FACTORS AFFECTING INITIAL SEEDLING ESTABLISHMENT OF *HOODIA GORDONII* IN !KHOB !NAUB CONSERVANCY, NAMIBIA, AFRICA

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BY

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A thesis submitted in partial fulfilment of the requirements for the degree of

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DECLARATION

This thesis represents the original work of the author and has not been submitted in any form for any degree to another University. Where use has been made of the work of others, it has duly been acknowledged in the text.

Signature……..………………………………

Melvin Mickson Lisao

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Windhoek, Namibia

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ABSTRACT

It is envisaged that population numbers of the species *Hoodia gordonii* will decline rapidly because of its commercial potential. To ensure that genetic materials are not lost due to lack of information, the research was conducted in order to examine the translocation potential of *Hoodia gordonii,* by assessing various factors affecting its initial establishment and survival. The study was carried out in the !Khob !Naub conservancy, located in the southern arid region of Namibia.

Local seeds from the conservancy were germinated and seedlings translocated into the natural environment. A total of 320 seedlings were transplanted in a 2 x 2 x 2 factorial plot with shade, water and protection against predation as factors. The treatment combinations (shade, water and protection against predation each at two levels, present or absent) were applied to two different age groups. The study found that due to adaptation of the species and harsh environmental conditions in which it occurs, a combination of factors were required for the seedlings to survive and establish. Neither shade nor water prevented mortality resulting from predation. However their effect promoted plant vitality and growth due to their role in preventing abiotic stress (moisture loss, high temperature, evaporation, heat, and radiation). While protection against predation played an important role in the prevention of predation (biotic pressure), its role in plant vitality and growth was minimal.

Translocations of seedlings at three months led to higher mortalities as a result of abiotic stress, while those that were transplanted at the age of one year appeared to be more resistant to abiotic stress. In both age groups predation affected seedling survival.

All factors (shade, water and protection against predation) in combination, appeared to facilitate the necessary support for early establishment of the seedlings. Given growing evidence that edaphic differences shape plant communities in arid environments, it is suggested that *Hoodia gordonii* can be translocated successfully using already established plant species as shields against biotic and abiotic pressure.

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ABBREVIATIONS

CBNRM	Community Based Natural Resource Management
CITES	Convention on International Trade of Endangered Species
CIIR	Catholic Institute for International Relations
CSIR	Council for Scientific and Industrial Research
DAAD	German Academic Exchange Service
DSS	Directorate of Scientific Services
ICEMA	Integrated Community-Based Ecosystem Management Project
IUCN	International Union for the Conservation of Nature and Natural Resources
MET	Ministry of Environment and Tourism
NBRI	National Botanical Research Institute
NACSO	Namibia Association of CBNRM Support Organisation
SCSA	San Council of Southern Africa
SPAN	Strengthening the Protected Area Network

CHAPTER ONE

INTRODUCTION

1.1 Background

Biodiversity is a concept that is widely accepted and used by those concerned with the survival of our planet. It refers to the variety of life forms on earth**,** their genetic variability, variety and abundance of species and complex ecological interrelationships between them (Spellerberg, 1996; Caughley and Sinclair, 1994; CIIR, 1993). In recent years the importance of biodiversity has gained prominence among concerned conservationists, scientists and politicians. In support, Brownlie *et al.* (2005) indicate that biodiversity conservation has become an integral part of our economy, livelihoods and quality of life. Namibia forms part of the world's richest regions as far as biodiversity is concerned. The South Western Cape is the region in which over two-thirds of the plant species and seven plant families are endemics (Brown and Botha, 2004). Additionally, it is a regional and world centre of succulent concentration (Günster, 1992b).

However the world's biodiversity is declining at unprecedented rates (Spellerberg, 1996; Caughley and Sinclair, 1994; CIIR, 1993). Succulents in Namibia and elsewhere in the world are not spared from the extinction crisis, like most other life forms, they are threatened by the fast-growing human populations and its various consequences. The word succulent is defined by Von Willert *et al.* (1992) as those plants adapted to dry environments and possessing quality tissues that appear to have high water content and are fleshy or juicy.

According to Sauer (1995) humans have historically underestimated the magnitude of their impact on existing ecosystems and they also continue to underestimate what needs to be done in order to reverse the damage caused. On the other hand, people do not only cause ecological disruptions, but are part of the environment in which they depend, make decisions and take action that determines the state of the environment now and in future. In the quest to halt species loss, improving reproduction and production has become a priority. Many different methods of halting species loss have been developed, including restoration ecology. Anderson *et al.* (2004) defines restoration ecology as the intentional alteration of a site with the aim of restoring the degraded system to some form of cover that is protective, productive, aesthetically pleasing, or valuable in a conservation sense. This study recognises that commercially valuable succulents such as *Hoodia gordonii* in Namibia are threatened by extinction in their natural environment. Furthermore, it recognises that our knowledge of such plants and their environments is riddled with large gaps that need to be addressed.

1.2 Rationale

Hoodia gordonii has attracted attention and has made international headlines in terms of commercial importance (social and economic). However, as far as conservation of the species is concerned, there has been little attention. Whilst a lot of work has been done on other individual succulent species in previous studies worldwide, little is known about the ecology of *Hoodia gordonii*. In the absence of such knowledge, the threat to the genetic materials remains unknown. At the same time, there is a continued interest to harvest seeds from wild plants for artificial propagation. Similarly, illegal harvesting and trade of plant material also continue to increase.

1.3 Purpose of the study

The research is an experimental based study which seeks to describe, analyse and explain the factors influencing the recruitment, performance and survival of the species within its natural environment. The purpose of the study is based on the realisation that *Hoodia gordonii* is under increasing threat and therefore faces a constrained future, with unknown consequences; including the possibility of reduced recruitment due to possible inadequate supply of seeds resulting from direct human activities, i.e. seed harvesting and illegal harvesting of productive plants. The overall goal is therefore to support conservation, sustainability and prudent management of local genetic material, while improving income generation and contributing to the social wellbeing of the people.

1.4 Scope of study

The possibility of initiating a recovery process of the species (*Hoodia gordonii)* is investigated. Whilst seeds are identified as a critical life history stage in plant development, seed germination and initial seedling development are equally important as seedlings may often be subjected to high mortality (Zheng, 2004). As the basis on which the success of a restoration or recovery process can be improved, the research investigates the initial establishment, survival and factors affecting the establishment of seedlings planted out.

1.5 Study area

The study was carried out in Namibia, an arid country located on the south western part of the African sub-continent. Namibia lies between latitudes 17° 00" and 22° 00" South and longitudes 11° 00" and 25° 00" East (Mendelsohn *et al.*, 2002). Namibia experiences variable, unpredictable and limited rainfall (Günster, 1994). As a result, it is a driest country and more vulnerable to drought than any other country in Sub-Saharan Africa (Mfune and Ndombo, 2005; Sweet, 1998; Booth *et al*., 1994). Large parts of Southern Africa are subjected to regular and unpredictable droughts, and this may be one reason why a succulent life style has diversified extensively in the region (Smith and Crouch, 2004).

1.5.1 Study site

The study was carried out within two locations; the germination of seeds was conducted in the green house of the National Botanical Research Institute (NBRI) in Windhoek, the capital city of Namibia. The translocation trial was conducted at Blaukehl located at S: 26° 14’ 021” E: 18° 27’ 788” within the !Knob !Naub conservancy in the Karas region. The !Knob !Naub conservancy is 2 747 square kilometre in size located about 40 kilometre North East of Keetmanshoop (Fig. 1). It is found in the Nama Karoo biome. This biome supports a varied assemblage of plant communities ranging from deciduous shrub vegetation to perennial grassland and succulent shrubs. The area falls within the semi-desert savannas of the Karas region receiving about 150mm of rain per year (NACSO, 2004; Mendelsohn *et al.*, 2002). The average maximum temperatures are 34-36 ˚C during the hottest months of the year, while average minimum temperatures range within 4-6 ˚C during coldest months of the year. Not only is the area among those that receive the least rain on average, but it also falls within the areas of highest average rates (more than 2660 mm per year) of evaporation (Mendelsohn *et al.*, 2002).

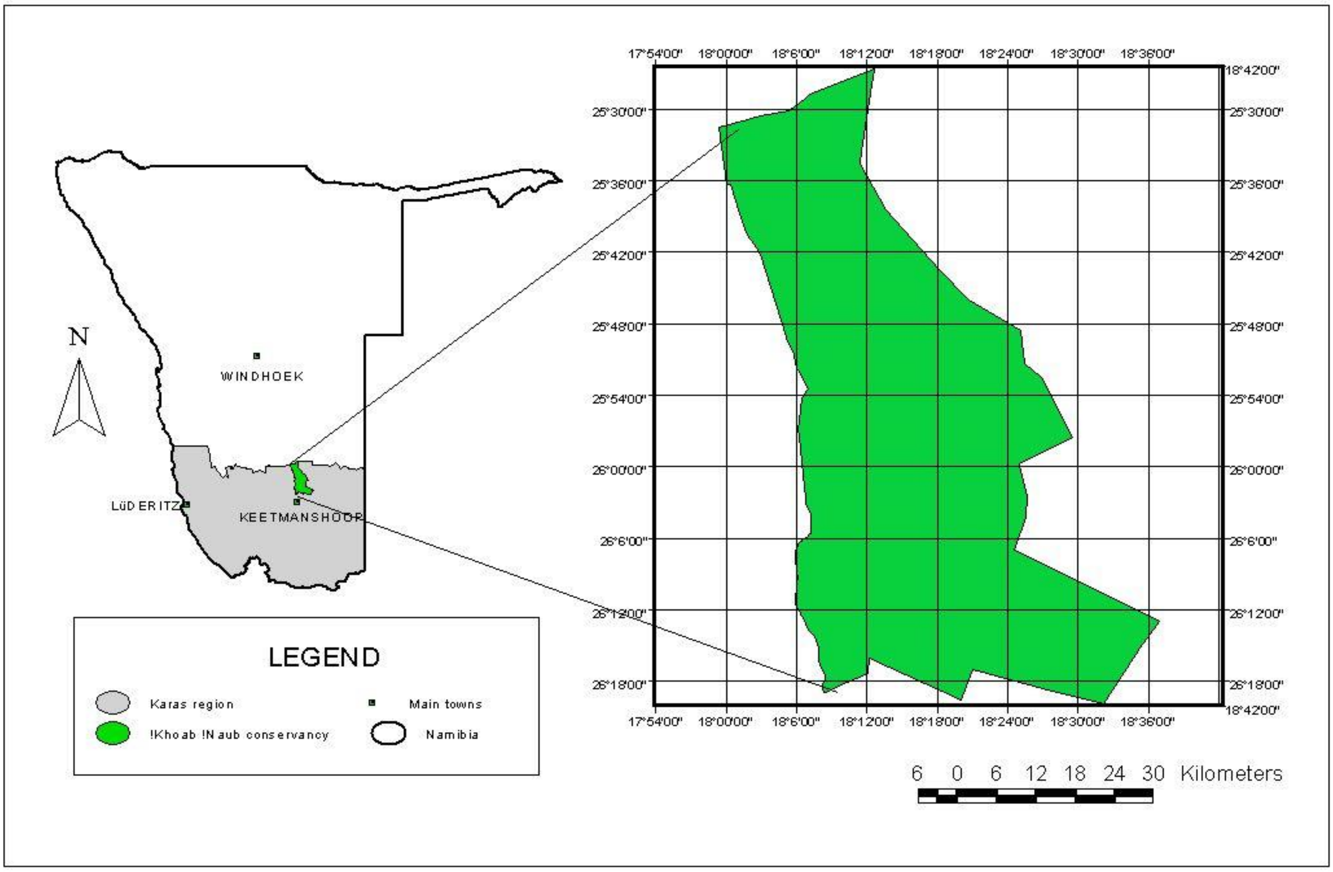

Figure 1: Map showing the location of !Khob !Naub Conservancy in Namibia.

1.5.2 Soil

Soil provides the growing medium for most plants. According to Mendelsohn *et al.* (2002) the support which soil provides depends on the depth, structure, and chemical composition. In turn, these qualities determine how much water the soil can retain, the depth to which plant roots may extend and how much plant nutrition the soil can provide to the plant. The combination of these qualities in one area determines the presence and absence of species, thus, the diversity and structure of the vegetation and plant community.

Mendelsohn *et al.* (2002) describe the Namibian soils to vary greatly, with deep sand in the Kalahari, clayey and salty soils in the Cuvelai, and shallow, mica-rich soils found in many rocky areas. The soil that is considered being the most fertile in Namibia is not rated highly on the world scale as far as soil potential is concerned. In addition most of these soils occur in areas where rainfall is low (Sweet, 1998). This is one of the reasons why most of the Namibian soils are not suitable for crop growth. In general, there is a great degree of local variation in the properties of soils in Namibia, where small patches of fertile soils are often surrounded by large areas of infertile grounds (Mendelsohn *et al*., 2002).

The soil formation found within the study site is Leptosols. This is the dominating soil type found in southern and north-western Namibia. These are course-textured soils characterised by their limited depth caused by the presence of continuous hard rock, a highly calcareous or cemented layer within 30cm of the surface. Much of Leptosols contains gravel and is considered as the shallowest soils in Namibia. As a result their water holding capacity is low and vegetation in areas in which they occur is often subjected to droughts. Moreover, there are normally high rates of water runoff and water erosion when heavy rains fall (Mendelsohn *et al*., 2002).

1.5.3 Climate

1.5.3.1 Temperature

In general, Namibia is considered to be a hot country, but temperatures vary greatly on a spatial scale, seasonally and within a single day. Therefore most Namibian plants and animals have evolved to tolerate a wide range of temperature variation. Accept for the much cooler coastal belt, most of Namibia have average maximums of over 30°C during the hottest months of the year. The study location falls within the central areas of southern Namibia, which are considered to be the hottest part of the country, with an average maximum of more than 36°C. Most parts of the country have an average minimum temperature less than 10°C (Mendelsohn *et al*., 2002). According to the data obtained from Namibia Meteorological Services (2006), the mean minimum and maximum temperature for the study area in April 2006 was 14.8°C and 27.8°C respectively, while the minimum and maximum temperature for August 2006 was 6.6°C and 22.7°C respectively.

1.5.3.2 Rainfall

Namibia is a dry country, in which rainfall is low, extremely variable and unpredictable, while droughts are frequently experienced. The amount of rain varies in a rather smooth gradient from the wettest most tropical areas in the north-east to the extremely arid desert in the west (Mendelsohn *et al*., 2002; Sweet, 1998). The highest annual average rainfall is approximately 700 mm received in the East-Caprivi (Sweet, 1998). The south western part of Namibia (along the coast) receives the lowest annual average rainfall less than 50 mm (Mendelsohn *et al*., 2002). The gradient of average rainfall fades off towards the west and south of the country.

The study site is located in areas where the average rainfall ranges between 100-150 mm per annually. Most rains recorded in the area falls between October and May, while a significant amount between January and March (Mendelsohn *et al*., 2002).

1.5.3.3 Humidity

Namibia is generally a dry country, and lack of moisture in the air results in reduced cloud cover and rainfall. Average humidity in the study area including most parts of the country is 20% from September to November. These months are less humid because of radiation and higher temperatures. The study was not affected by this climatic condition because it was conducted outside the dry period. In most humid months, the average humidity values are higher than 80% for northern Namibia, compared to 50-60% for southern Namibia (Mendelsohn *et al*., 2002).

1.5.3.4 Evaporation

Rates of water loss through evaporation in Namibia is determined by several factors including temperature, humidity, wind speed, atmospheric pressure and surface areas of open waters. Whilst most of Namibia has high average temperatures and low relative humidity a volume of the little rainfall that it receives is evaporated (Sweet, 1998), resulting in less water available to plants and animals. Although the rates of evaporation are high through the country the southern parts loose more water through evaporation than north-eastern and coastal areas (Mendelsohn *et al.,* 2002).

1.5.4 Biodiversity and people in Namibia

On a global level, it is estimated that more than one species go extinct daily (Baillie, 2004). Important ecological roles and possibilities for future use that existed within these species are normally lost with them. It has been widely argued that the nature and extent of environmental problems have increased dramatically over the last century. Reasons for this expansion are many and often linked to increased pressure on resources, increased population, unsustainable practices, ideas of progress and global inequality among others. All these and their causes are linked to social, cultural and economic practices. According to Mendelsohn *et al.* (2002), Namibia depends to a greater extent on resources derived directly from the land; with approximately 71% of the people living in rural areas dependent to a greater or lesser extent upon it for their livelihoods.

Namibia is a lower middle-income country with perennial food deficits. It relies on importing food (about 50 percent of cereals) from outside the country to meet food requirements of its population (Namibia Early Warning and Food Information System, 2006; Sweet, 1998). Food shortage in rural areas during periods of droughts is a major concern. While the country's economy is heavily dependent on the mining sector, the agricultural sector is the main source of employment and livelihood for the population. Approximately 85% of Namibia's surface area is utilized for various sectors of agriculture or related activities. The agricultural sector is one of the major contributors to the Gross Domestic Product. It currently contributes more than 6.5% to the overall economic activity (Sweet, 1998).

People in rural areas engage in subsistence agriculture for their livelihood. Extensive livestock production is thus the major agricultural activity. According to Mendelsohn *et al.* (2002), the livestock population in Namibia has dropped in commercial land, while the opposite has happened on communal land, with cattle numbers almost doubling and goats increasing up to 40% between 1988 and 2000. This is attributed to the availability of income that could be invested in large herds as capital investment.

In contrast to income generated and capital investments, heavy grazing by livestock is considered to be among the main threats to floristic diversity of arid and semi-arid regions world wide (Riginos and Hoffman, 2003). Milton and Dean (1995) indicate that sustained heavy grazing in succulent Karoo tends to result in increased cover of unpalatable perennials with decreases in succulent perennial cover and diversity. However, it is not known how grazing negatively affect stem and leaf succulents. It is inevitable that all these factors are directly or indirectly affecting *Hoodia gordonii*.

1.5.5 Biodiversity and the Namibian legislation

Namibia is one of the few countries that embrace the protection of biodiversity in its constitution. Biodiversity or natural resources in Namibia are protected by three legislations, the Nature Conservation Act No. 4 of 1975, the Forestry Ordinance No. 37 of 1952, and the Forestry Act No. 72 of 1968. For many years the Namibia people were denied rights to utilise and manage resources within their areas (Owen-Smith and Jacobsohn, 2003). In conjunction with population increase, the situation led to an accelerated poverty and loss of biodiversity. Curtis and Mannheimer (2005), note that enforcement of certain regulations to protect protected species has been insufficient until now.

Since Namibia became independent from South Africa's colonial rule in 1990, there has been a paradigm shift with regard to biodiversity policies and legislation. The shift started with the introduction of Chapter XI, Article 95 (I) of the Namibian constitution stating that: "the state shall actively promote and maintain the welfare of the people by adopting, *inter alia*, policies aimed at maintenance of ecosystems, essential ecological processes and biological diversity of Namibia and utilisation of living natural resources on a sustainable basis for the benefits of all Namibians, both present and in future". Since then, Cabinet has approved the establishment of the legislations that allow communal people the rights to use and benefit from wildlife (Owen-Smith and Jacobsohn, 2003). This led to the amendments of the existing legislations and the introduction of the active Community Based Natural Resource (CBNRM) programme in Namibia. The Amendment Act of the Nature Conservation Ordinance No. 4 of 1975, allows for creation of communal conservancies with rights to use and benefit from wildlife. The Forestry Act No. 21 of 2001 allows the creation of the community forest and aims to reconcile rural development with the conservation of biological diversity by empowering farmers and local communities to manage forest resources.

Els and Bothma (2000), state that linking sustainable utilisation to sustainable development has become an important part of wildlife management policy all over the world. As with most ideas in recent years, biodiversity conservation depends on direct interaction with local communities (Crowe, 1995). According to Owen-Smith and Jacobsohn (2003), the CBNRM programme in Namibia has developed a new vision where users of the local resources take full responsibility for management of those resources. !Khob !Naub conservancy is a registered conservancy in which local management systems provide mechanism for sustainable management of biodiversity. *Hoodia gordonii* is presently prioritised as a critical management and conservation option in the conservancy.

CHAPTER TWO

LITERATURE REVIEW

2.1 Diversity of succulents in Southern Africa

In general terms, Southern Africa is extremely rich in plant and animal life. According to Smith and Crouch (2004) Southern Africa has the most diverse succulent flora in the world. It has the richest temperate flora in the world by far and is accepted as the mega-diversity region of the world (Smith, 2004).

An estimated 10 000 plant species are generally recognized as succulents (Baillie *et al.*, 2004), and 4 674 taxa in 58 families have been recorded in the List of Southern African Succulent Plants (Smith and Crouch, 2004; Smith *et al*., 1997). According to Biggs (2003), South Africa and Namibia together have approximately 4 000 species of succulents.

The Succulent Karoo Biome stretching over parts of both Namibia and South Africa is a centre of succulent diversity (Burke, 2004). Here succulents make up a significant proportion of the plant species present (up to 70 % of species in some places) (Biggs, 2003). According to Günster (1992a) Namibia alone is home to 2 500 different species of succulent plants-more than anywhere else in the world.

