies done to determine the number of flowers and subsequent follicle and seed production. Low insect activity was observed in flowering *A. swazicum* populations although a fast flying *Sphingidae* Linnaeus 1758 (Hawk Moth) was observed at *A. swazicum* flowers after dusk, just before complete darkness and it is likely that this is one of the pollinators of *A. swazicum.* The percentage of sampled plants that produced flowers in 2010 was significantly different between the four populations, with 92% of the sampled plants in population A producing flowers compared to population B in which only 32% of sampled plants flowered. In the same year, population A, which had the largest floral display, produced 221 follicles from 3439 flowers, but due to follicle and seed predation, only 19 of these remained intact. Fruit (follicle) set (percentage of flowers which produced follicles) in 2010 was the highest in population B (11%) compared to populations A (6.4%), C (6.2%) and D (1%). However, population B produced significantly more intact (unpredated) follicles ($X^2_3 = 25.69$; $P < 0.001$) than the other three populations in 2010. At the same time, population D failed to produce any seed in 2010 and it is possible that isolation as well as small population size resulted in very low pollinator activity and therefore reduced reproductive success for 2010. Despite the higher number of intact follicles recorded in population B in 2010, population C released an estimated 2363 viable seeds compared to far fewer in populations A (257), B (242) and D (0).

Tetrazolium tests showed that seed viability was significantly lower in population C compared to seed from population A (ANOVA: $F_{2,16} = 58.29$; $P < 0.001$) and since population C was located more than 25km away from the nearest known population of *A. swazicum*, it is possible that inbreeding depression and/or reduced fitness resulted in low seed set and seed viability. High seed germination success was achieved at temperatures between 25°C and 35°C which is consistent with studies conducted for other savanna species (Hoffman et al. 1989; Baskin & Baskin 1998; Stevens et al. 2014). The cultivation of *A. swazicum* from seed under ambient nursery conditions was highly successful, with the different soil media and depth of planting having no apparent influence on seedling emergence and establishment. However, the emergence of seedlings was highly dependent on the availability of moisture, while shade was essential for seedling establishment/survival.

6.2 CONSERVATION RECOMMENDATIONS FOR *ADENIUM SWAZICUM*

6.2.1 Legislation

A common misconception is that providing an endangered species with legal protection will save the species (Van Dyke, 2003). The failure of legislation to protect plant species in South Africa has been illustrated in medicinal plants (Botha et al. 2004b) and cycads (Donaldson and Bosenberg 1999; Golding and Hurter 2001; Raimondo & Donaldson 2003; Retief et al. 2014). *Adenium swazicum* has been protected since 1983 under Schedule 11 of the Transvaal Nature Ordinance (No. 12 of 1983), and subsequently under Schedule 12 of the Mpumalanga Nature Conservation Act (Act No. 10 of 1998) as well as Section 56 and Section 57 of the National Environmental Management: Biodiversity Act (Act 10 of 2004) (NEMBA). According to the Threatened or Protected Species Regulations (TOPS), which were published under Section 57(1) of NEMBA (Regulation Gazette Number R.8638), the principle requirement of TOPS is: "A person may not carry out a restricted activity involving a specimen of a listed threatened or protected species without a permit". In addition to this, nurseries and wildlife traders wishing to trade in any species listed on TOPS were required to register the breeding facility (nursery). Although the protection of *A. swazicum* has been well legislated, lack of enforcement resulted in the continued decline of *A. swazicum* due to harvesting and habitat destruction with one population reaching critically low levels between 2008 and 2010 (pers. obs.). Legislation also makes it increasingly difficult to obtain permits, which tends to discourage research on protected species, often having a significant impact on the effective conservation of these species (Ralls & Brownell 1989). This was also applicable to *A. swazicum* since essential research questions regarding regeneration and propagation were dependant on "restricted activities" which included the following:

- Picking parts of any specimen of a listed or protected species (collection of seed);
- Exporting from the Republic (e.g. export of seed to the Kew Millennium Seed Bank project for research and seed banking);
- Having in possession or exercising physical control over any specimen (*ex situ* collections at the LNBG used for propagation and disturbance experiments); and
- Growing, breeding or in any other way propagating a specimen (propagation activities at LNBG and Skukuza Nursery).