2.2 Species description

Hoodia gordonii belongs to the family Apocynaceae. It is a slow-growing perennial, spiny stem succulent, endemic and highly adapted to the drier parts of Southern Africa (Downs *et al.*, 2005; Carr, 2004; Archer and Victor, 2003). Although this species is among the wide spread and abundant species that belong to the genus *Hoodia*, it is sparsely populated and patchily distributed within the area of its distribution. The patchy distribution is believed to be natural and attributed to sedentary habits and the spatial heterogeneity of its natural environment. However, human activities have exacerbated the patchy distribution in certain areas (Downs *et al.*, 2005).

There are approximately 14 species and four infra-specific taxa belonging to this genus (Barsch and Madisa, 2005; Downs *et al.*, 2005). According to Downs *et al.*, (2005), a broader taxonomic interpretation of the genus *Hoodia* recognizes 26 species. Carr (2004) points out that ten species occur in Namibia; five of these are categorized as vulnerable due to their small population number, one is endangered, and four, (including *Hoodia gordonii* and *Hoodia parviflora*) are presently categorized as not threatened. Although *Hoodia gordonii* is categorized as a non-threatened species, it remains protected under the Namibian law and has been listed on CITES Appendix II (Carr, 2004). According to my knowledge no published records exists of its detailed distribution or ecology within the region. Based on general observations and data from the herbarium records of the National Botanical Research Institute, it is believed that *Hoodia gordonii* is mostly distributed in the southern parts of Namibia.

2.3 The commercial potential of *Hoodia gordonii*

Based on traditional knowledge of the Khoisan people, the extracts from *Hoodia gordonii* are reputed to have powerful appetite suppressant qualities (Feord, 2005; Archer and Victor, 2003). The ingredients are believed to work like super powered glucose, which sends messages to the hypothalamus that the stomach is full (Scott, 2006). In recent years the active ingredient in *Hoodia gordonii* that suppresses the appetite has been identified and isolated, a result of over 30 years of research by the CSIR (Council for Scientific and Industrial Research) in South Africa (Carr, 2004; Archer and Victor, 2003).

Since the CSIR in South Africa sold the exclusive rights to a large international pharmaceutical company for further research and development of the products (Anon, 2006; Britton, 2006; Feord 2006; Archer and Victor, 2003), the actual market value of *Hoodia* is still volatile, characterised by high prices for plant material. Despite claims of effectiveness of *Hoodia gordonii* in suppressing appetite and limited research information to support this, there are controversies as to its effectiveness and safety within the field of herbal (botanicals) and food science. According to Scott (2006), some scientists believe that the weight management claims are based on frugal scientific evidence. Similarly claims of finding the ingredient that tricks the brain into thinking that the stomach is full, are obviously not straight forward and are unsubstantiated (Scott, 2006).

However, there are many *Hoodia* products currently on the market in the USA (Britton, 2006) and Europe (Feord, 2005). Britton (2006), note that most of these products are deceitful, especially when claims of efficacy around extraction of the active ingredient are concerned. In support Scott (2006) state that many of these products contain adulterated material and therefore potentially damage the reputation of a fledgling industry. Unilever, a functional food research and product developer, together with their partner Phytopharm, remain confident in the potential of the product. They are currently working on a 21 million US dollar project to develop the extract for an application to weight loss (Britton, 2006; Scott, 2006). According to Britton (2006), a developed product could be on the market by 2007.

Presently, there are some controversies regarding benefit sharing, the intellectual property rights and indigenous knowledge, since Khoisan people have and are still using *Hoodia* (Archer and Victor, 2003). Inspired by richness in terms of biological diversity, Southern Africa is making deliberate efforts to recognise the indigenous San as the holder of the traditional knowledge according to the International Agreement on Biodiversity (Anon, 2006). Efforts are being made to improve the social wellbeing of the local people by sharing royalties from potential sales of the products derived from *Hoodia gordonii*. Anon (2006), states that the first agreement to give the holder of the traditional knowledge a share of the potential profits was signed between the San Council of Southern Africa (SCSA) and the CSIR. Similarly, steps to stop countries who are selling unauthorised *Hoodia* products in order to honour the obligations of the Biodiversity Convention are underway.

2.4 Ecological and economical importance of *Hoodia gordonii*

Succulent plants are of great ecological (Wolf and del Rio, 2003) and economic significance (Lisao and Musiyalike, 2006, Archer and Victor, 2003), particularly in arid and semi-arid parts of the world. Halting the growing extinction (crisis) is a major concern for conservationists, scientists and politicians. According to Anderson *et al*. (2004) the cost caused by the loss of biodiversity on earth are not yet known. However, the loss of ecosystem functioning frequently result in considerable economic cost.

Although little scientific information regarding the ecological importance of *Hoodia gordonii* is available, it is obvious that these plants provide abundant resources in the form of shelter, nectar, fruit, nutrients and water for different types of animals. According to Wolf and del Rio (2003) the functional ecological importance of most succulents as resource providers in arid environments has not been quantified, despite the fact that they produce abundant flowers and fruit. Their evolutionary structure allows them to store an immense amount of water, thereby playing an important role in structuring food webs in ecosystems.

2.5 Interactions and co-existence in the semi-arid environment

Despite the fact that biotic factors e.g. herbivory and trampling, have negative effects on plants (Riginos and Hoffman, 2003), other biotic factors can also act as resources in arid environments (Umbanhower and McCann, 2005; Riginos *et al.*, 2005; Carrick, 2003). Blignaut and Milton (2005) suggested that plants in the Karoo shrubland are often arranged in multispecies clumps on fertile islands. While the reason for this is currently under debate, Riginos *et al*. (2005) attribute this to the fact that the abiotic stress is a determinant of facilitation between co-existing plants.

Patchy availability of water and nutrients, predator - grazing pressure, life stage and other abiotic stresses are amongst the factors that are thought to control plant-plant interactions. In addition a study conducted by Lenz and Facelli (2003) on the invasive stem succulent *Orbea variegate*, found that shade was the main mechanism determining the plant-plant interactions. Mycorrhizal fungus is another factor that has been proposed to mediate co-existence (Umbanhower and McCann, 2005). Although some of these factors are not investigated in the present study, an understanding of their role in determining survival of target species will inform a desirable translocation practice.

2.6 Environmental stress and plant development

Environmental factors temporarily or consistently constrain the growth, development and reproduction of plants (Grime, 1984). According to Schemske *et al*. (1994), ecological interactions between plants and their environment does influence population growth rates due to their effects on fecundity, growth or survival of individuals. Consequently, it is responsible for the continuous evolution and adaptation of plant communities. Succulents in arid and semi arid environments appear to be widespread due to environmental stress (Smith, 2004).

According to Schemske *et al*. (1994), to ensure continued survival of a species, identifying the biological information that influences survival of a target species, is required. One approach to establish biological information on plants is suggested by Grime (1984). It is based on developing a framework to identify plant responses to stress. This does not only recognise stress responses, but provides the basis for understanding the mechanisms by which resources and environmental stress influence the structure and functioning of a plant species or community. As there is presently no biological information on the factors that influence the establishment and survival of *Hoodia gordonii,* this study follows more or less the same strategy in the quest of investigating the response of the current species to environmental stress.

2.7 Restoration in context

While humans are dependent on natural systems, they have to act as guardians at the same time. Cairns (1997) states that human society as we know it today, would not survive if ecological restoration and preservation are not widely practised. Moreover, there are also ethical responsibilities for both the future generations and other species with which humans share the planet.

Macdonald *et al.* (2002) suggest that the requirements of restoration are very much the same for both animals and plants; the end result must be a self sustaining population or community. Cairns (1997) believe that if the basic planning in a restoration programme is done properly, ten years or less is enough for nature to take over.

There are a number of ecological factors that needs to be taken into account before a restoration programme is implemented. Starting with identification and removal of ecological stressors or cause of the population decline, Schemske *et al*. (1994) suggest two more steps towards the development of a recovery effort for an endangered species. Namely identify critical life history stages, and biological causes of variation in demographically sensitive life history stages. The first step involves identifying stages in plant life cycles that contribute to the population dynamics. This will allow conservation biologists to investigate biological processes that affect those stages and design effective recovery measures. Subsequent to knowing which stages in the life history have the greatest impact, experiments and observations are needed to estimate the relative importance of the genetic or ecological factors that affects these stages. Schemske *et al*. (1994) further suggest that the most efficient approach is to investigate the contribution of a number of factors contributing to variation in a particular parameter. This should be done simultaneously and in the absence of obvious external threats.

2.8 Effects of seed viability and germination

Seed germination is a critical stage in the life history of plants (Zheng *et al*., 2004) as many factors can influence their ability to germinate. These include amongst others dormancy, viability and their rate of germination. In many species dormancy mechanisms prevent the germination of viable seeds until suitable conditions arise (Ooi *et al.*, 2004). According to Ellis *et al*. (1985), a number of difficulties can arise in deciding why a particular seed lot has not germinated in a particular test.

A surviving seed represents the future of a plant population and community; therefore its biological traits should be known. Baskin and Baskin (1998) noted that determining whether poor germination is explained by unknown dormancy mechanisms or by low levels of viability is an essential part of understanding how species persist in the landscape. Similarly, it is important for restoration ecologists who need to know the germination potential of a seed lot (Ooi *et al.*, 2004). Testing the viability of seed lots is a primary step when assessing the effectiveness of dormancy breaking treatments. A viable seed is defined by Ooi *et al.* (2004) as a seed that has the potential to germinate.

Environmental and biological conditions such as temperature (Zheng *et al*., 2004; Mennan, 2003; Ellis *et al.*, 1985); light quality and intensity (Zheng *et al*., 2004; Mennan, 2003); photoperiod and nutrients (Mennan, 2003); moisture (Baskin and Baskin, 1998; Pake and Venable, 1996; Copeland and McDonald, 1995; Ellis *et al*., 1985) and seed position in the soil (Mennan, 2003; Moles *et al.*, 2003); all contribute to the successes of seed germination and seedling establishment. Moreover, in arid environments plants are believed to have developed various mechanisms to ensure that seeds remain dormant and protected until environmental conditions coupled with sufficient moisture supply is favourable for germination (Baskin and Baskin, 1998; Pake and Venable, 1996; Günster, 1992a, 1994). However not all plant species are able to persist for years in both soil (Moles *et al.,* 2003) and serotenous seed banks (Baskin and Baskin, 1998). According to Moles *et al*. (2003), seeds of other species are unable to persist for even moderate periods and must either geminate at the first available opportunity or perish.

One important biological factor which may help in the understanding the recruitment and performance of the species in the natural ecosystem is the influence of seed size on the growth and survival of seedlings. According to Moles *et al*. (2003), small seeded species produce a large amount of seeds on less amount of energy compared to large seeded species. On the contrary, seedlings developing from large seeded species are believed to be stronger competitors. Although they are considered to be slow growers after germination compared to small seeded species, they do tolerate a wide range of environmental stresses (Moles *et al*., 2003; Jurado and Westoby, 1992). The species under present investigation is considered to be small seeded with no signs of seed dormancy.

CHAPTER THREE

THEORETICAL FRAMEWORK

3.1 Problem statement

According to Baillie (2004), there are approximately 10 000 estimated succulent species worldwide, 2 000 of these are threatened with global extinction in their natural environment. Many more are threatened with extinction at a regional and local level. In Namibia and South Africa the threats to indigenous succulents involve habitat destruction through mining, farming, and urban expansion (Loots, 2005; Biggs, 2003). Blignaut and Milton (2005) confirm that there are rich deposits of diamonds, gypsum, titanium, marble, and zircon in shallow soils beneath the succulent Karoo. This has resulted in a decrease in species numbers due to mining.

Collecting *Hoodia gordonii* for international trade poses an even more serious threat, as compared to the threats mentioned above. The commercial potential of *Hoodia gordonii* as a natural appetite suppressant agent has led to a dramatic increase in the level of interest. Illegal, destructive harvesting of *Hoodia* species, especially *Hoodia gordonii*, where the active ingredient was initially identified are of high concern (Barsch and Madisa, 2005; Carr, 2004; Van Niekerk, 2004).

According to Carr (2004) and Downs *et al.* (2005), the demand for this plant material has triggered concern that the resulting uncontrolled harvesting of plants *in situ* will lead to devastating consequences for the populations. Evidence of over-collection of

Hoodia has been reported in Botswana (Barsch and Madisa, 2005) and Namibia (Lisao and Musiyalike, 2006). Reports of illegal harvesting, buying, selling and smuggling of *Hoodia gordonii* have been on the increase in Southern Namibia. These reports are supported by criminal cases. Although the species is protected under the Namibian law, controlling illegal activities has proven difficult, due to the vastness of the areas and the ingenious means used by illegal cross boarder smugglers. The current laws are failing to deter offenders, probably due to low fines and sentences in relation to the potential value of the smuggled material (Lisao and Musiyalike, 2006).

Despite the fact that the future of species is threatened by continued exploitation in its natural environment, little is known about its growth requirements, distribution and population status. Most work done on *Hoodia* has concentrated on artificial propagation for commercial purposes (Carr, 2004). Consequently, the threat resulting from the increasing exploitation of the species remains high.

3.2 General objectives

1. To explore and understand the growth requirements of *Hoodia* in its natural environment.
2. To better understand how the recovery efforts of wild populations of *Hoodia gordonii* can be improved.
3. To contribute to the protection, conservation and future propagation of *Hoodia gordonii* in Namibia and elsewhere.

3.3 Specific objectives and key questions

1. To determine the role of protection against predation in the establishment of *Hoodia gordonii* seedlings in the natural environment

Key question

Does protection against predation play a role in the establishment and growth of Hoodia gordonii *seedlings transplanted in the natural environment?*

2. To examine the role of shade in the establishment of *Hoodia gordonii* seedlings when planted out into their natural environment

Key question

Does shade play a role in the establishment and growth of Hoodia gordonii *seedlings transplanted from a nursery into their natural environment?*

3. To determine the role of moisture in the establishment of *Hoodia gordonii* seedlings in their natural environment

Key question

Does moisture play a role in the establishment and growth of Hoodia gordonii *seedlings transplanted in the natural environment?*

4. To evaluate the significance of age of age at transplant in the establishment of

the transplanted *Hoodia gordonii* seedlings

Key question

Does age at the time of transplanting influence the establishment and growth of Hoodia gordonii *seedlings transplanted into their natural environment?*

5. To determine the effect of a combination of shade, moisture and protection against predation on the initial establishment of *Hoodia gordonii*

Key question

Does the initial establishment of Hoodia gordonii *require an interaction between shade, moisture and protection against predation?*

3.4 Research hypothesis

1. Unprotected seedlings are expected to establish poorly in comparison to the protected seedlings, due to the influence of predation. However, differences in performance will also depend on how seedlings that are partially browsed recover when given other treatments i.e. shade and moisture.

2. Shaded seedlings are expected to perform poorly compared to un-shaded seedlings. This is expected as a result of adaptability of the species to harsh environments and the advantage of improved photosynthetic rates in un-shaded areas. In contrast shaded plants may retain more moisture compared to un-shaded plants.

3. Irrigated seedlings are expected to perform better compared to non-irrigated ones due to moisture availability, at least in the short term, while plants are recovering from the stress brought about by the transplanting process. Since succulents are known to survive under extreme conditions of moisture stress, the differences of seedling performance as a result of moisture is not expected to affect establishment. Nonetheless, irrigated seedlings will show more rapid growth, and one would expect this advantage to persist and be reflected in general growth rates.

4. There will be a significant difference in the survival rate of transplanted seedlings due to age at the time of transplanting. One would expect seedlings transplanted at three months of age to perform better than those transplanted at one year. This would be the case because young seedlings have a less developed root system than older ones, and as a result easily recover from damage during transplanting. However, vulnerability of younger seedlings to pathogens like fungal infection may affect their establishment and survival rate.

5. Differences in seedling performance are generally expected between and within treatments in the field. One would expect a treatment combination of protected, shaded, irrigated seedlings to do better compare to seedlings not protected against predation, not shaded and not irrigated. Differences would be brought about as a result of removed negative effects caused by predators, reduced stress of the harsh environment e.g. heat and limited moisture.

CHAPTER FOUR

FACTORS AFFECTING THE SURVIVAL OF SEEDLINGS

4.1 Material and methods

4.1.1 Germination of seeds

Seeds were collected by removing seed pods from wild plants in the !Khob !Naub conservancy during 2005 seeding season. Local seeds from the conservancy were recommended for the research in order to maintain the local gene pool and avoid genetic contamination during the transplanting experiment. The seeds were sown in January 2006. All seeds were sterilised by placing them in a 10% fungicide mixture for 5 minutes in order to kill antigens before being germinated.

Individual trays containing the standardised mixture of river sand and sterilised natural potting soil was used as a growing medium. This was prepared by mixing one part course and one part fine river sand. The mixture was sifted to separate and remove stones and debris using a 1000 micrometer sift. Subsequently, ¼ of potting soil was added and carefully mixed before filling individual trays. For each tray forty two (42) seeds were sown in a row of 6 and columns of 7, by placing them on top of the growing medium and thinly covering them with a layer of sand. Trays were irrigated on a daily basis during the first two weeks to allow germination of seeds and establishment of seedlings; subsequently trays were irrigated once every two days. Local tap water was used in quantities sufficient to induce drainage. The seeds were monitored for 12 weeks, and subsequently, transported to the conservancy for the translocation study. One year old seedlings were obtained from the nursery at the conservancy. These seedlings were germinated from the seeds collected from the local population in February 2005. The standard medium and the method discussed above was used to germinate the seedlings at the nursery.

4.1.2 Experimental design for the translocation experiment

A fairly homogenous area where *Hoodia gordonii* naturally grows was selected. Since it is known that soil fertility in arid area varies considerably, a 2 x 2 x 2 factorial experiment in a randomised block design with twenty replicate blocks was designed to eliminate the effects of nutrient rich and poor soils. According to Zar (1999), a particular treatment can be assigned to the most fertile parts of the soil by chance and wrong conclusion may be drawn if the experimental site is not uniform.

Each block was in a 10 x 10m grid with five columns and four rows. Water, shade and protection against predation, each at two levels (present or absent) were combined to produce eight different treatments which were applied in the experiment (Appendix 1). The eight treatments were applied to seedlings at two different ages i.e. three months and one year at transplant, resulting into sixteen treatments within each 10 x 10m replicate block (Appendix 1). A total of 320 seedlings were included in the experiment, resulting in 20 replicates and 19 degrees of freedom per treatment combination.

4.1.3 Transplanting and monitoring

The 15 x 15cm wide and 15cm deep holes were dug in advance, and randomly allocated with a specific treatment combination within each replicate block. Specified treatments were colour coded and marked using metal and 15mm x 4.5 PVC insulated colour tapes. Seedlings were then transplanted into pre-dug holes, watered, shaded and protected according to prescribed treatment combinations in a single day. Protection was done by surrounding the seedlings with a 13 mm size hexagonal wire. Shade was provided using 80% shade nets. Treatment combinations that required water were irrigated three times per week during the first two months and twice a week for the remainder of the period. For each irrigation time, local tap water was given in quantities sufficient to induce drainage. Treatments with protection, shade, and water were monitored periodically to ensure that they were not interfered with.

The first measurements were taken two weeks after transplant. These included survival (surviving *versus* dead plants), factors causing deaths measured by identifying cause of death, plant growth measured in stem height and diameter, and plant vitality of the surviving plants measured by using a subjective scoring method. All response variables (plant vitality, causes of mortalities, and survival) with the exception of growth, were continuously monitored, from April up to August 2006.

To determine the effects of shade, water, protection against predation and age on the survival of the plants the number of dead and living plants were counted in all 20 blocks. Plants were monitored continuously in order to determine the cause of deaths when this occurred. The total number of seedlings that died and survived at the end of the period was considered as the final survival within each treatment combination.

4.1.4 Statistical analysis

Survival data were recorded as binomial data (dead or alive) according to the experimental design described in 4.1.3. The data were subjected to the chi-square test for contingency tables, with shade or no shade, water or no water, protection or no protection each at ages three months or one year at transplant as factors. Pearson's loglinear analysis was used to compare the observed and expected frequency of plant survival as a result of the highest order interaction of all factors and see if their removal significantly affects the fit of the model (Field, 2005).

The Loglinear analysis was followed up by separating factors (shade, water, protection and age); the data were then subjected to a 2 x 2 contingency table with survival at two levels (dead or alive) subjected to single factor at two level (present or absent) at a time. The Pearson's chi-square test was used to compare the relationship between survival and each individual factor. The Odds ratio method as explained by Field (2005) was used to calculate the effect size for each factor within the 2 x 2 contingency table.

4.2 Results and Discussion

The five way loglinear analysis produced a final model (Appendix 2), that retained all effects. The likelihood of this model was χ^2 (0) = 0, p = 1, therefore the model perfectly predicts the data (Appendix 3). This indicates that the highest order interactions (age x shade x water x protection x survival) was significant χ^2 (15) = 106.1, $p < 0.01$ (Appendix 4). The evaluation of the final model indicates that expected values generated by the model is a good feat of the data, the likelihood ratio statistics is χ^2 = 3.314 (10) = 0.973, $p > 0.05$.

To break down the interaction effect, chi-square tests were conducted to see the effects of shade, water, protection and age on survival separately. Of the total 320 seedlings that were transplanted a total of 115 (35.9%) died, where as a total of 205 (64.1%) survived (Fig. 2).

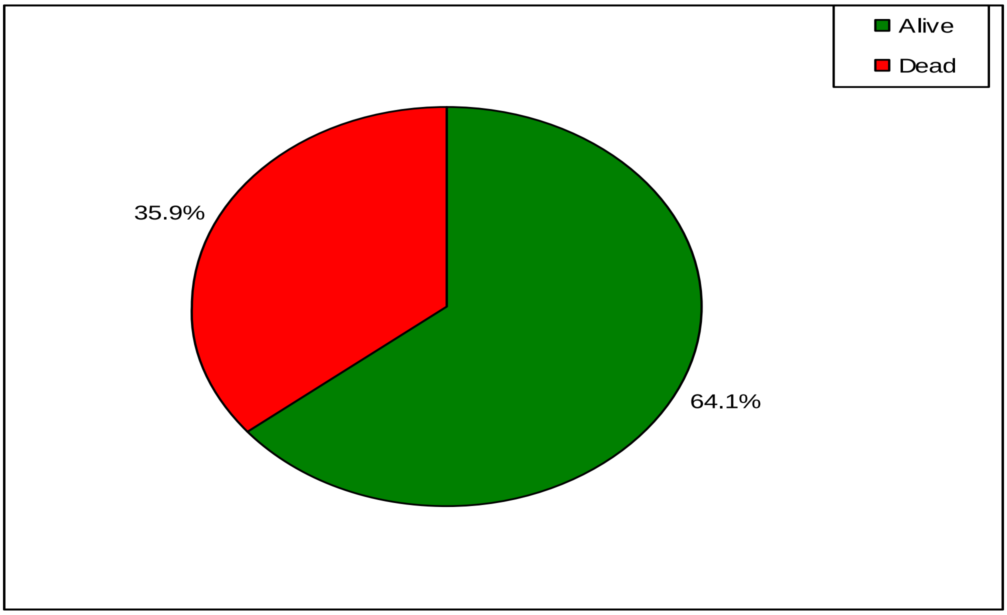

Figure 2: The total percentage of plants that died in comparison to the total number of plants that survived.

4.2.1 Effect of shade

From the total number of plants that were shaded, 48 plants (30%) died, while a staggering 117 plants (70%) survived (Appendix 5). Within the total 160 un-shaded plants a total of 67 plants (41.9%) died, and only 93 plants (58.1%) survived (Fig.3).

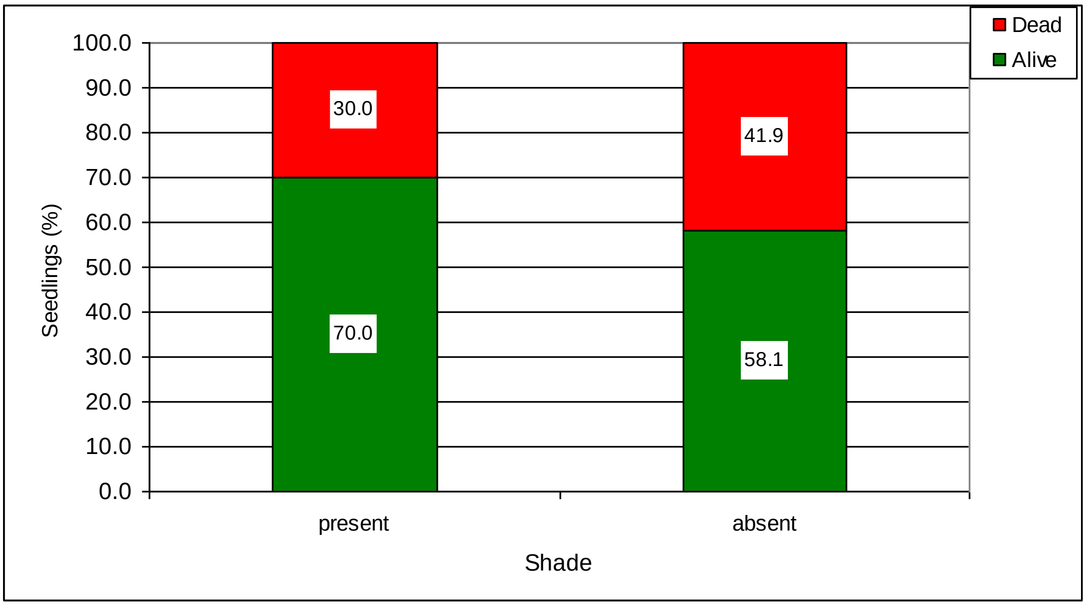

Figure 3: Recorded proportions of dead and living seedlings in percentage resulting from the presence and absence of shade.

There was a statistically significant association between shade (shade or no shade) and survival χ^2 (1) = 4.900, p = 0.036 < 0.05 (Appendix 6). Based on Odds ratio, shaded seedlings were 1.67 times more likely to survive compared to those not shaded (Appendix 7).

The high frequency of surviving plants in the shaded category compared to the non shaded category, indicates that *Hoodia gordonii* requires shade to survive (Appendix

5). There are many factors that dictate the survival of the plants under shade; similarly there are many factors that dictate increased deaths of plants under none shaded category. These factors will be discussed in subsequent chapters.

4.2.2 Effect of watering

Within the total of 160 watered plants 107 plants (66.9%) survived and 53 (33.1%) died. From a total 160 un-watered plants 98 plants (61.3%) survived where as 62 plants (38.8%) died (Fig. 4).

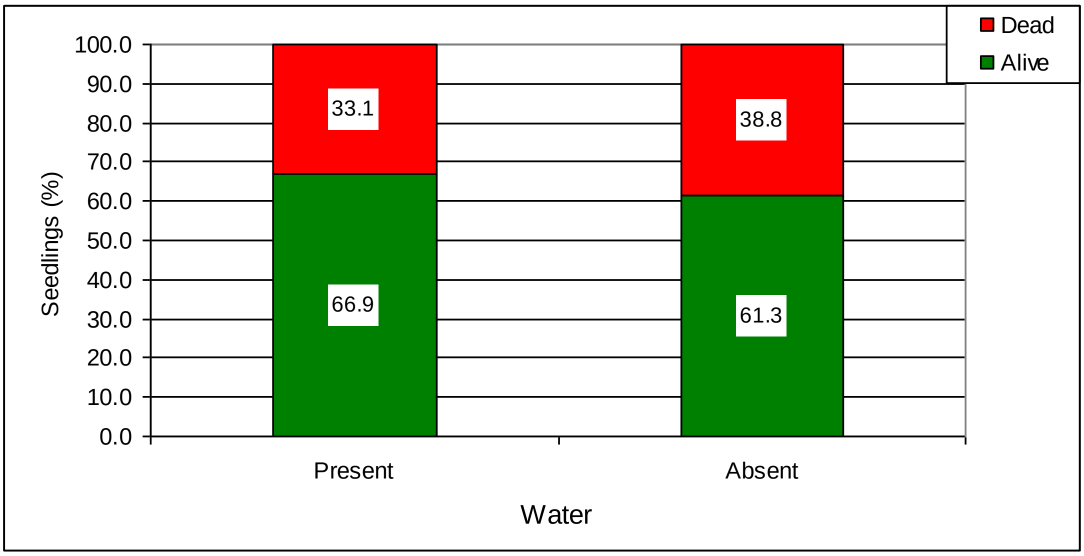

Figure 4: Recorded proportions of dead and live seedlings in percentage resulting from the application and none application of water.

There was no statistical significant association between survival and whether water was present or absent χ^2 (1) = 1.099, p = 0.351 > 0.05 (Appendix 6). Based on the Odds ratio, plants were only 1.26 times more likely to survive when watered than when un-watered (Appendix 7). Therefore water had no significant influence on the survival of seedlings. Although water is generally known to be an important factor in the survival of both plants and animals, *Hoodia gordonii* has adapted to survive in extremely dry areas with limited moisture availability. It is therefore assumed that the succulent life form of the seedlings contributed to sustained survival amongst non watered category. However a reduced frequency of plant survival in the watered category is not consistent and is attributed to factors other than the actual effect of water. These factors are discussed in detail in subsequent chapters.

4.2.3 Effect of protection against predation

Protection against predation surpassed other factors in terms of the number of plants that survived (Appendix 5). Of the total of 160 plants that were protected against predation, a remarkable 138 plants (86.3%) survived and only 22 plants (13.8%) died. Within the un-protected category, 93 plants (58.1%) died and only 67 plants (41.9%) survived (Fig. 5). These numbers indicate a very strong relationship between protection against predation and survival (Appendix 5).

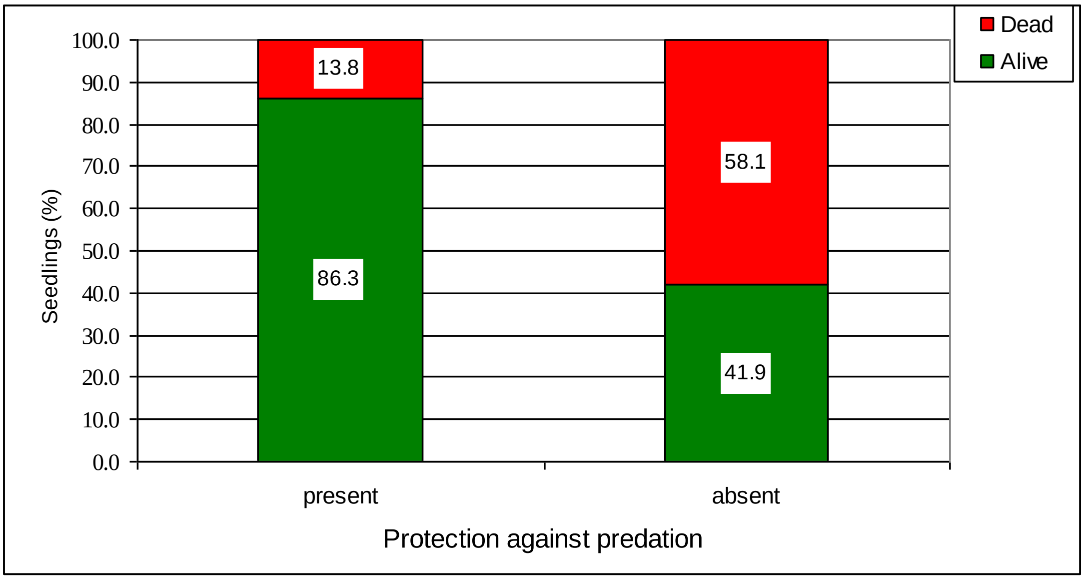

Figure 5: Recorded proportions of dead and live seedlings in percentage resulting from the presence and absence of protection.

There was a highly significant association between survival and whether protection against predation was present or absent χ^2 (1) = 68.425, p = 0.000 < 0.001 (Appendix 6). The percentage of dead plants decreased significantly within protected plants, whereas survival increased significantly (Fig.5). The percentage of dead plants was significantly higher amongst plants that were not protected (Appendix 5).

Of all the factors (shade, water and age), protection against predation produced the highest effect size; based on the Odds ratio, seedlings were 8.69 times more likely to survive when protection against predation was present than when it was absent (Appendix 7). The high frequency of surviving plants in the protected category compared to the non protected category indicates that seedlings required protection against predation to survive. There are many factors that dictate the survival of the plants that were protected against predation; similarly there are also other factors that dictate increased deaths of plants when not protected against predators. These factors are discussed in subsequent chapters.

4.2.4 Effect of age at transplant

Amongst seedlings that were transplanted at the age of three months, 61 plants (38.1%) died while 99 plants (61.9%) survived. From seedlings that were transplanted at the age of one year, 54 plants (33.8%) died where as 106 plants (66.3%) survived (Fig. 6). The results show that there is no relationship between age at transplant and survival (Appendix 5).

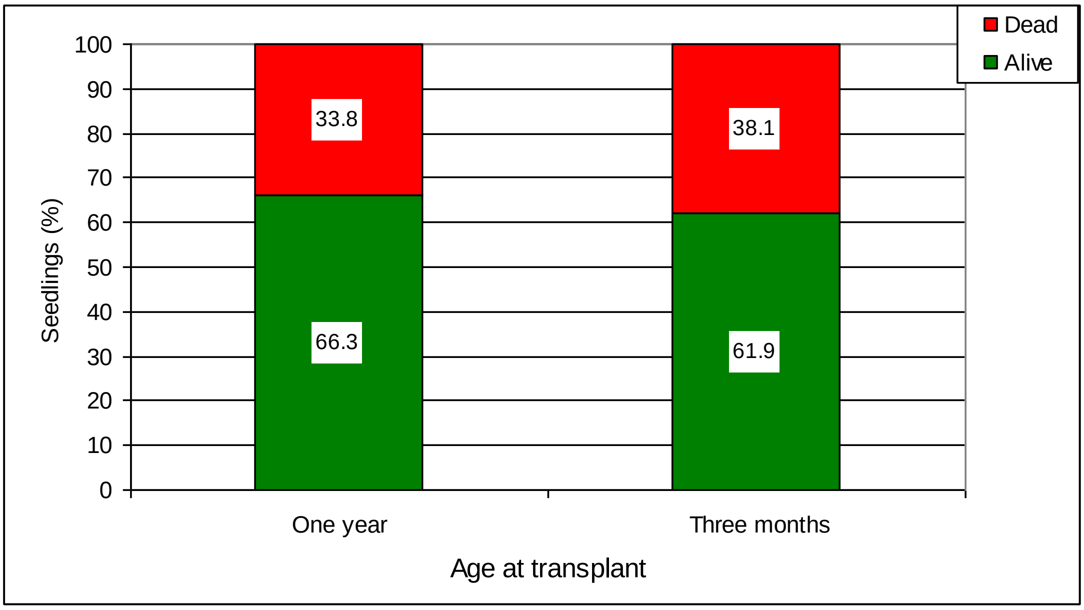

Figure 6: Recorded proportions of dead and live plants in percentage recorded from seedlings at three months and one year of transplant.

Seedlings transplanted at the age of one year show an increase in survival and slight decrease in deaths compared to plants transplanted at the age of three months. The association between survival and age at transplant (three months or one year) is χ^2 (1)

= 0.665, $p = 0.415 > 0.05$ (Appendix 6). Although the association between survival and age was not significantly different, the Odds ratio indicates that plants transplanted at one year were 1.22 times more likely to survive than seedlings transplanted at three months (Appendix 7).

Overall, age had the lowest effect size on survival compared to the effects of protection, shade and water. Despite this low effect size, factors that contributed to survival amongst seedlings (transplanted at the age of one year and at the age of three months) are discussed in subsequent chapters.

4.2.5 Interactions between factors

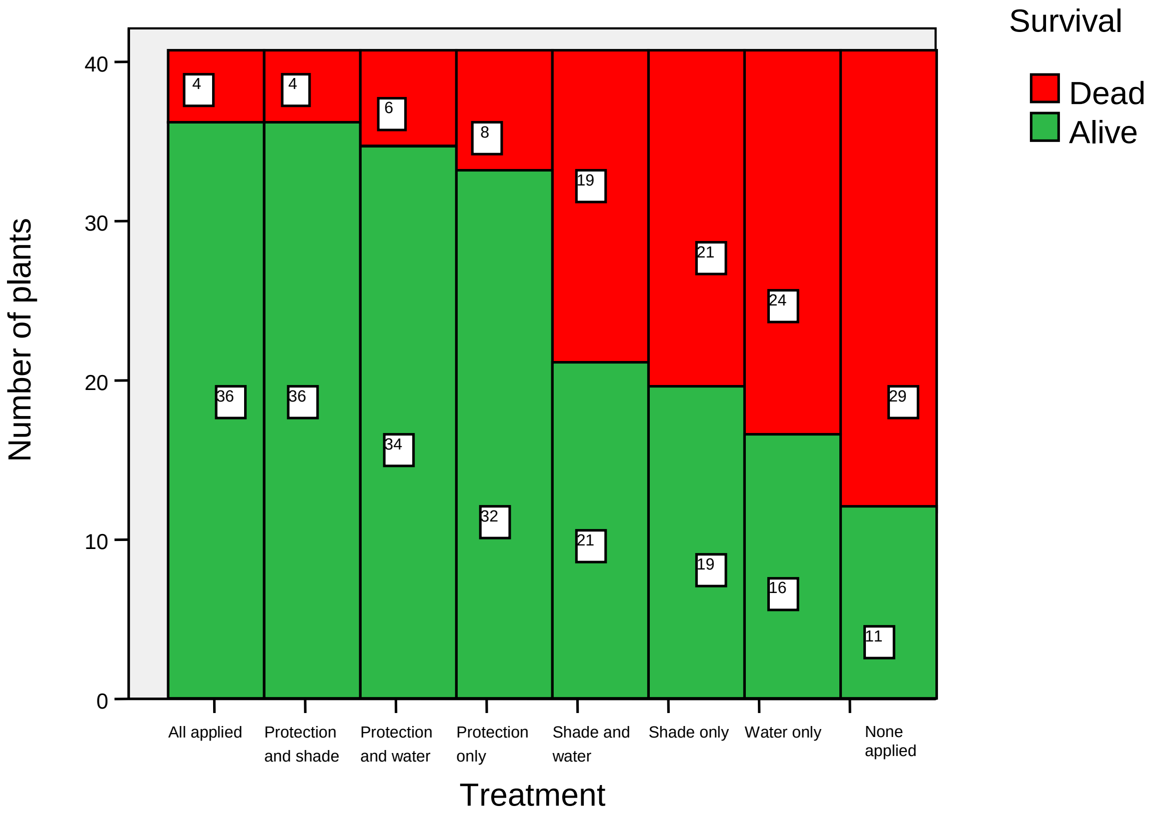

Figure 7: Proportions in numbers of seedlings that died *versus* the seedlings that survived as a result of treatment combination between water, shade and protection.

The highest proportions of live plants were recorded in treatment combinations where protection against predation was present (Fig. 7). Treatment combinations without protection against predation show an increasing proportion of dead plants (Fig. 7). The plants that received water and those that received shade, show a decrease in the number of plants that survived. However, when water and / or shade were combined with protection against predation the number of live plants significantly increased. Thus if their application were separated from protection their relationship with survival of seedlings diminished considerably.

These results show that the survival of *Hoodia gordonii* depends on the interaction of different important factors such as shade, protection against predation and water. Although protection against predation appears to be the major factor that determined survival of the seedlings, shade and water may be important factors that contribute to the successes of transplant. The interactions of factors that lead to improved survival of seedlings are discussed in the subsequent chapters.

CHAPTER FIVE

THE IMPACT OF PREDATION AND ABIOTIC STRESS ON TRANSLOCATION SUCCESS

5.1 Materials and methods

In order to determine the impact of predation and abiotic stress on the initial establishment of transplanted seedlings, seedlings were monitored continuously from April - August 2006. Factors that contributed to the deaths of the seedlings were identified and recorded in all 20 blocks. Other factors such as the effect of trampling were reduced by controlling animal movements. This was necessary due to the fact that the study site was located close to a homestead where trampling is quite intense as a result of animal movements to and from the water point.

The total number of factors recorded at the end of the period was taken as the final number of factors within each treatment combination. Three major factors that contributed to the deaths of seedlings were identified. (1) Abiotic stress; a plant was considered to have died of abiotic stress if it lost vigour and eventually die without interference from biotic factors such as insects and rodents. (2) Insects; a plant was considered to have died because of insects if there was clear evidence of bites that eventually lead to loss of vigour and deaths. (3) Rodents; a plant was considered to have died from rodent attack if it was uprooted or if the above ground plant parts were totally removed. The comparisons and analysis of factors was done at different ages and different treatment combinations.

5.2 Statistical analysis

Data concerning the factors impacting translocation successes were recorded as binomial data at four levels (plants that were alive and plants that died due to: stress, insects, and rodents) according to the experimental design described in 4.1.2. The data were subjected to the chi-square test for contingency tables, with shade or no shade, water or no water, protection against predation or no protection and age at three months or one year at transplant as factors. Pearson's loglinear analysis was used to compare the observed and expected frequency of disturbance as result of the highest order interaction of all factors, and see if their removal significantly affects the fit of the model (Field, 2005).

The Loglinear analysis was followed up by separating the factors (shade, water, protection and age). As plants that died due to fungi and trampling had very low frequency, removing the outlier from the analysis improved the contingency table and avoided the violation of a statistical assumption. A total of 320 seedlings were used to analyse the factors that contributed to the improved survival of seedlings. The data were then subjected to a 2 x 2 contingency table with factors impacting translocation success at four levels (alive, stress, insects and rodents) and each independent factor at two levels (present or absent) at a time. The Pearson's chi-square test was used to compare the relationship between factors impacting translocation success and the individual factor.

The Odds ratio was calculated for the 2 x 2 contingency table in order to determine the effect size for each variable that caused deaths. The odds for the presence or absence of each variable causing deaths (stress, insects and rodents) were calculated against the numbers of dead plants within each (Appendix 14-16).