Research activities on *A. swazicum* for this MSc were conducted under the auspice of the South African National Biodiversity Institute (SANBI) and research permits were therefore applied for through the Department of Environmental Affairs (DEA). However, according to M. Boshoff (Deputy Director Policy Development: TOPS regulations and CITES) (pers. comm. 2009), DEA was not able to process the permit application for *A. swazicum* since TOPS did not make provision at that stage, for research activities conducted by other governmental organizations. Lengthy discussions, which included using alternative research subjects, resulted in the issuing of a permit for research activities on *A. swazicum* by the Mpumalanga Tourism and Parks Agency in 2009.

Ex situ approaches for conserving rare plant species include commercial propagation and trade (Affolter 1997) for various reasons, one of which is to satisfy the horticultural demand (Winter & Botha 1992; Shirey et al. 2013). In contrast to this, many conservationists believe that trade in endangered species will be a threat to that species, and when uncertainty exists concerning the impact of trade, a precautionary approach is taken (Ginsberg 2004). The LNBG through its threatened plants project has been propagating threatened plants species, mostly *Encephalartos* since the early 1990's, and through this highly successful propagation programme, more than 8 000 *Encephalartos* seedlings have been sold between 2006 and 2011 (pers. obs.). Since *A. swazicum* was considered threatened due to collection for horticultural purposes (TSP 2008), large numbers of propagated seedlings have been made available to the public since 2006. However, failure to register the LNBG under Section 57(1) of NEMBA as a trading nursery for *A. swazicum* has resulted in the withdrawal of this species from the market. Subsequently, amendments to the TOPS regulations has been published for comment on the 16^{th} of April 2013 (Government Gazette No. 36375), in which restricted activities make a distinction between <u>artificially propagated</u> and <u>wild collected</u> specimens and it is proposed that artificially propagated specimens can be traded. Since my results show that *A. swazicum* can easily be propagated in large numbers and impact from the horticultural market was considered minimal at the time of the surveys, it supports the trade in artificially propagated specimens of *A. swazicum.*

6.2.2 *Ex situ* conservation

Ex situ conservation is considered a tool to ensure the survival of a wild population and should preferably be established within the distribution range or region of the taxa (Rempel 1992; Guerrant et al. 2004). Irrespective of the locality of the *ex situ* collection it should be managed so ensure minimal loss of capacity for expression of natural behaviours and loss of ability to later again thrive in natural habitats (IUCN SSC 2002; Taylor 2007). *Ex situ* collections play a critical and fundamental role in combating extinctions (Maunder & Byers 2005) and the management of *ex situ* populations must therefore minimize any deleterious effects associated with *ex situ* conservation such as loss of genetic diversity, artificial selection, pathogen transfer and hybridization (Taylor 2007).

There are various *ex situ* conservation methods which include field genebanks (living collections), *in vitro* storage methods and pollen banks (Laliberte 1997; Li & Pritchard 2009; Shirey et al. 2013). With more than 2700 Botanic Gardens around the world, the contribution made by horticulturists towards discovering, documenting and conserving plant diversity is substantial (Havens et al. 2006). In South Africa's National Botanical Gardens, there has been an effort to conserve threatened indigenous plants since the establishment of Kirstenbosch National Botanical Garden in 1913 (Willis & Van Wyk 2006). The LNBG, which was established in 1970, collected 120 rare and endangered plants in 40 years (Botha et al. 2000). According to W. Froneman (pers. comm. 2014), the total number of rare and endangered plant species currently in LNBG is 150. One of these is *A. swazicum*, which was first collected as seed from wild populations in 2003 with more than 100 of these adult plants now growing in field gene banks in the LNBG. The use of *ex situ* living collections/genebanks as conservation collections have been questioned due to the high risk of hybridization, introduction of pathogens and inaccurate record keeping resulting in the loss of original collection and/or locality information which is essential for reintroduction (Guerrant et al. 2004). In addition to this, it has been argued that plants in living collections may evolve naturally and shift their genetic makeup into a modified form of its wild ancestor due to fertilization, pest control and artificial watering regimes (Rempel 1992; Guerrant et al. 2004; Havens et al. 2006). Despite this, baseline data collected in botanic gardens can help develop an understanding of plant behaviour, development and growth under various conditions, which could be useful to understand plant biology and direct restoration efforts (Ali & Trivedi 2011).