5.3 Results and Discussion

The four-way loglinear analysis produced a final model that retained all effects (Appendix 8). The likelihood ratio of this model was χ^2 (0) = 0, p = 1 (Appendix 9). This indicates that the highest-order interaction between variables causing deaths and shade x water x protection on both ages was highly significant χ^2 (1) = 20.31, $p < 0.000$ (Appendix 10).

The effect was then broken down by chi-square tests carried out separately on shade, water and protection and age at transplant. Of the 320 seedlings that were transplanted, a total of 205 (64.1%) were alive. Rodents were the single highest cause of plant losses which accounted for a total of 58 plants (18.1%), 38 plants (11.9 %) were lost due to stress, while insects was the lowest cause of plant loss with a total 19 plants (5.9 %) lost (Fig. 8).

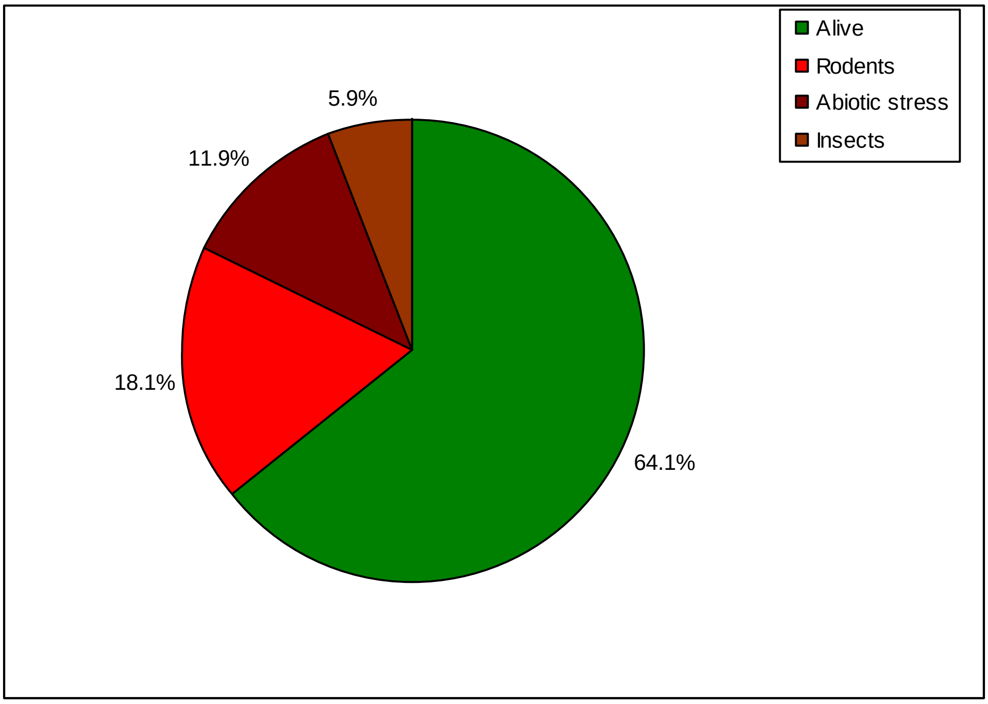

Figure 8: The percentage of plants that were alive and percentage of the aspects that caused deaths of transplanted seedlings.

5.3.1 Effect of protection against predation

Amongst the total of 160 plants that were protected against predation, 138 (86.3 %) were alive and the remaining 22 plants (13.8 %) were lost due to stress. No plant under protection was lost due to insects or rodents. Whereas only 67 of the total 160 plants (41.9 %) which were not protected from predators were alive, a total 16 plants (10.0 %) were lost due to stress, 19 (11.9 %) died due to insects and 58 (36.3%) were lost due to rodents (Fig. 9). In summary, the numbers shows that protection against predators played a highly significant role in preventing plant mortalities.

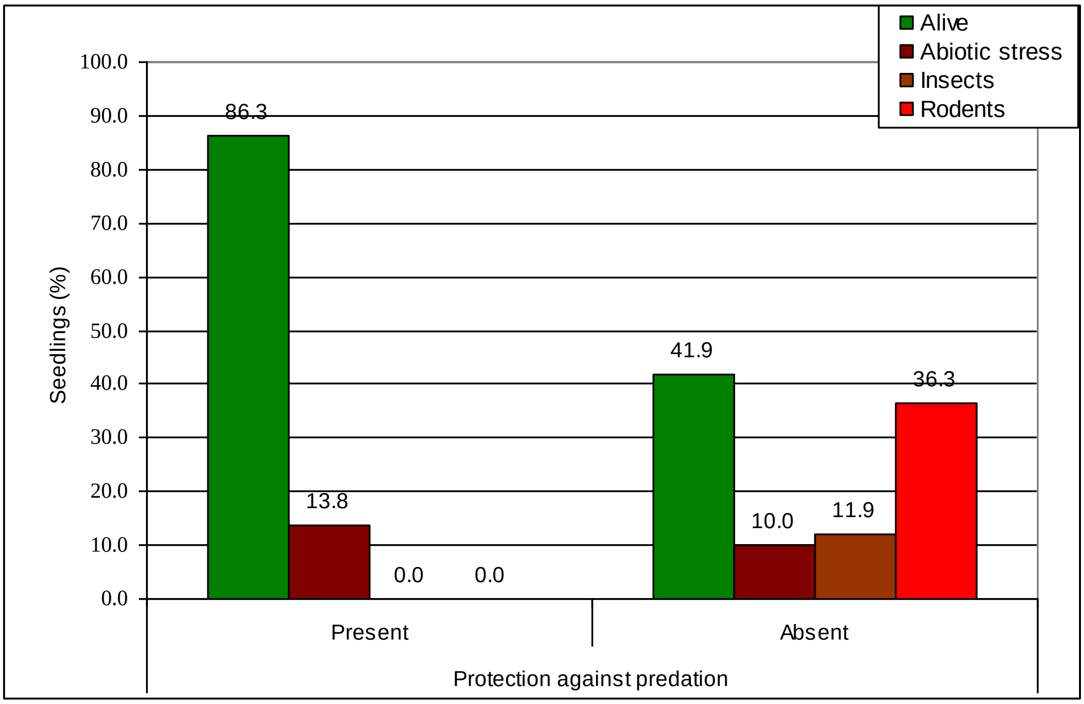

Figure 9: Percentage of seedlings that were alive and the frequencies of different factors causing deaths as a result of the presence and absence of protection against predation.

According to the results there was a highly significant negative association between factors that caused deaths and whether protection against predation was present or absent χ^2 (3) = 102.538, p < 0.000 (Appendix 12). Seedlings were more likely to be alive when protected against predation. While protection against predation completely removed rodent and insect attacks, it did not prevent deaths due to stress (Fig.9). However, the Odds ratio indicates that plants were 0.67 times more likely to die of stress under protections than when they were not protected against predation (Appendix 13).

A marked difference in the total percentage of live plants was recorded between seedlings protected against predation and those that were not protected against predation (Fig. 9). Accept for stress, which was the major contributor of mortalities under protected plants, attacks by insect and rodent were prevented 100 percent; in the non protected category attacks by insects and rodents were prevalent (Fig.9).

Amongst all factors, protection against predation was the only category that had removed deaths caused by rodents and insects completely. Since rodent attacks were the biggest contributor to plant losses overall (Fig. 9), there is justification why there was an exceptionally high survival frequency in the protected category compared to shade, water and age. It is however clear that the effect of protection against predation on the survival of seedlings only had an impact on the prevention of biotic stress (predation pressure) and not abiotic stress (climatic pressure).

5.3.2 Effect of shade

From the total of 160 shaded plants 112 (70.0%) were alive, rodents remained the highest cause of plant losses with a total of 26 (16.3 %) followed by stress which accounted for a total 15 plants (9.4 %) while insects only accounted for 7 deaths (4.4%). Amongst the 160 un-shaded plants 93 plants (58.1%) were alive, rodents and stress were the highest causes of plant losses in this category and accounted for 32 plants (20.0%) and 23 plants (14.1%) respectively, where as insects accounted for 12 (7.5%) plant losses (Fig 10). Availability of shade appears to have had a marked impact as far as prevention of death resulting from stress (Appendix 11).

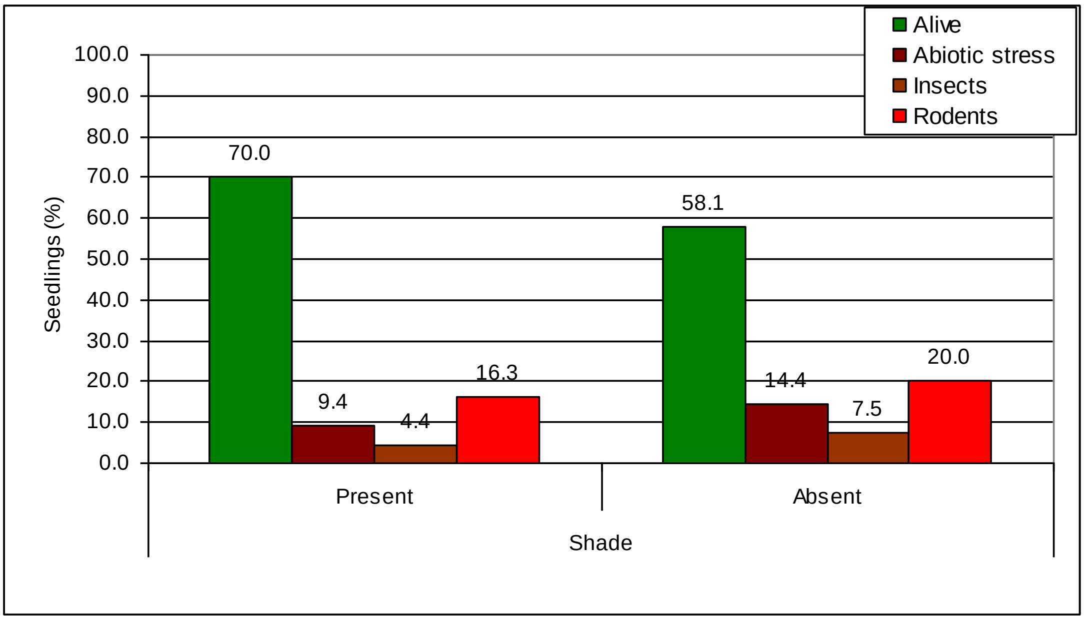

Figure 10: Percentage of seedlings that were alive and the frequencies of different factors causing deaths as a result of the presence and absence of shade.

Although there was a significant relationship between shade and survival (Fig. 3), the statistical analysis found no significant association between factors causing deaths and whether shade was present or absent. At 95% confidence level χ^2 (3) = 5.382, p = 0.146 therefore $p > 0.05$ (Appendix 12). This indicates that whether shade was present or absent, the factors that contributed to the plant deaths would likely have the same effect. Shade is therefore not a determining factor as far as causes of plant mortality is concerned.

Although, rodent attacks were the highest single cause of deaths (Fig. 10), there were very slight variations in total percentage of deaths between shaded and un-shaded categories. The Odds ratio indicates that plants were 0.67 times more likely to die of rodent attacks when shaded than when un-shaded (Appendix 15).

Contrary, there was a marked variation in deaths as a result of abiotic stress and insects. Shade reduced deaths of plants due to stress and insects (Fig. 10). The Odds ratio indicates that plants were only 0.54 times more likely to die of stress and 0.48 times more likely to die of insect attacks when shaded than when un-shaded (Appendix 13-14).

The above results indicate that unlike protection, the effect size of shade on factors causing death was negligible on aspects such as rodents, but it was greater on stress. It is therefore suggested that the effect of shade on the survival of seedlings was geared to the prevention of abiotic stress (climatic pressure) rather than biotic stress (predation pressure). The positive effect of shade on insect attacks was not expected and is surprising. In essence, it is attributed to the fact that seedlings that were partially browse in shaded category recovered at a high rate compared to seedlings in un-shaded category.

5.3.3 Effect of watering

Within a total of 160 plants that were watered, 95 plants (59.4 %) were alive; while rodents were again the highest cause of plant losses and accounted for 32 plants losses (20.0%). Losses due to stress and insects were almost a tie with 17 plants (10.6%) and 16 plants (10.0%) lost respectively (Appendix 11). Among un-watered plants, 91 (56.9%) were alive; stress was the highest cause of plant loss in this category and accounted for 28 plants (17.5%). This was followed by rodents with a total of 25 plants (15.6%), while insects accounted for the least losses in the category with 16 plants (10.0%).

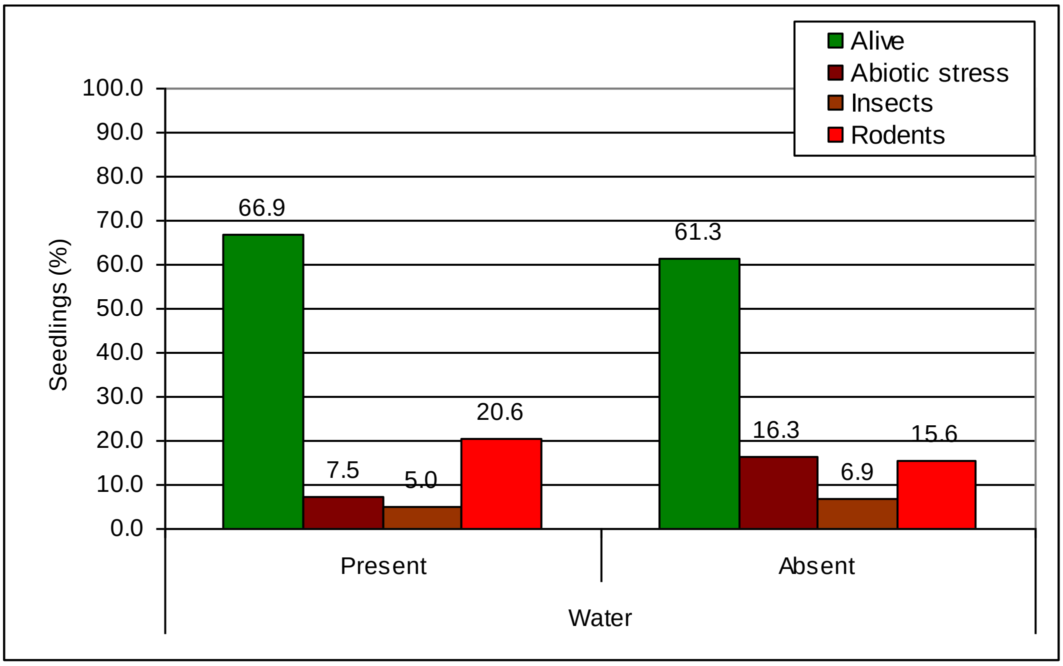

Figure 11: Percentage of seedlings that were alive and the frequencies of different factors causing deaths as a result of the presence and absence of moisture.

The difference between the number of live plants in both the watered and non watered plants was negligible and only varied with 5.6% (Fig 11). Again the results show no significant association between factors causing deaths and whether water was present or absent χ^2 (3) = 7.130, p = 0.068, p > 0.05 (Appendix 12). It seems as if whether water was applied or not, there would be no differences in factors that contributed to plant mortalities.

There was no profound differences in deaths resulting from insects between watered and un-watered categories (based on the Odds ratio plants were only 0.67 times more likely to die of insects when watered than when un-watered) (Appendix 14). The analysis seems to reveal that rodents had a strong effect size in watered plants compared to non watered plants. Deaths resulting from rodents had increased when water was applied compared to when not applied (Fig.11). The Odds ratio indicates that plants were 1.21 times more likely to die of rodents when irrigated than when not irrigated (Appendix 15).

Stress had a negligible effect size in the watered category compared to the non watered category. As in the case of shade, the application of water seems to have reduced plant deaths resulting from stress (Fig. 11). The Odds ratio indicates that plants were only 0.42 times more likely to die of stress when watered than when they did not receive water (Appendix 13). This is consistent and shows that water had a similar effect on abiotic stress as shade. Even though shade had slightly reduced deaths resulting from biotic pressure (predation), results obtained from the effect of water show that water encouraged deaths resulting from rodents.

In comparison to shade (Fig.3) water showed a marginal impact as far as prevention of deaths is concerned (Fig.4). This is because there was an increase in incidents of attacks by rodents where water was applied compared to where it was not applied. On the contrary, mortality due to stress under water regime appeared to have been reduced dramatically (Appendix 11). Ultimately, the effects of water and shade are oriented towards reducing climatic pressure (abiotic stress) that tends to have negative effects on the establishment and survival of seedlings.

5.3.4 Effect of age at transplant

From the total 160 seedlings that were transplanted at the age of one year, 106 plants (66.3 %) were alive. Rodents were the biggest cause of plant losses with a total of 30 plants (18.8%) lost, insects and stress accounted for the loss of 10 plants (6.3%) and 14 plants (8.8%) respectively. Among the total of 160 plants that were transplanted at the age of three months, 99 plants (61.9%) were alive. Rodents and stress were the biggest cause of plant losses and accounted for 28 plants (17.5%) and 24 plants (15.0%). Insects were the least cause of plant loss with a total of 9 plants lost (5.6%) (Fig. 12).

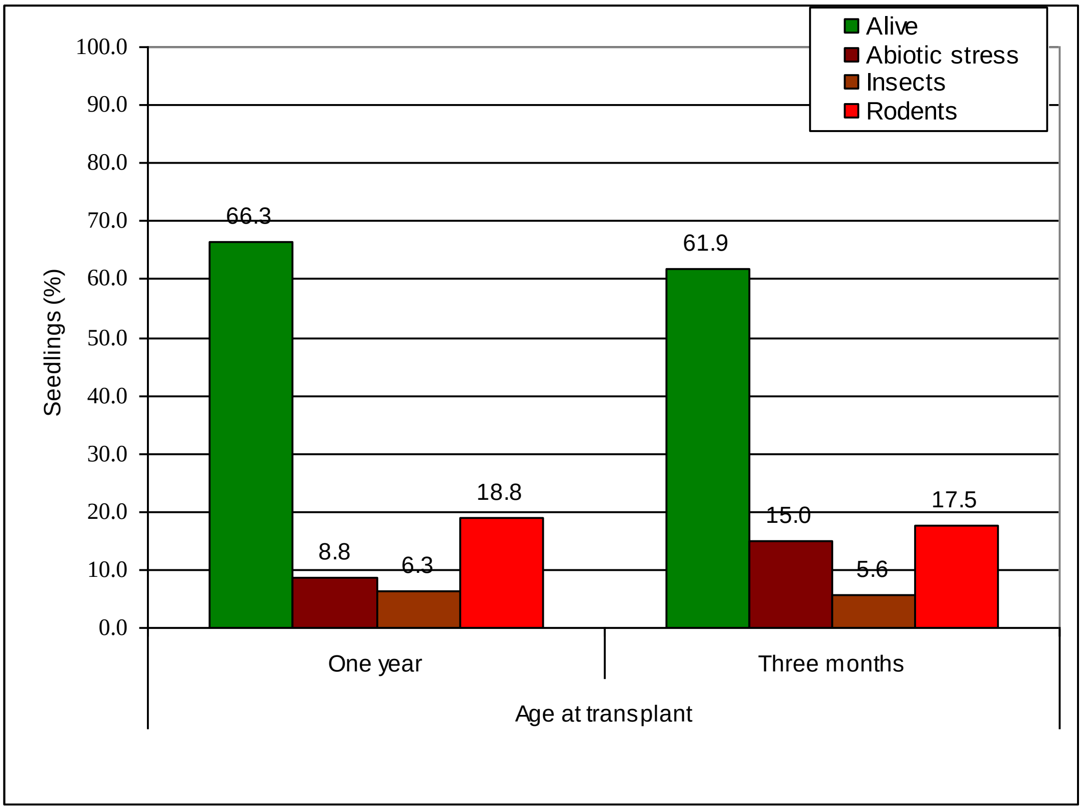

Figure 12: Percentage of seedlings that were alive and the frequencies of plant deaths with its associated cause resulting from differences in age at transplant.

The statistical analysis shows no significant association between factors causing deaths and whether the age at transplant was three months or one year χ^2 (3) p = 2.992 p = 0.393 therefore $p > 0.05$ (Appendix 12). This represents the fact that whether seedlings were transplanted at three months or one year, there would likely be no difference in the number of mortalities.

Deaths resulting from insects did not vary between plants that were one year old at transplant, compared to plants that were three months old at transplant. The Odds ratio indicates that one year old plants at transplant were 1.04 times more likely to die of insects attacks (Appendix 14). As in the case of the deaths resulting from insects, rodents did not discriminate between plants that were transplanted at the age of three months and those that were transplanted at the age of one year. According to the Odds ratio, plants that were one year old at transplant were 1.00 times more likely to die of rodents attacks (Appendix 15).

Deaths caused by stress were negligible among seedlings that were transplanted at one year and thus, contributed to the higher survival. The Odds ratio indicates that one year old seedlings were only 0.54 times more likely to die of stress, than seedlings that were three months old at the time of transplant (Appendix 13).

Generally, with the exception of stress which contributed to plant survival by preventing mortalities among one year old plants, differences in deaths resulting from rodents and insects were not significant and appeared to affect both plants that were transplanted at the age of one year and those that were transplanted at the age of three months. Therefore, plants that were transplanted at three months were more vulnerable to climatic pressure (abiotic stress) compared to plants transplanted at one year. The effect of predation did not vary among age groups (Fig.12).

5.3.5 Interaction between factors

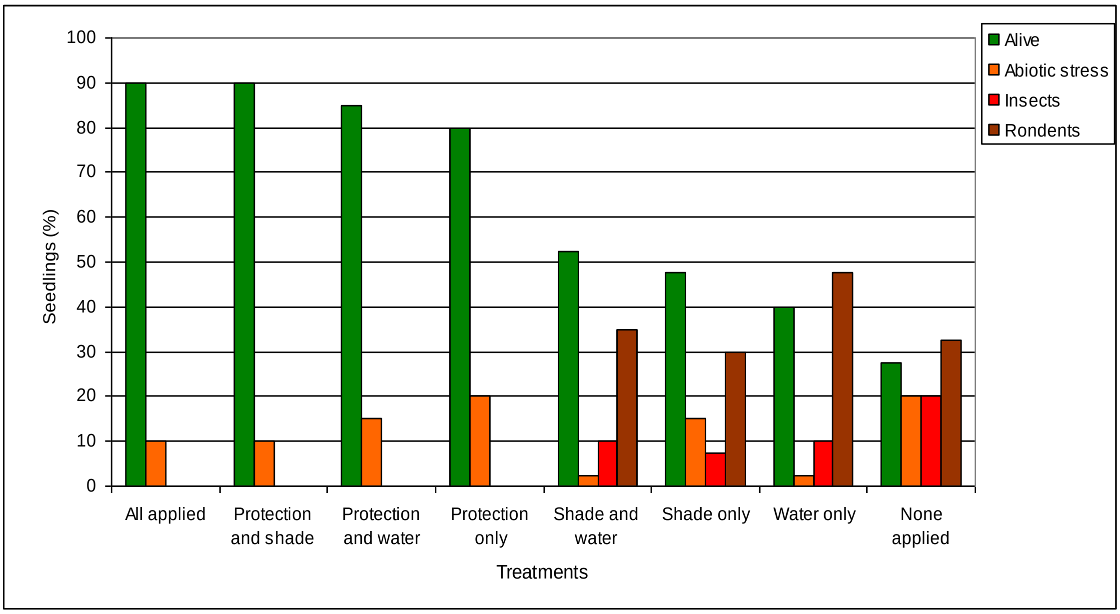

Figure 13: Frequencies of live plants and frequencies of plant mortalities with its associated cause resulting from treatment combination between water, shade and protection.

Treatment combinations with protection against predators show very high numbers of live seedlings (Fig. 13). Except for stress, all other causes of plant losses were eliminated (Fig 13). Rodent attacks followed by insect bites seem to be the highest cause of mortalities in treatment combinations that do not include protection against predation (Fig. 13). Overall predation (insects and rodents combined) accounted for more than 66.8% of the total death amongst the plants.

CHAPTER SIX

FACTORS IMPROVING PLANT VITALITY

6.1 Materials and methods

The vitality of plants described in 4.1.2 was determined by using a scoring method from 0 to 4:

with: 0 = seedling that died

1= seedling with little chance of recover

2= seedling with good chance of recovery if given improved conditions

3= seedling with good vigour, but not growing

4 = actively growing seedling

The scoring methods were in three major categories: dead plants, the plants that have lost vigour (1 and 2) and the plants that maintained vigour (3 and 4). The vitality of plants at different ages during transplant, different treatment combinations, as well as the effects of water, protection against predation and shade on vitality, were analysed and compared.

6.2 Statistical analysis

Vitality data were recorded as binomial data at five levels (dead, low chance of recovery, good chance of recovery, good vigour but no growth and actively growing) according to the experimental design described in 4.1.2. The data were then subjected to the chi-square test for contingency tables, with shade or no shade, water or no water, protection against predation or no protection against predation and age of three months or one year at transplant as factors. Pearson's loglinear analysis was used to compare the observed and expected frequency of plant vitality as a result of the highest order interaction of all factors and to see if their removal significantly affects the fit of the model (Field, 2005).

The Loglinear analysis was followed up by separating the factors (shade, water, protection against predation and age). The data were then subjected to a 2 x 2 contingency table with vitality at five levels (dead, low chance of recovery, good chance of recovery, good vigour but no growth and actively growing) and each factor at two levels (present or absent) at a time. The Pearson's chi-square test was used to compare the relationship between vitality and the individual factor.

The Odds ratio was calculated for the 2 x 2 contingency table in order to determine the effect size for each factor (shade, water, protection and age) on a specific vitality category. The odds for the presence or absence of factors were calculated against the number of plants that died in each category.

6.3 Results and Discussion

The five-way loglinear analysis produced a final model that retained all effects (Appendix 16). The likelihood ratio of this model was χ^2 (0) = 0, p = 1 (Appendix 17). This indicates that the highest-order interaction between vitality and shade x water x protection on both ages was highly significant χ^2 (1) = 20.31, $p < 0.000$ (Appendix 18).

To break down the interaction effect, chi-square tests were conducted separately on shade, water, protection and age in order to see their effects on plant vitality.

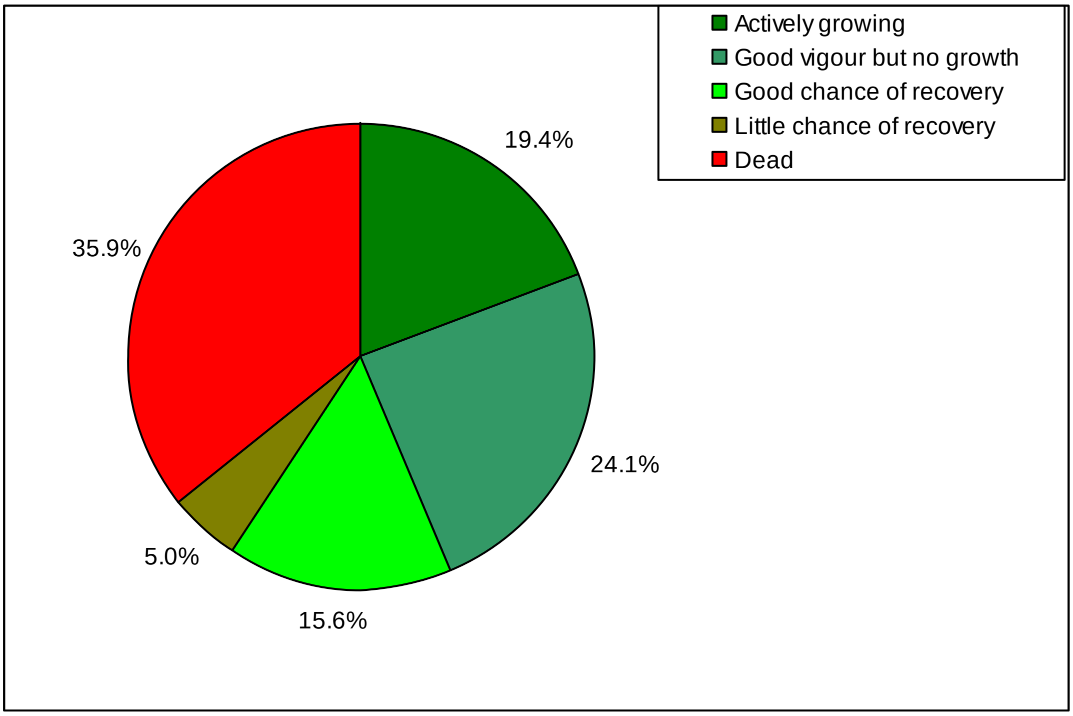

Figure 14: The percentage of plants that died and the total percentage of plants within each vitality category.

Amongst the total 320 seedlings that were transplanted, a total of 115 plants (35.9%) died, while a total of 16 plants (5.0%) were categorized as having little chance of recovery and a total of 50 plants (15.6%) were categorised as having a good chance of recovery. The majority of plants, with a total of 77 (24.1%), were categorised as having good vigour but no growth, while a total of 62 plants (19.4%) were still growing actively at the end of the study period (Fig 14).

6.3.1 The effect of shade

From the total of 160 plants that were shaded, 48 plants (30%) died. The majority of live plants were categorised as actively growing with a total of 57 plants (35.6%); followed by good vigour but no growth with a total of 33 plants (20.6%). Plants that were categorised as having a good chance of recovery were 16 (10%), whereas plants categorised as having little chance of recovery were only 6 (3.8%).

Amongst the total of 160 plants that were un-shaded 67 plants (41.9%) died. The majority of live plants (44) were categorised as good vigour but no growth (27.5%) and good chance of recovery 34 (21.3%); 10 plants (6.3%) had little chance of recovery and only 5 plants (3.1%) were actively growing (Fig. 15). Generally it appears that shade had dramatically improved the vitality of seedlings (Appendix 19).

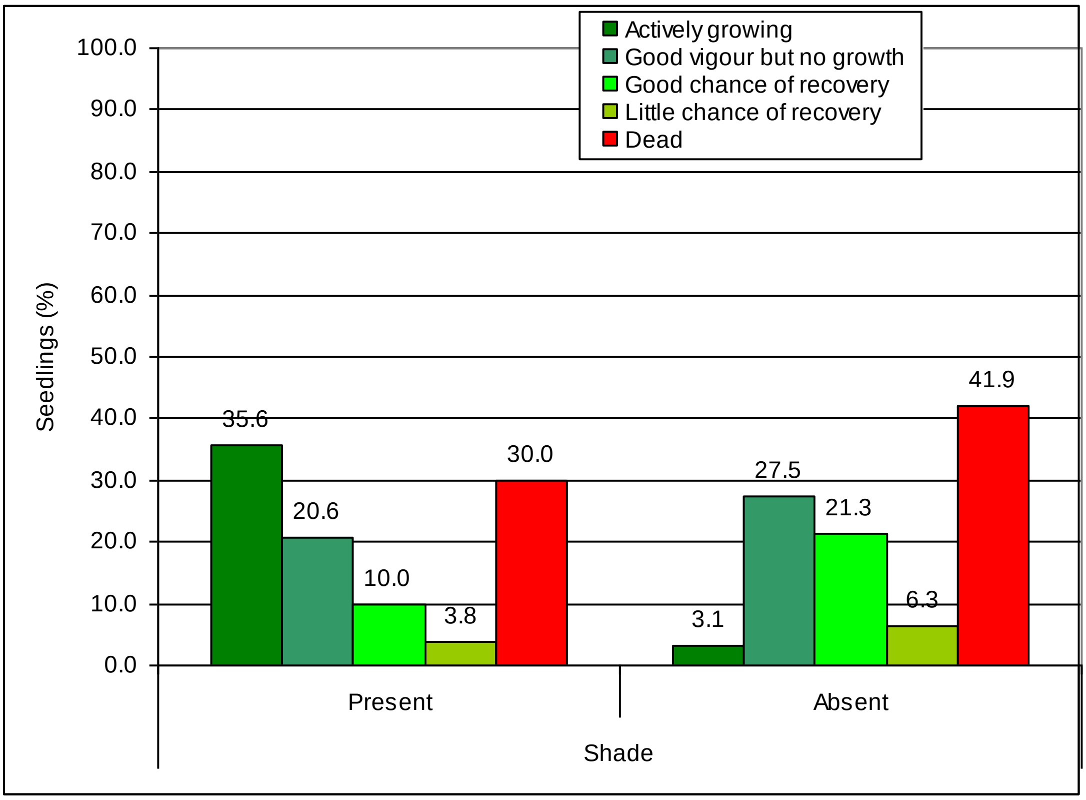

Figure 15: Percentage of seedling vitality as a result of the presence and absence of shade.

There was a highly significant positive association between vitality and the presence of shade χ^2 (4) = 55.803, p < 0.000 (Appendix 20). This seems to represent the fact that seedlings were more likely to have better vitality when shade was present than when it was absent. The Odds ratio indicates that the effect size resulting from the presence of shade, were lower in the first two categories (these seedlings generally lost their vigour), but were higher in the latter two categories (these seedlings maintained or improved their vigour) (Appendix 21-24). This is a confirmation that plants would have better vitality if shaded than when not.

On the contrary, there was very high number of plants that had lost their vigour in the un-shaded category. The results seem to be consistent with the fact that shade promoted survival by reducing climatic pressure (abiotic stress). Moreover, it is clear that *Hoodia gordonii* requires shade for it to be established and survive within its natural environment.

6.3.2 Effect of watering

Of a total of 160 plants that were watered, 53 plants (33.1%) died. Again the majority of live plants were categorised as actively growing with a total of 45 plants (28.1%) followed by the category of good vigour but no growth with a total of 38 plants (23.8%). Nineteen plants were categorised as having a good chance of recovery (11.9%), while only 5 (3.9%) were categorised as having little chance of recovery. Amongst the total of 160 plants that were not watered, a total of 62 plants (38.8%) died. The majority of live plants fell in the two middle categories where 39 plants (24.4%) were categorised as having good vigour but no growth and 31 plants (19.4 %) had a good chance of recovery. The remaining 17 plants (10.0%) were actively growing, while only 11 plants (6.9%) had little chance of recovery (Fig. 16).

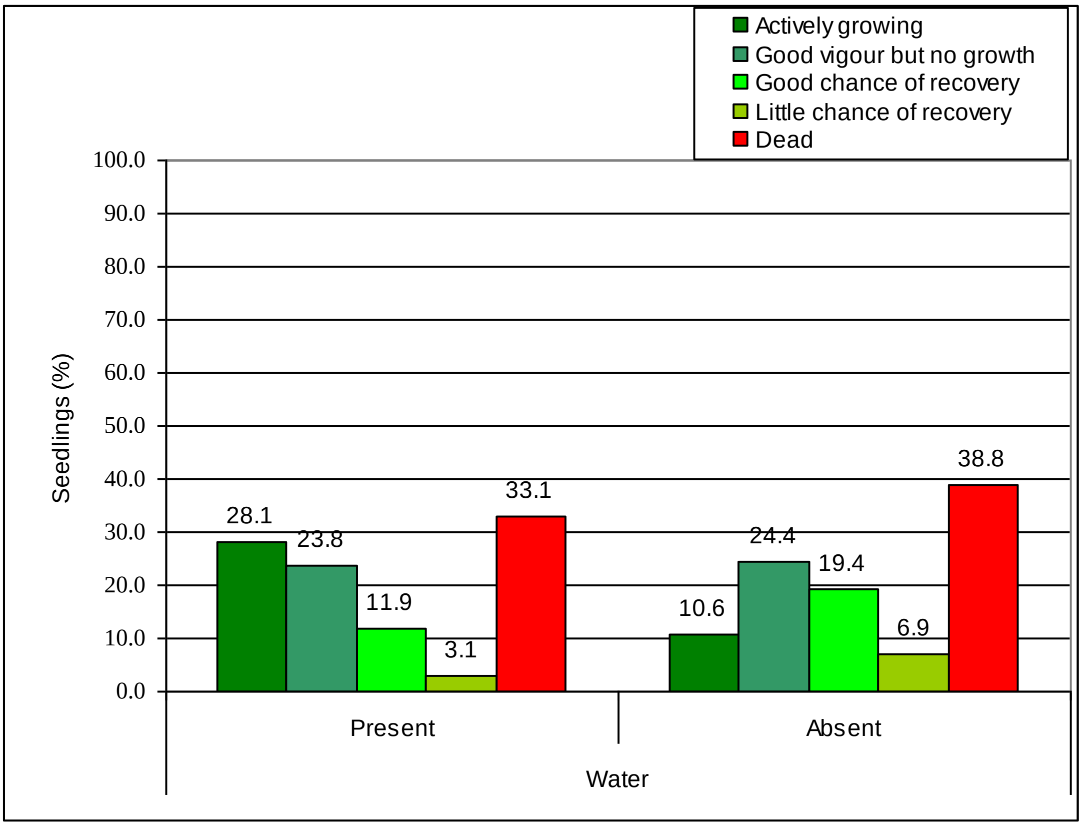

Figure 16: Percentage of seedling vitality as a result of the presence and absence of water.

There was a highly significant statistical association between vitality and the presence or absence of water χ^2 (4) = 18.492, p < 0.001 (Appendix 20). Seedlings were more likely to have a better vitality when water was applied than when not applied. The Odds ratio indicates that the effect sizes resulting from the application of water on plant vitality, were lower in the first two categories (low vigour), but were high in the latter categories (maintained or improved vigour) (Appendix 21-24). This is a confirmation that plants would have better vitality if watered compared to those that do not receive water.

The results seem to be consistent with the fact that both shade and water tend to promote survival by reducing climatic pressure (abiotic stress). The mechanisms by which these factors contribute to the survival and better establishment of *Hoodia gordonii* are discussed in Chapter 8.

6.3.3 Effect of protection against predation

From a total of 160 plants that were protected against predation, 22 (13.8%) died. The majority of plants that survived were categorised as actively growing (52 plants; 32.5%), and good vigour (46 plants; 28.8 %). Plants with a good chance of recovery accounted for 32 (20.0%), where as only 8 plants (5.0%) had little chance of recovery (Fig.17).

Among the total of 160 plants that were not protected against predation, 93 (58.1%) died. The majority of live plants were categorised as good vigour which accounted for 31 plants (19.4%), followed by 18 plants with a good chance of recovery (11.3%), while 10 plants (6.3%) were actively growing and another 8 (5.0%) had little chance of recovery (Fig.17)

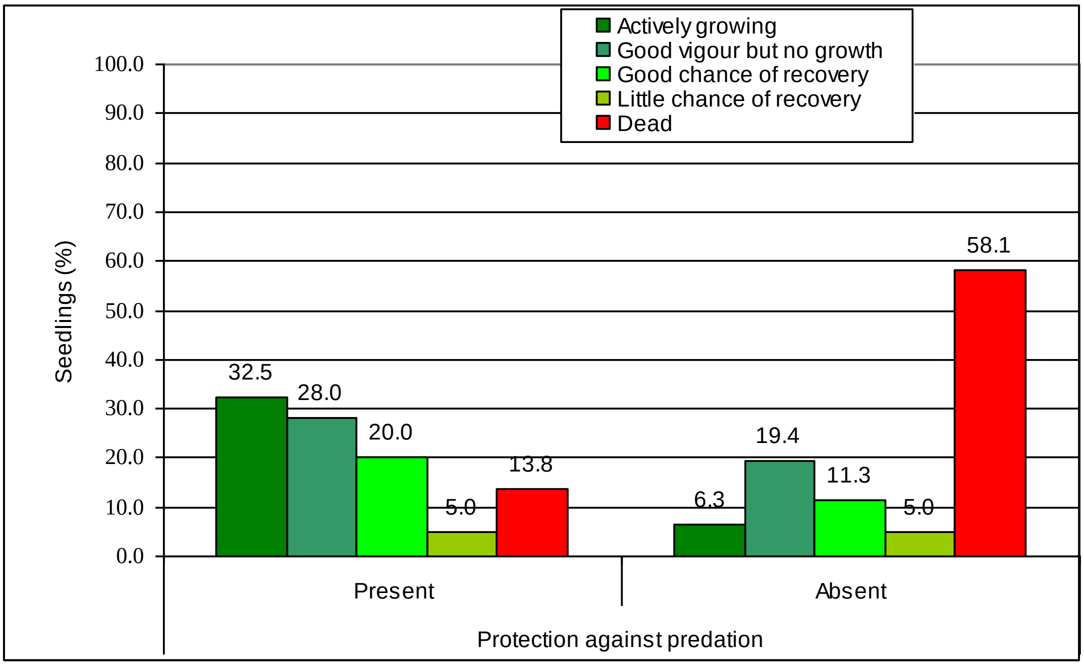

Figure 17: Percentage of seedling vitality as a result of the presence and absence of protection against predation.

Overall there is a very good relation between protection against predation and plant vitality (Appendix 19). The statistics show a significant positive association between plant vitality and protection against predation with χ^2 (4) = 79.128, p < 0.000 (Appendix 20). Seedlings were more likely to have better vitality when protected against predation than without protection. Although there was a statistically significant relationship between vitality and protection against predation it is believed that this was brought about as a result of the effect of factors such as water and shade. The Odds ratio indicates that the effect size of both the plants that lost vigour and those that maintained vigour was strong (Appendix 21-24). This is contrary to the results revealed by shade and water; in these instances the Odds ratio was only stronger in plants that maintained vigour and not those that lost vigour. This supports a suggestion that a positive association between protection against predation and vitality was influenced by factors other than actual plant protection against predation.

6.3.4 Effect of age at transplant

From a total of 160 plants that were transplanted at the age of one year, 54 plants (33.8%) died. The majority of live plants were categorised as good vigour but no growth (50 plants 31.3%), followed by the category of actively growing (39 plants; 24.4%). Eleven plants (6.9%) were categorised as having a good chance of recovery, while only 6 plants (3.8%) had little chance of recovery. Among the total of 160 plants that were transplanted at three months, 61 plants (38.1%) died. The majority of live plants were categorised as good chance of recovery which accounted for 39 plants (24.4%); good vigour no growth with total 27 plants (16.9%) and actively growing which accounted for 23 plants (14.4%). Only 10 plants (6.3%) had little chance of recovery (Fig. 18).