The *ex situ* collection of *A. swazicum* at LNBG provided valuable insight into the effect of herbivory and fire on *A. swazicum* as well as establishing germination parameters which explained limitations on seed germination and seedling establishment in the wild. However, since field genebanks/living collections require a great deal of space as well as substantial financial investment linked to intensive management, future *ex situ* conservation activities for *A. swazicum* should rather focus on seed banking and augmenting field populations so that they have at least minimum viable populations. The Kew Millennium Seed Bank Project in England is the largest collection of *ex situ* wild species and currently holds more than 27 000 species (Ali & Trivedi 2011), 1 500 of these are of threatened, endemic or useful species from South Africa (Willis & Van Wyk 2006). Seed banking provides a low cost, effective and efficient way to protect a wide range of diversity (Havens et al. 2006), however Guerrant et al. (2004) caution against one-time collecting efforts for seed banking since the genetic characteristics of stored seed will change with time. Seed collections spread over several seasons, will capture more genetic diversity since each year the weather conditions will favour some genotypes over others, however seed collection should not further endanger wild populations (Guerrant et al. 2014). Follicle and subsequent seed production of *A. swazicum* in 2009 and 2010 was significantly different between populations but also varied between the years in the same population. It is not considered that seed collection, spread over several seasons will have a detrimental effect on *A. swazicum* in the wild, if the total number of seed collected at any time does not exceed 10% of the seed produced. Furthermore, the banking of seed collected from populations outside of protected areas should be prioritized since these populations are faced with high levels of habitat destruction and exploitation.

6.2.3 Recommendations for populations in protected areas

Based on the findings of this research study, it is likely that the survival of *A. swazicum* in the wild will depend on the effective conservation of populations in formally protected areas. Although exploitation of *A. swazicum* in formally protected areas was not recorded during this study, it is likely that medicinal plant harvesters will focus on protected areas once the populations on communal and private land have been depleted. It is therefore essential that anti-poaching patrols be conducted in areas, which contained large populations of *A. swazicum*. In addition to this, the following is recommended:

- Continued monitoring of *A. swazicum* with special reference to follicle production and seed set;
- Annual population counts to determine population trends over time; and
- Seed collection (not more than 10% of the viable seed) for seed banking spread over several seasons for all populations.

6.2.4 Recommendations for populations on private and communal land

Populations outside of formally protected areas were impacted by habitat destruction as well as harvesting for medicinal purposes. Many populations and localities of various plant species in South Africa have been extirpated for medicinal and household use (Botha et al. 2004b; Williams et al. 2013) and *A. swazicum* was no different. One population (P) decreased by 80% in two years with other populations also showing signs of harvesting. Botha et al. (2004a) found that there was a large informal traditional medicine market in Mpumalanga, with vendors operating mainly near taxi ranks and at pension markets. Although Botha et al. (2004a) did not quantify the use of *Adenium* species (*A. multiflorum* and *A. swazicum*) within Mpumalanga; vendors did trade in these species and indicated that there was a higher demand for fresh plants. It is therefore unlikely that the harvesting of *A. swazicum* will cease and since plants on communal land cannot be secured, enhancement or restoration of these populations is not considered a viable option at this stage. However, as part of Project MGU – Useful Plants Project, twenty adult *A. swazicum* plants were donated to the Mvagatini Primary school adjacent to population P, which has been decimated by medicinal harvesters. The adult plants were grown from seeds collected from population O in 2003, and it is therefore likely that these plants will produce and disperse seed into the natural areas surrounding the school.

Population C was located on a private sugarcane farm, which was surrounded by communal land, and although no plants were harvested in 2009 and 2010, numerous snares were collected within the area where *A. swazicum* grew, and it is likely that this population will be targeted in the near future. In addition to this, low seed viability was recorded in seeds collected from this population in 2009 and 2010. Although further research is needed to confirm this, it is possible that this population could be suffering from inbreeding depression due to the decimation of surrounding populations.

Population D which was located within a residential development need to protected through home owner education. In addition to this, more research is required to determine if the reproduction failure recorded in this study is a long term concern.

6.3 FUTURE AREAS OF RESEARCH

From this study, several areas or further research have been identified:

- Pollinator studies for *A. swazicum* to identify the primary pollinator(s) and ensure its effective conservation;
- Monitoring of populations to determine reproduction over time;
- Genetic studies to determine the extent of inbreeding in small, isolated populations located outside of formally protected areas;
- Determine the effect of fire on seedlings and juvenile plants less than 24 months old;
- Distribution and population sizes of *A. swazicum* in Mozambique and Swaziland.

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APPENDIX A: DEVELOPING PROPAGATION PROTOCOLS FOR MEDICINAL PLANTS IN MPUMALANGA FOR CONSERVATION AND EDUCTION (PROJECT M.G.U)

APPENDIX B: MEDICINAL PLANTS PROJECT GARDEN: A PRODUCT OF THE UPP – PROJECT M.G.U