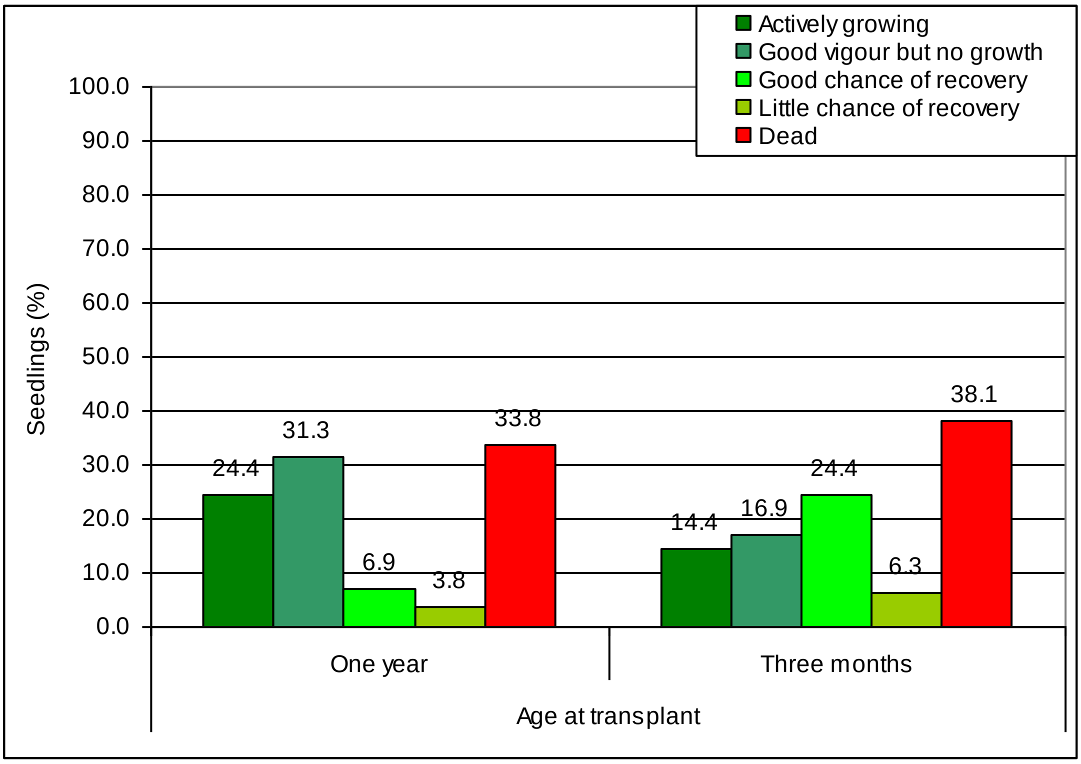

Figure 18: Percentage of seedling vitality as a result of the ages at transplant

There is a very strong relationship between age at transplant and vitality (Appendix 19). The vast majority of seedlings that were transplanted at the age of one year had highly improved vitality, compared to the plants transplanted at the age of three months (Fig. 18).

There was a highly significant association between the age at transplant (χ^2 (4) = 28.105, $p < 0.000$) and vitality (Appendix 20). This indicates that seedlings were more likely to have better vitality when transplanted at one year. The Odds ratio indicates that the effect size of age at transplant was low amongst plants that lost vigour, but was strong amongst plants that maintained vigour (Appendix 21-24). This is a confirmation that plants would have better vitality if transplanted at one year compared to three months.

6.3.5 Interactions between factors

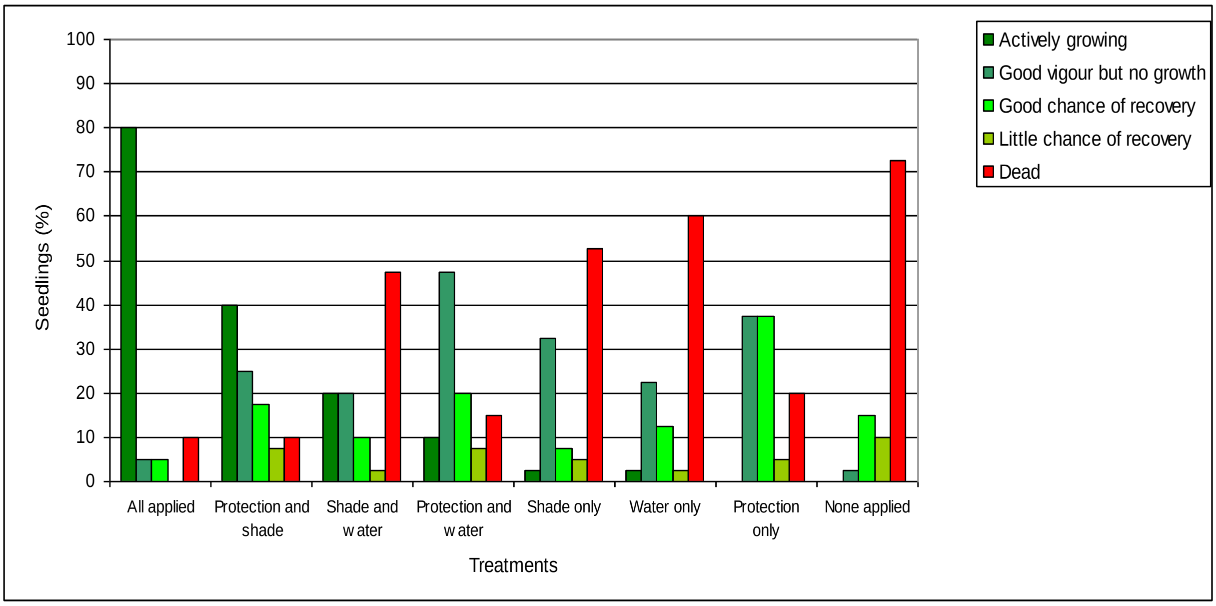

Figure 19: Plant vitality frequencies within each treatment as a result of treatment combinations between water, shade and protection.

Actively growing plants seem to be prominent in treatment combinations including shade, followed by the combinations that include water (Fig. 19). Treatments that does not include either shade or water, seems to result in a high number of plants categorised with low vigour (Fig.19).

A further discussion on how shade, water, protection and age may affect or induce plant vitality in *Hoodia gordonii* is included in chapter eight.

CHAPTER SEVEN

FACTORS DETERMINING PLANT GROWTH

7.1 Materials and methods

In order to determine the growth of the plants, the initial heights of the plants were measured from the soil surface to the highest point of the seedling using a 30cm ruler soon after transplanting. Initial seedling diameters were also measured on a marked point about half way on the stem using a vernier calliper. Plants were measured again at the end of the study period.

7.2 Statistical analysis

The Kolmogorov-Smirnov normality test was carried out if the data were normally distributed. As data deviated from normality, it did not meet the ANOVA assumptions (Appendix 27) and the Kruskal Wallis (rank test) test for non-parametric data was carried out to determine the effect of each factor (shade, water, protection and age) on plant growth. The SPSS statistical package was used to conduct the tests.

The Kruskal-Wallis test was followed by a non-parametric *post hoc* procedure using Mann-Whitney test, to test the effect of all the factors together. The *Bonferroni correction* method as described by Field (2005) was used to adjust the critical value in order to avoid inflating the type I error. This method ensures that the type I error does not inflate the critical value.

7.3 Results and discussion

7.3.1 Effect of shade

Shade improved the growth of seedlings from 79.18 mean ranks to 123.5 mean ranks (appendix 26). The test statistics *H* indicates that the growth rate (height) between seedlings that were shaded in comparison to un-shaded seedlings was significantly different, ($H(1) = 30.923$, $p < 0.001$ (Appendix 27).

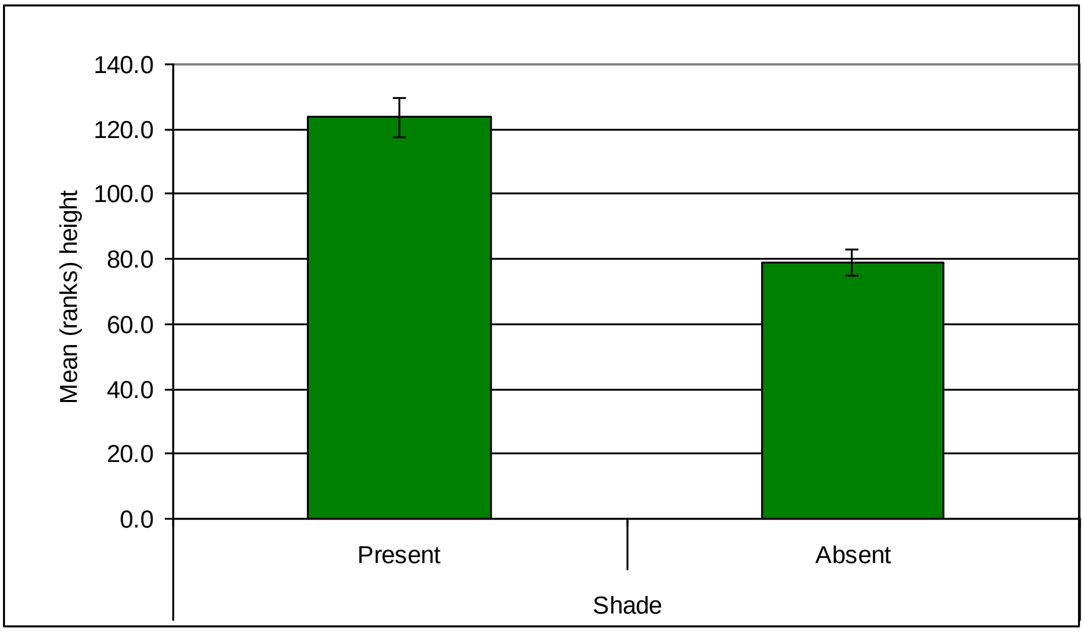

Figure 20: Differences in seedling height (in mean ranks) as a result of the presence or absence of shade.

Similarly, the mean ranks in diameter between shaded (127.7 mean ranks) and un-shaded (74.7 mean ranks) seedlings differed (Appendix 26). The test statistics *H* shows significant differences in diameter between shaded and un-shaded seedlings ($H(1) = 42.101$, $p < 0.001$ (Appendix 28).

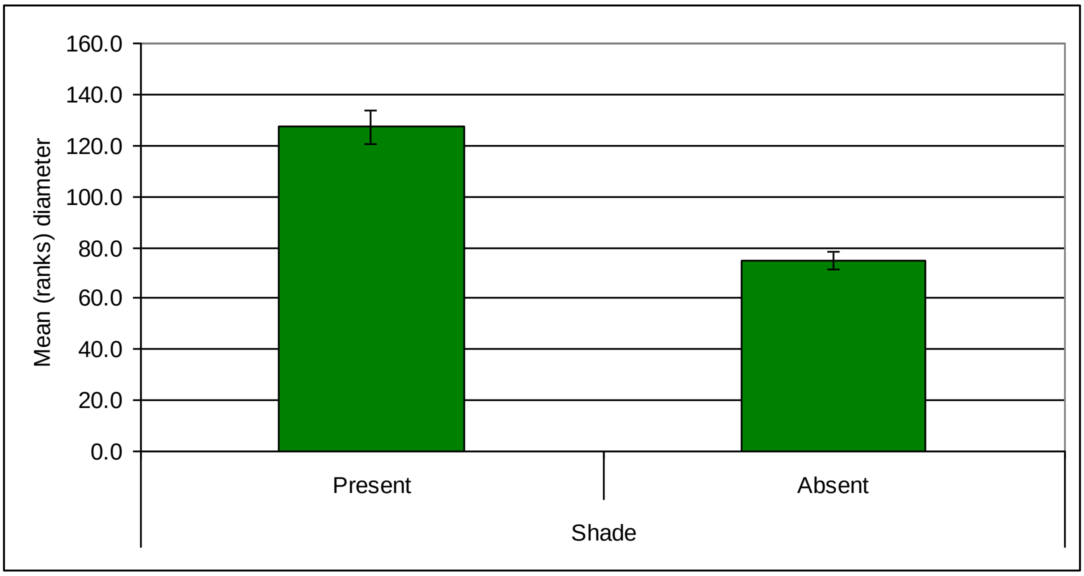

Figure 21: Mean height of seedlings as a result of treatment combination between water, shade and protection.

Considering the fact that vitality amongst shaded seedlings was good, it is not surprising that their growth was exceptionally good. The results are consistent and suggest that unlike plants in moist savannas, *Hoodia gordonii* has adapted to survive better, under shaded areas compared to open areas. This appears to be an important ecological adaptation, given the harsh abiotic stress and limited moisture availability in the arid environments.

7.3.2 Effect of watering

Water improved seedling growth both in height and diameter. Irrigated seedlings were 117.37 mean ranks in height compared to 88.21 mean ranks in height when water was not applied (Appendix 26). The test statistics *H* indicates that there was a highly significant difference (height) between the seedlings that were irrigated compared to those that were not irrigated (H (1) = 10.248, $p < 0.001$ (Appendix 27).

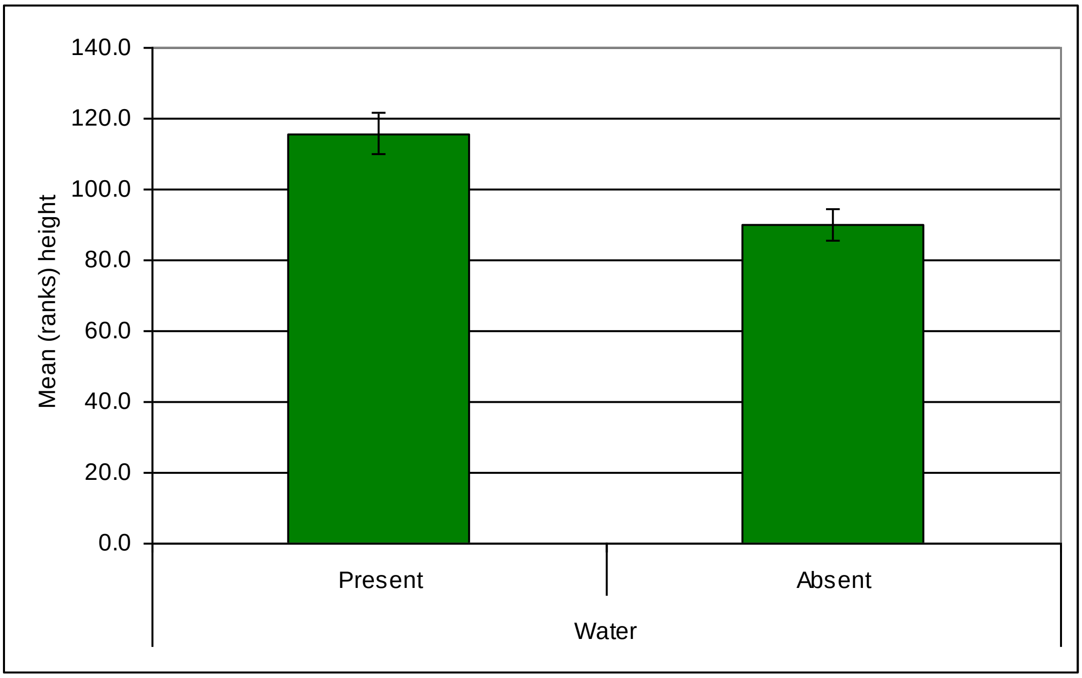

Figure 22: Differences in mean plant height as a result of the presence or absence of water.

The growth of seedlings in diameter was 120.94 mean ranks when watered and 84.28 mean ranks when water was not applied (Appendix 26). The test statistics *H* indicate that there were significant differences (diameter) between the seedlings that were irrigated compared to those that were not irrigated (H (1) = 20.736, $p < 0.000$ (Appendix 28).

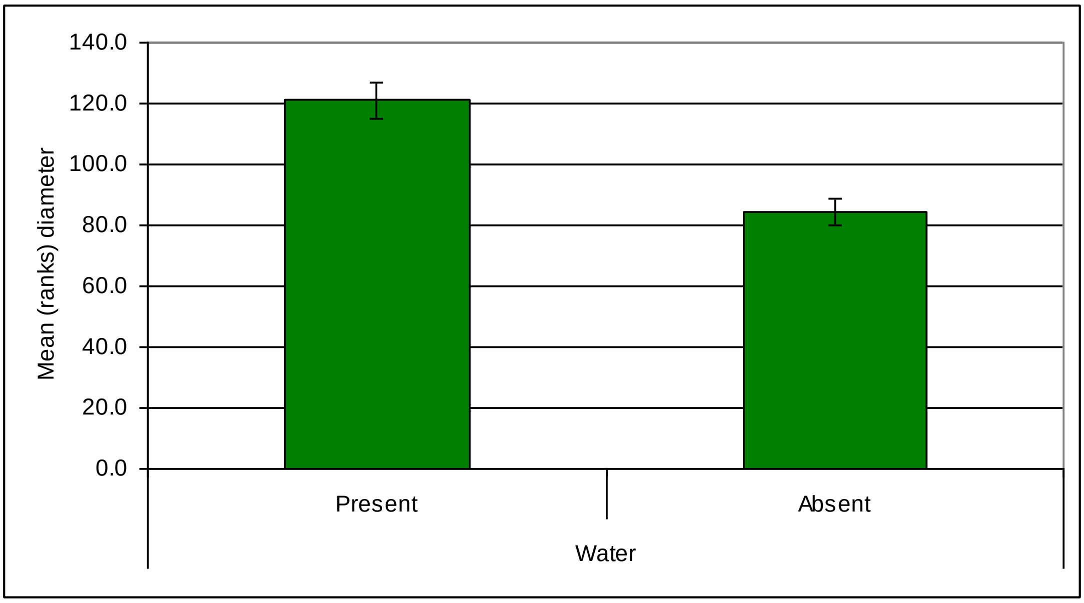

Figure 23: Differences of mean plant height as a result of the presence or absence of protection against predation.

Water contributed largely towards the growth of seedlings both in height and in diameter (Fig. 22-23). Although there was no significant difference in survival between irrigated seedlings compared to non irrigated seedlings, water significantly contributed towards growth and vitality of seedlings.

7.3.3 Effect of age at transplant

The seedlings that were transplanted at the age of one year had high mean ranks (114.43) compared to the seedlings that were transplanted at the age of three months (91.92 mean ranks) (Appendix 26). The test statistics *H* showed that there was a highly significant difference (height) between the seedlings that were transplanted at the age of one year and those that were transplanted at the age of three months (H (1) = 10.240, $p < 0.001$ (Appendix 27).

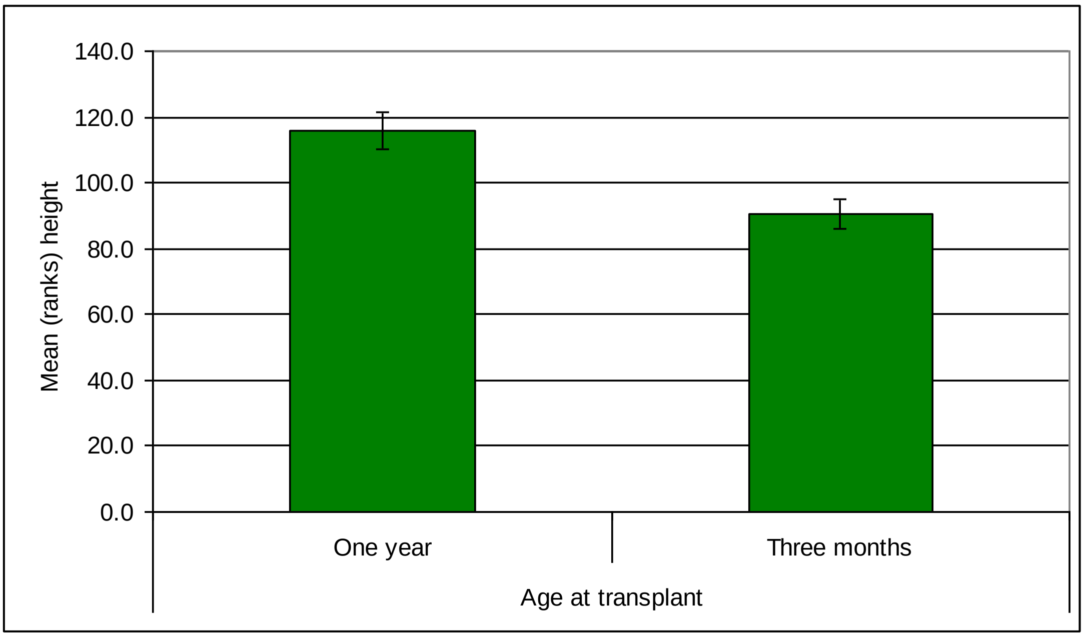

Figure 24: Differences in mean seedling height in ranks as a result of the ages at which seedlings were transplanted.

The results obtained from the growth of seedlings in diameter were similar to the growth in height (Fig. 24-25). There was a variation in growth rate between seedlings that were transplanted at one year of age 116.97 (mean ranks) compared to 89.22 (mean ranks) for the seedlings that were transplanted at the age of three months (Appendix 26).

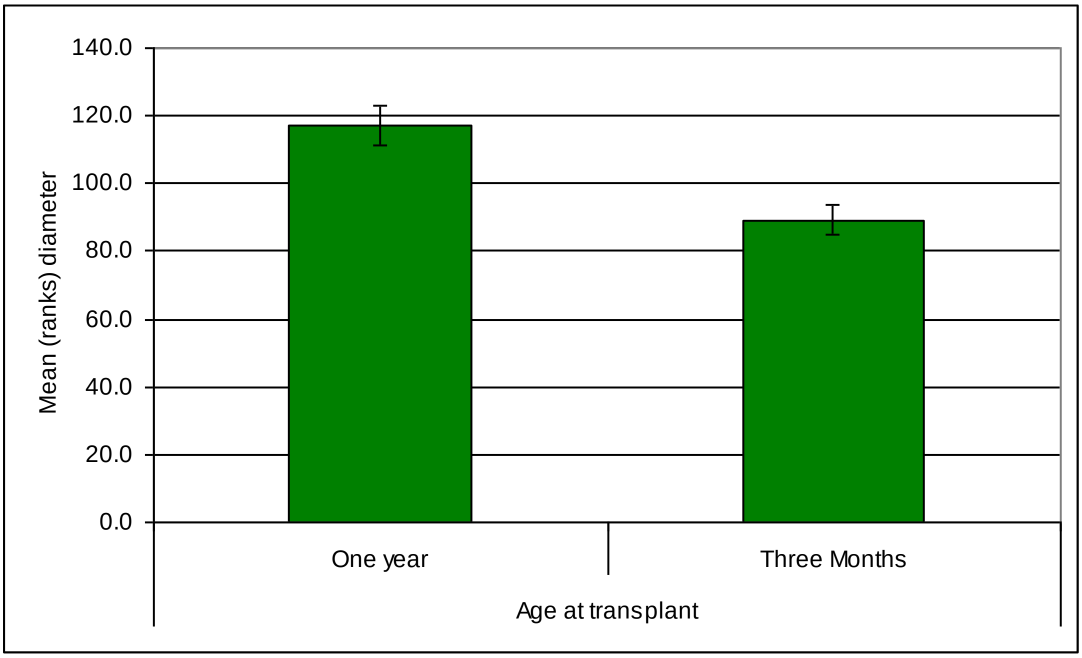

Figure 25: The mean growth in diameter of seedlings as a result of various combinations of water, shade and protection.

The differences between means of seedlings transplanted at the age of one year and those that were transplanted at three months were highly significant, (*H* (1) = 11.895, $p < 0.001$ (Appendix 28).

Biologically, seedlings transplanted at the age of three months were expected to show higher growth in comparison to seedlings that were transplanted at the age of one year. However, it is believed that the growth of seedlings transplanted at the age of three months was grossly suppressed by abiotic stress, at the age of one year; seedlings are believed to have improved resistance to harsh climatic stress. The effect of abiotic stress on different ages (Fig. 12) and the role of age at transplant on vitality (Fig. 18) are consistent and support this notion.

7.3.4 Effect of protection against predation

The mean growth rate in height amongst seedlings that were protected against predation was 109.42 (mean ranks) compared to 91.48 (mean ranks) amongst un-protected seedlings (Appendix 26). The test statistics *H* indicates that growth of seedlings that were protected against predation and those that were not protected differed significantly (*H* (1) =3.448, p = 0.035, $p < 0.05$ (Appendix 27).

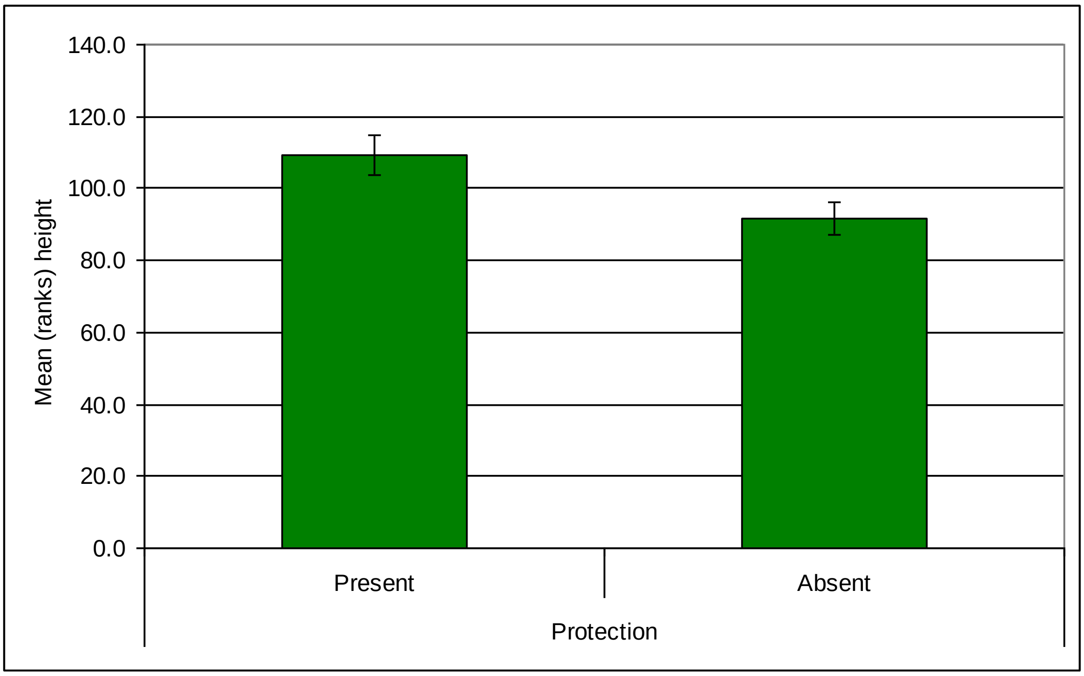

Figure 26: Differences in mean seedling growth (ranks) in diameter as a result of the presence or absence of shade.

The growth in diameter only varied from 96.60 (mean ranks) amongst non protected seedlings to 106.90 (mean ranks) amongst protected seedlings (Appendix 26). The test statistics *H* indicates that this difference is not significant statistically (*H* (1) = 1.452, p = 0.228, therefore $p > 0.05$ (Appendix 28). The result reveals that seedlings did not grow in diameter compared to how much they grew in height. This is attributed to the fact that, the stem of *Hoodia gordonii* would normally shrink if a plant loses vigour.

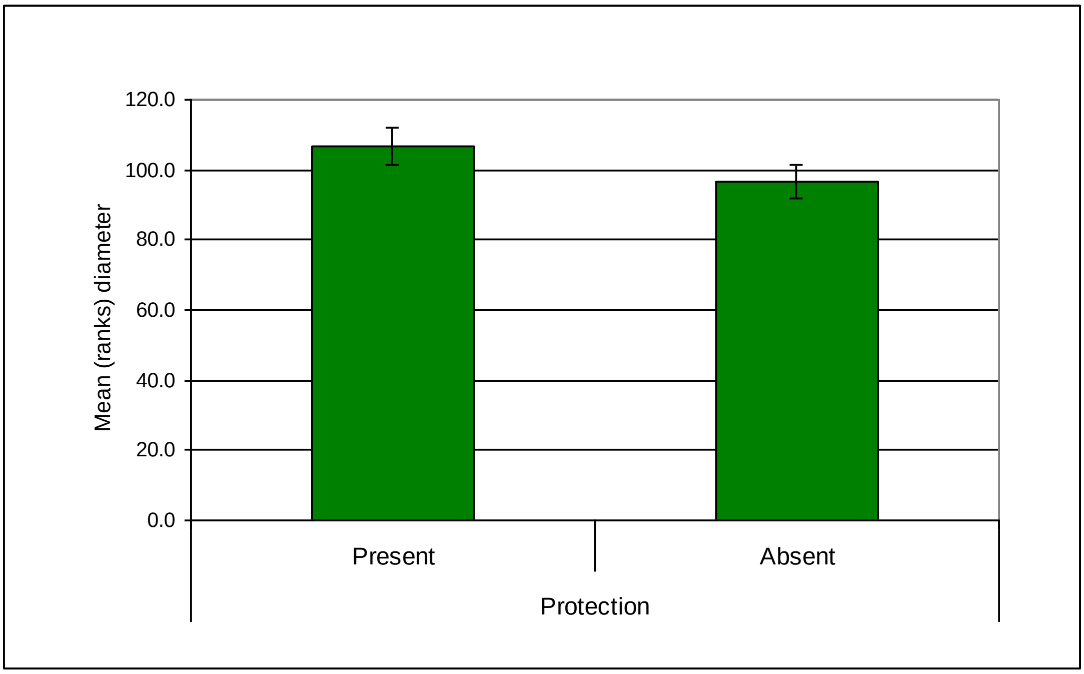

Figure 27: Differences of mean plant growth in diameter as a result of the presence and absence of water.

In comparison to shade and water, protection appeared to have the highest number of plants with low vigour (Fig. 17). Contrary, most plants with low vigour remained more or less at the same height, whilst their diameter was significantly reduced. Nevertheless, since there was no significant relationship between protection against predation and growth attributed to reduced vigour, it seems as if seedlings do not depend on protection against predation to grow.

7.3.5 Interaction between factors

Treatment combinations at the highest interaction level, resulted in a dramatic increase in growth (Fig. 28-29). At the lowest level, protection only had the lowest effect on the growth of seedlings and was significantly different in comparison to shade and water (Fig.28). On the other hand the treatment where none of the three factors were applied showed that growth differed significantly from all other treatment combinations (Fig. 28)

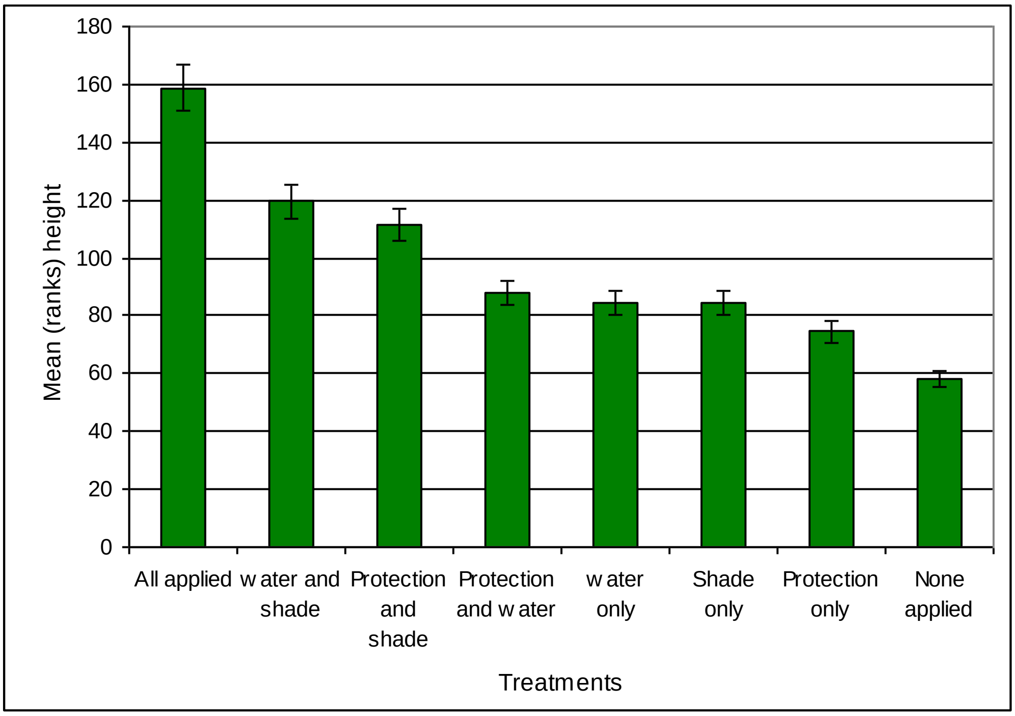

Figure 28: Show differences of mean plant growth in height as a result of presence and absence of protection against predation.

In response to treatments, seedlings showed a similar growth pattern in diameter, as in height (Fig. 29). Although water and shade alone improved the growth rate in both height and diameter, seedlings were most responsive to an interaction of all factors (Fig.28-29).

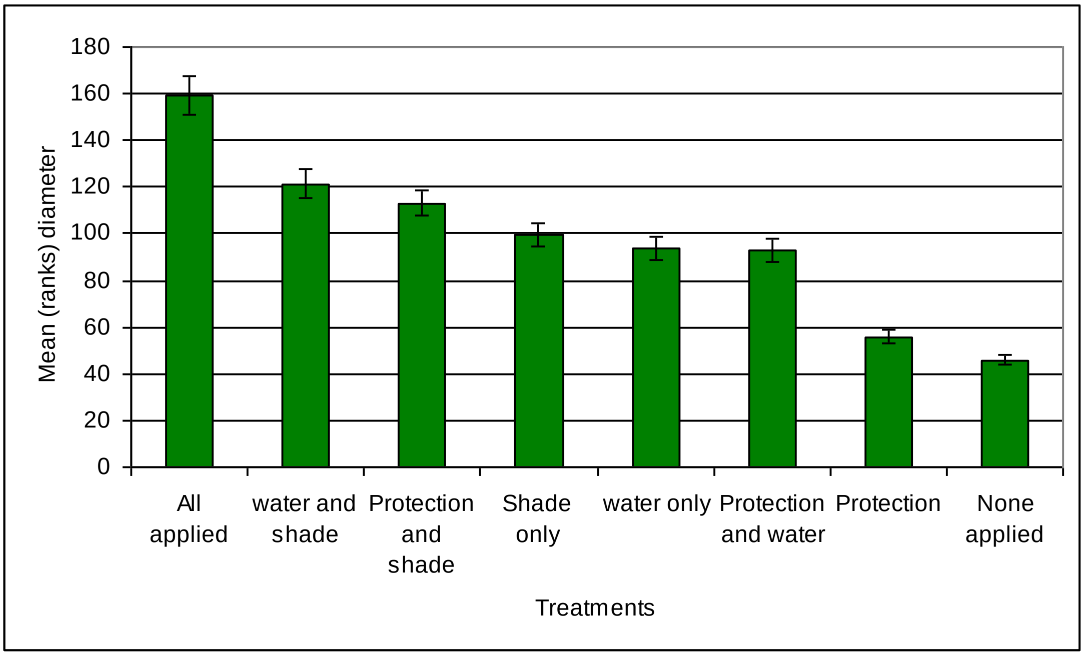

Figure 29: Differences in seedling height in ranks as a result of the age at which seedlings were transplanted at (three months and one year).

An observed positive response of seedlings to a combination of factors appears to facilitate necessary support for the establishment of seedlings. This is assumed to be an important evolutionary advantage of the species to survive in harsh environmental conditions.

CHAPTER EIGHT

ANALYSIS, RECOMMENDATIONS AND CONCLUSIONS

8.1 The role of protection against predation on initial seedling establishment

The key question posed under hypothesis three states that: “Does protection against predation play a role in the establishment and growth of *Hoodia gordonii* seedlings transplanted in the natural environment?” Since there was a very strong positive association between survival of the seedlings and protection against predation, the tested hypothesis in this study is substantiated. *Hoodia gordonii* seems to be heavily dependent on protection against predation in order to survive in nature.

The strong dependence of seedlings on protection against predators for survival is supported by a strong negative association between mortality and the availability of protection. The results show that mortalities were significantly reduced among plants that were protected.

Although there is little information about the functional ecology of the genus *Hoodia*, several authors have studied and confirmed that there is a positive plant-plant interaction in arid environments (Blignaut and Milton, 2005; Riginos *et al.*, 2005; Flores and Jurado, 2003). Observations in the study area and other parts of Namibia showed a general strong association of *Hoodia gordonii* and other plant species. Blignaut and Milton (2005) confirm that plants in the Karoo shrub lands are often arranged in multispecies clumps on fertile islands. In addition, there is a suggestion that stem succulents are vulnerable to predation since they are believed to play an important role as far as energy, nutrient and water budgets for consumers in arid environments are concerned (Wolf and del Rio, 2003). CAM plants store abundant resources in the form of water (Mauseth, 2004; Jochen Schenk and Jackson, 2002) and produce abundant flowers and fruit crops that is used by many animals in arid environment (Wolf and del Rio, 2003).

Despite the role of edaphic stress in determining the relative importance of facilitation in arid plants (Armas and Pugnaire, 2005; Blingnaut and Milton, 2005; Riginos *et al.,* 2005; Flores and Jurado, 2003); biotic factors (i.e predation pressure) has been highlighted (Riginos *et al*., 2005; Carrick, 2003; Lenz and Facelli, 2003; Wolf and del Rio, 2003). The results of this study are therefore consistent and seem to suggest that despite edaphic influence, other factors (mostly biotic) resulting from the lack of protection against predation may have a marked influence on the survival of *Hoodia gordonii* in its natural environment.

The effect of protection on plant growth and vitality is less important in comparison to its role in preventing mortality and promoting survival. Although there was a significant positive association between protection and growth/vitality, the association was less significant in comparison to the association observed in survival and in factors contributing to seedling mortality. Moreover, the number of surviving plants in the protected category was significantly higher than those in non-protected category. In contrast high discrepancy between the comparative categories (protected and un-protected), is a justification that the results were influenced by other factors. In addition, a high number of seedlings that maintained vigour were lost due to predation in the non protected category. These plants could have contributed immensely to the assessment of growth and vitality within the category.

8.2 The effect of shade on seedling establishment

The key question posed under hypotheses four was to examine the effect of shade on the establishment of *Hoodia gordonii* in its natural environment. The results obtained in this study show that shaded seedlings performed better than un-shaded seedlings, contrary to the hypothesis as stated in 3.4. There was a significant positive association between shade and the survival of the seedlings. This implies that initial establishment of this species requires shade.

Although the margin of association between shade and survival is low in comparison to the one brought about as a result of protection, it is clearly explained by the lack of association between shade and the factors that contributed to seedling mortalities (Fig.10). This study found no significant association between shade and causes of deaths, indicating that seedlings survival would not be affected, despite the presence or absence of shade. However, an in-depth analysis of the 2 x 2 contingency table shows that although shade had little or no influence on causes of deaths brought about as a result of predation (biotic pressure), it had a marked influence on stress (abiotic pressure), and reduced deaths due to stress by almost half. Since there was reduced effect of abiotic stress amongst shaded seedlings, it is assumed that a high number of seedlings that were partially browsed by insects recovered, where as the majority of those that was un-shaded died. Although the difference in plant mortalities due to rodents between shaded and un-shaded categories was negligible in comparison to the impact of abiotic stress and insects, mortality was still less amongst shaded seedlings. This explains the reason why shade was positively associated with seedling survival.

The results on plant growth and vitality further confirm that shade is a critical factor as far as initial establishment of *Hoodia gordonii* is concerned. In both cases there was a highly significant difference in seedlings that were shaded compared to those that were not. Growth and vitality of seedlings were both positively associated with shade, indicating that seedlings required shade in order to have good vigour and grow.

Many authors have highlighted the adaptation mechanisms that allow succulents to survive in shaded areas (Escudero, *et al.,* 2005; Riginos, *et al.,* 2005; Carrick, 2003; Lenz and Facelli, 2003; Wolf and del Rio, 2003). According to Lenz and Facelli (2003) the stem succulents *Orbea variegate* (family Asclepiadaceae) appear to grow vigorously and contain less red pigments underneath other plants, than when growing in the open. The results obtained in this study suggest that *Hoodia gordonii* may employ a similar strategy to *Orbea variegate* in Australia. It should however be noted that the present study was restricted to the early life stages of *Hoodia gordonii* and that these processes may not necessarily operate across all life stages.

The findings of this study seem to be consistent and explain why plants in arid environments have diverged through adaptation to survive under shaded areas where they receive protection against predation. Unlike protection against predation, where biotic pressure was the major factor determining survival, the role of shade seems to suggest that abiotic pressure such as temperature, radiation (Carrick, 2003; Lenz and Facelli, 2003) and evaporation (Martorell and Patiño, 2006) are the major factors determining survival and establishment of *Hoodia gordonii*. According to Martorell and Patiño (2006), succulents frequently grow in cool microenvironments because they are prone to overheating due to their massive organs and lack of daytime transpiration. Carrick (2003) also suggests that in the Nama Karoo young plants grow under other established plants in order to avoid excessive radiation and heating.

8.3 The effect of water on initial seedling establishment

The key question posed under this hypothesis was to determine the effect of moisture on the establishment of *Hoodia gordonii* in its natural environment. The results obtained from the analysis of survival show that there was no association between survival of seedlings and water. Under the conditions of the present trial, survival of the seedlings was independent of moisture. This seems to suggest that the hypothesis as stated is unsubstantiated, and whether seedlings were irrigated or not there would be no difference in survival.

In contrast, an in-depth analysis of a 2 x 2 contingent table, revealed that as in the case of shade, provision of water reduced plant loss due to abiotic stress, but tends to promote loss of plants due to biotic pressure (rodents). This seems to explain why water did not have an overall influence on seedling survival. Based on observations, a possible explanation is that the presence of water (through the smell) tends to attract animals, which resulted in an increased loss of seedlings within the irrigated category. Although not confirmed, it is suggested that as a result of animals in search of moisture, in conjunction with the seasonal timing of the experiment (dry season), plants that were irrigated fell victims to predation more often than plants that were not irrigated.

It should be acknowledged that the seasonal timing of the research was a limitation in this regard and contributed to low number of plant survival amongst irrigated seedlings. Even though, there was high mortalities due to predation amongst irrigated seedlings compared to non irrigated seedlings, succulent life form of *Hoodia gordonii* also contributed to sustained survival amongst non watered seedlings.

Despite the role of predation and resistance of the seedlings to moisture stress, the results show that there was a significant association between water and plant vitality as well as plant growth. This seems to suggest that initial establishment (through good vigour and plant growth) of *Hoodia gordonii* is highly dependent on moisture. The results are consistent and explain why stress was the only variable that was positively associated with the provision of water.

It is widely known that water is a critical factor on which both plant and animal life depend. Arid environments have extremely variable soil moisture in space and time (Mauseth, 2004; Martorell and Ezcurra, 2002) and as a result plant life has adapted in order to survive (Curadi *et al.*, 2005; Schwining and Ehleringer, 2001). Several authors have highlighted mechanisms such as characteristics of succulent structure (Mauseth, 2004; Martorell and Ezcurra, 2002; Schwining and Ehleringer, 2001) and establishment under nurse plants (Montorell and Patiño, 2006; Flores and Jurado, 2003; Schwining and Ehleringer, 2001) as strategies to survive limited availability of moisture in arid environments.

According to Mauseth (2004), succulence is an adaptive mechanism that allows arid plants to store excess amounts of water, which can then be used over extended periods of time. Moreover, the anatomy of stem photosynthetic succulents in many species including those that belong to the family Apocynaceae, are believed to be structured in such a way that loss of water due to transpiration is minimised.

Since water is a limiting resource in arid environments, growing under established plants does not only favour the provision of shade and protection, but involves several other factors including the retention of soil moisture. According to Flores and Jurado (2003) soils in open arid areas dry out very quickly, where as under established plants, moisture is more likely to be retained for an extended time period due to shade, lower temperature, less evaporation and greater stability of soil structure.

8.4 The importance of age on initial seedling establishment

The key question posed under hypothesis four states that: "Does age at the time of transplanting influence the establishment and growth of *Hoodia gordonii* seedlings transplanted into their natural environment?" As far as seedling survival is concerned, the results show that overall there was no significant difference in survival between seedlings transplanted at the age of three months and those that were transplanted at the age of one year. The analyses of a 2 x 2 contingent table indicates that there was a very high percentage of losses as a result of stress amongst seedlings that were transplanted at the age of three months in comparison to those that were transplanted at the age of one year.

However, the assessment of plant vitality between the age groups revealed that the majority of seedlings that were transplanted at the age of one year maintained vigour where as the majority of those that were transplanted at the age of three months had lost vigour. Although mortality due to predation did not vary considerably, more seedlings transplanted at the age of one year died due to insects and rodents compared to those transplanted at the age of three months. Two explanations are suggested: Plant predators may favour bigger plants because they have higher moisture and nutrient contents compared to smaller plants. Presently there is no available information on the role of *Hoodia* in providing nutrients and water to animal consumers. However, a study conducted by Wolf and del Rio (2003) suggest that many succulent species found in the arid environments are important providers of energy, moisture and nutrients to animals. The link between good plant vitality and predation was also clearly observed in the results obtained on the role of water.

There was a highly significantly difference in growth between the seedlings that were transplanted at the age of one year compared to the seedlings that were transplanted at the age of three months. These results are consistent and confirm that seedlings transplanted at the age of one year had established better and quicker than those that were transplanted at three months. Several authors have highlighted the fact that species that depend on others to establish and survive, are more vulnerable at a younger age than at maturity (Martorell and Patiño, 2006; Escudero *et al*. 2005; Flores and Jurado, 2003; Lenz and Facelli, 2003).

The overall survival of plants in the two age groups was not significantly different. However, it is clear that at a young age *Hoodia gordonii* is more vulnerable to abiotic stress. Although there is no conclusive evidence, this seems to suggest that the early life stages of *Hoodia gordonii* may require the availability of other plants (nurse plants) to minimise the effect of abiotic stress such as temperature, radiation and evaporation coupled with protection from predation. Moreover, as the seedling grows older abiotic stress becomes less important compared to biotic stress.

8.5 Interaction

Under the conditions of the present study, separate treatments on their own may not amount to much, but their interactions are additive. In all dependent variable (survival, vitality and growth) seedlings were most responsive to complete combination of factors (shade, protection against predation and water). Positive response of seedlings to a combination of factors is assumed to facilitate necessary support for establishment and appears to be an adaptive mechanism for *Hoodia gordonii* to survive in arid environments. At lower level individual factors appeared to have positive influence on specific dependent variable, however their impact would be short lived since the seedlings required all factors to establish.

8.6 Recommendations and conclusions

The findings of this study indicate that cultivated *Hoodia gordonii* can be successfully translocated into its natural habitats. The results cover many attributes which can be interpreted by allocating factors affecting the establishment of this species as follows:

1. The influence of biotic factors (predation) brought about by the lack of physical protection against predation.
2. The influence of abiotic factors (temperature, radiation, evaporation) brought about by the lack of shade and subsequently limited moisture availability to plants.
3. The resilience of plants against abiotic stress (high temperature and lack of moisture) brought about as a result of age at transplant.

The influence of edaphic factors are not quantified in this study; however many authors have shown that soil has a major influence on plant structure in semi arid environments (Armas and Pulgnaire, 2005; Blignaut and Milton 2005; Umbanhower and McCann, 2005; Carrick, 2003; Lenz and Facelli, 2003; Otto, *et al.*, 2001). Water has been identified as one of the most limiting factors in arid environments. In addition, arid zones are known to have high evaporation rates, radiation and transpiration, due to high temperatures and dry air.

The study presents evidence that overall, a combination of physical protection, shade and moisture is critical and determines the survival and establishment during the early life stages of *Hoodia gordonii*. Although this study did not investigate the importance of these factors at all life stages, the findings are consistent with the suggestion based on observation that, “nurse plant syndrome” is common in *Hoodia gordonii* which is found to co-exist with other established species against the simple interpretation of the principle of competitive exclusion.

Based on the results of this study and growing evidence that edaphic differences shape the structure of plant communities in arid environments, it is suggested that translocation of *Hoodia gordonii* can successfully be done by using other plant species already established to act as shields against biotic and abiotic pressure. There has been growing evidence suggesting that seedlings growing under canopies of other plants species benefit from cooler temperatures during hot days, higher water infiltration and less evaporation, more nutrients in the soil, protection from grazing and trampling, physical support, less soil compaction and less soil erosion (Mortorell and Patiño, 2006; Armas and Pulgnaire, 2005; Umbanhower and McCann, 2005; Mauseth, 2004; Carrick, 2003; Lenz and Facelli, 2003; Otto, *et al.*, 2001). However, it is not known whether nurse plants have the ability to attract seed dispersers or capture seed dispersal, which might be a contributing factor leading to common establishment of seedlings under canopies. Further studies may explore the role of nurse plants and shed some light on their role on seed dispersal. In addition, rehabilitation of plant species using seeds seems to be less expensive and in some cases successful (Zheng *et al.*, 2004). This is also a field of study that will need to be explored in order to contribute to the conservation and protection of this commercially important species.

It should be noted that the rehabilitation of plant species into natural environments *via* intensive intervention presents a major challenge and requires an enormous investment of time, money and expertise. Presently, the extent to which *Hoodia gordonii* has been lost in its natural habitat can not be quantified to warrant such an effort. However, the trend brought by a combination of human activities is frightening and may have led to local extinction of the species in some areas. In the same way the species may go extinct in a wide range of areas sooner than anticipated. Until the extent to which the species is being affected by the recent development of human activities is quantified, preparation for active management of genetic materials should not be overlooked.

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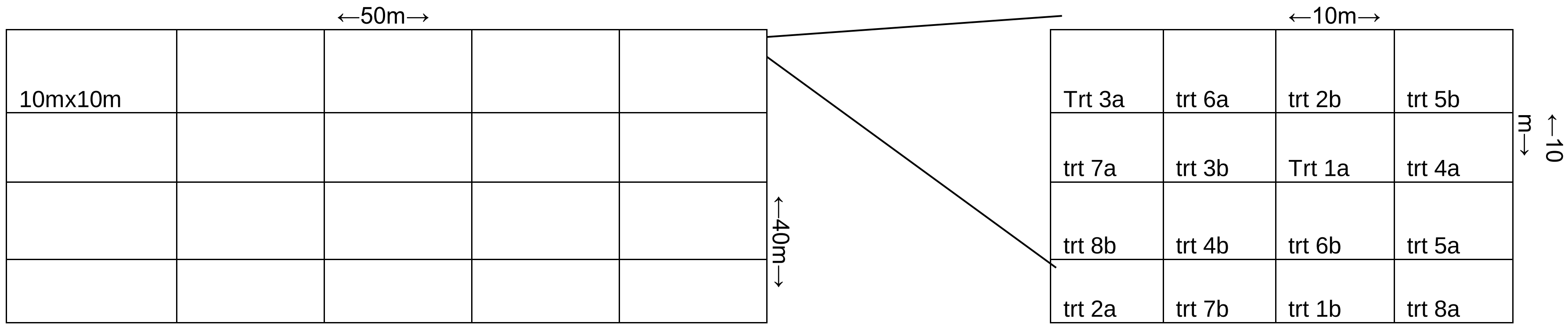
Appendix 1: The layout and the design of the experiment conducted at !Khob !Naub Conservancy. A total area of 50m x 40m was divided into 20 blocks each block equal to 10m x 10m. Plants were in two different ages: three months and one year old, each age was given all eight treatments combination in one block resulting in a total of 16 plants per block. Each treatment combination at each age was replicated one in each block resulting in N=20 and df=19.



A 2x2x2 factorial experiment

Factors include	**Combined into eight treatments**
Water	1. All not applied
Shade	2.All applied
Protection	3. Water only
	4. Shade only
	5. Protection only
	6. Water and shade only
	7. Water and protection
	8. Shade and water

Appendix 2: Loglinear analysis shows the observed and expected survival as result of the interactions between factors (shade, water and protection**.**

Survival	Shade	Water	Protection	Observed		Expected		Residuals	Std. Residuals
				Count(a)	%	Count	%		
Dead	Absent	Absent	Absent	29.500	9.2%	29.500	9.2%	.000	.000
			Present	8.500	2.7%	8.500	2.7%	.000	.000
		Present	Absent	24.500	7.7%	24.500	7.7%	.000	.000
			Present	6.500	2.0%	6.500	2.0%	.000	.000
	Present	Absent	Absent	21.500	6.7%	21.500	6.7%	.000	.000
			Present	4.500	1.4%	4.500	1.4%	.000	.000
		Present	Absent	19.500	6.1%	19.500	6.1%	.000	.000
			Present	4.500	1.4%	4.500	1.4%	.000	.000
Live	Absent	Absent	Absent	11.500	3.6%	11.500	3.6%	.000	.000
			Present	32.500	10.2%	32.500	10.2%	.000	.000
		Present	Absent	16.500	5.2%	16.500	5.2%	.000	.000
			Present	34.500	10.8%	34.500	10.8%	.000	.000
	Present	Absent	Absent	19.500	6.1%	19.500	6.1%	.000	.000
			Present	36.500	11.4%	36.500	11.4%	.000	.000
		Present	Absent	21.500	6.7%	21.500	6.7%	.000	.000
			Present	36.500	11.4%	36.500	11.4%	.000	.000

Appendix 3: The loglinear analysis shows the Goodness-of-Fit model for survival data.

	Chi-Square	df	Sig.
Likelihood Ratio	.000	0	INF
Pearson	.000	0	INF

Appendix 4: The loglinear analysis shows the K-Way and Higher-Order effects for interaction of factors.

			Likelihood Ratio		Pearson		
	K	df	Chi-Square	Sig.	Chi-Square	Sig.	Number of Iterations
K-way and Higher Order Effects(a)	1	31	114.073	.000	98.200	.000	0
	2	26	88.416	.000	79.148	.000	2
	3	16	7.577	.960	6.618	.980	5
	4	6	5.492	.482	4.152	.656	4
	5	1	3.224	.073	2.595	.107	3
K-way Effects(b)	1	5	25.657	.000	19.052	.002	0
	2	10	80.840	.000	72.530	.000	0
	3	10	2.084	.996	2.466	.991	0
	4	5	2.269	.811	1.558	.906	0
	5	1	3.224	.073	2.595	.107	0

Appendix 5: The cross tabulation showing the relationship between plant survival in the presence and absence of all factors. (shade, water, protection), and age at three months and one year at transplant.

Survivorship		Shade			Water			Protection			Age at transplant		
		Absent	**Present**	**Total**	**Absent**	**Present**	**Total**	**Absent**	**Present**	**Total**	**Three months**	**One year**	**Total**
	Count	67	48	115	62	53	115	93	22	115	61	54	115
	% within Survivability	58.3	41.7	100	53.9	46.1	100	80.9	19.1	100	53.0	47.0	100
	% within Factor	41.9	30.0	35.9	38.8	33.1	35.9	58.1	13.8	35.9	38.1	33.8	35.9
Dead	% of Total	20.9	15.0	35.9	19.4	16.6	35.9	29.1	6.9	35.9	19.1	16.9	35.9
	Count	93	112	205	98	107	205	67	138	205	99	106	205
	% within Survivability	45.4	54.6	100	47.8	52.2	100	32.7	67.3	100	48.3	51.7	100
	% within Factor	58.1	70.0	64.1	61.3	66.9	64.1	41.9	86.3	64.1	61.9	66.3	64.1
Live	% of Total	29.1	35.0	64.1	30.6	33.4	64.1	20.9	43.1	64.1	30.9	33.1	64.1
	Count	**160**	**160**	**320**	**160**	**160**	**320**	**160**	**160**	**320**	**160**	**160**	**320**
	% within Survivability	**50**	**50**	**100**	**50**	**50**	**100**	**50**	**50**	**100**	**50**	**50**	**100**
	% within Factor	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
TOTAL	**% of Total**	**50**	**50**	**100**	**50**	**50**	**100**	**50**	**50**	**100**	**50**	**50**	**100**

Appendix 6: Pearson's chi-square statistics shows the significance of association between variables (shade, water, protection and age at transplant) and survival.

Factors **Chi-Square Tests Survivorship**

		Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Shade	Pearson Chi-Square	4.900	1	0.027	0.036	0.018	
Water	Pearson Chi-Square	1.099	1	0.294	0.351	0.176	
Protection	Pearson Chi-Square	68.425	1	0.000	0.000	0.000	
Age	Pearson Chi-Square	0.665	1	0.415	0.485	0.242	
Shade	Continuity Correction(a)	4.398	1	0.036			
Water	Continuity Correction(a)	0.869	1	0.351			
Protection	Continuity Correction(a)	66.511	1	0.000			
Age	Continuity Correction(a)	0.489	1	0.485			
Shade	Likelihood Ratio	4.917	1	0.027	0.036	0.018	
Water	Likelihood Ratio	1.100	1	0.294	0.351	0.176	
Protection	Likelihood Ratio	72.266	1	0.000	0.000	0.000	
Age	Likelihood Ratio	0.665	1	0.415	0.485	0.242	
Shade	Fisher's Exact Test				0.036	0.018	
Water	Fisher's Exact Test				0.351	0.176	
Protection	Fisher's Exact Test				0.000	0.000	
Age	Fisher's Exact Test				0.485	0.242	
Shade	Linear-by-Linear Association	4.885	1	0.027	0.036	0.018	0.008
Water	Linear-by-Linear Association	1.096	1	0.295	0.351	0.176	0.054
Protection	Linear-by-Linear Association	68.211	1	0.000	0.000	0.000	0.000
Age	Linear-by-Linear Association	0.663	1	0.415	0.485	0.242	0.067
	N of Valid Cases	320					

Appendix 7: The Odds ratio comparing the effect size resulting from shade, water, protection and age on survival of plants

Odds ratio for factors	**Shade**	**Water**	**Protection**		**Age**
factor absent					
Odds factor absent = number that died in the absence of factor	67	62	93	Three months	61
number that survived in the absence of factor	93	98	67	Three months	99
Odds factor absent =	67 / 93	62 / 98	93 / 67		61 / 99
Odds factor absent =	0.72	0.63	1.39		0.62
factor present					
Odds factor present = number of that died in the presence of factor	48	53	22	One year	54
number that survived in the presence of factor	112	107	138	One year	106
Odds present =	48 / 112	53 / 107	22 / 138		
Odds factor present =	0.43	0.50	0.16		0.51
Odds ratio factor					
Odds ratio = Odds factor absent	0.72	0.63	1.39	Three months	0.62
Odds factor present	0.43	0.5	0.16	One year	0.51
Odds ratio =	0.72 / 0.43	0.63 / 0.50	1.39 / 0.16		0.62 / 0.51
Odds ratio =	1.67	1.26	8.69		1.22

Appendix 8: Loglinear analyses show the observed and expected dead and undisturbed plants as a result of the interactions between factors (shade, water and protection).

Shade	Water	Protection	Factors causing deaths	Observed		Expected		Residuals	Std. Residuals
				Count(a)	%	Count	%		
Absent	Absent	Absent	Undisturbed	9.500	3.0%	9.500	3.0%	.000	.000
			Stress	8.500	2.7%	8.500	2.7%	.000	.000
			Insect bite	11.500	3.6%	11.500	3.6%	.000	.000
			Rodent attack	12.500	3.9%	12.500	3.9%	.000	.000
		Present	Undisturbed	30.500	9.5%	30.500	9.5%	.000	.000
			Stress	10.500	3.3%	10.500	3.3%	.000	.000
			Insect bite	.500	.2%	.500	.2%	.000	.000
			Rodent attack	.500	.2%	.500	.2%	.000	.000
	Present	Absent	Undisturbed	13.500	4.2%	13.500	4.2%	.000	.000
			Stress	3.500	1.1%	3.500	1.1%	.000	.000
			Insect bite	6.500	2.0%	6.500	2.0%	.000	.000
			Rodent attack	18.500	5.8%	18.500	5.8%	.000	.000
		Present	Undisturbed	33.500	10.5%	33.500	10.5%	.000	.000
			Stress	7.500	2.3%	7.500	2.3%	.000	.000
			Insect bite	.500	.2%	.500	.2%	.000	.000
			Rodent attack	.500	.2%	.500	.2%	.000	.000
Present	Absent	Absent	Undisturbed	16.500	5.2%	16.500	5.2%	.000	.000
			Stress	6.500	2.0%	6.500	2.0%	.000	.000
			Insect bite	5.500	1.7%	5.500	1.7%	.000	.000
			Rodent attack	13.500	4.2%	13.500	4.2%	.000	.000
		Present	Undisturbed	36.500	11.4%	36.500	11.4%	.000	.000
			Stress	4.500	1.4%	4.500	1.4%	.000	.000

		Insect bite	.500	.2%	.500	.2%	.000	.000
		Rodent attack	.500	.2%	.500	.2%	.000	.000
Present	Absent	Undisturbed	14.500	4.5%	14.500	4.5%	.000	.000
		Stress	2.500	.8%	2.500	.8%	.000	.000
		Insect bite	10.500	3.3%	10.500	3.3%	.000	.000
		Rodent attack	14.500	4.5%	14.500	4.5%	.000	.000
	Present	Undisturbed	35.500	11.1%	35.500	11.1%	.000	.000
		Stress	5.500	1.7%	5.500	1.7%	.000	.000
		Insect bite	.500	.2%	.500	.2%	.000	.000
		Rodent attack	.500	.2%	.500	.2%	.000	.000

Appendix 9: The loglinear analysis shows the Goodness-of-Fit model for data on factors causing deaths.

	Chi-Square	Df	Sig.
Likelihood Ratio	.000	0	INF
Pearson	.000	0	INF

Appendix 10: The loglinear analysis shows the K-Way and Higher-Order effects for interaction of factors.

			Likelihood Ratio		Pearson		
	K	df	Chi-Square	Sig.	Chi-Square	Sig.	Number of Iterations
K-way and Higher Order Effects(a)	1	63	363.585	.000	356.400	.000	0
	2	56	198.790	.000	163.917	.000	2
	3	38	24.449	.957	21.781	.984	5
	4	16	9.214	.904	8.547	.931	4
	5	3	8.049	.045	7.420	.060	2
K-way Effects(b)	1	7	164.795	.000	192.483	.000	0
	2	18	174.341	.000	142.136	.000	0
	3	22	15.235	.852	13.235	.926	0
	4	13	1.164	1.000	1.126	1.000	0
	5	3	8.049	.045	7.420	.060	0

Appendix 11: The cross tabulation shows a relationship between the presence and absence of factor and causes of deaths.

Causes of deaths		Shade			Water			Protection			Age at transplant		
		Absent	Present	Total	Absent	Present	Total	Absent	Present	Total	Three months	One year	Total
Undisturbed	Count	93	112	**205**	98	107	**205**	67	138	**205**	99	106	**205**
	% within Disturbance factors	45.4%	54.6%	**100.0%**	47.8%	52.2%	**100.0%**	32.7%	67.3%	**100.0%**	48.3%	51.7%	**100.0%**
	% within Factor	58.1%	70.0%	**64.1%**	61.3%	66.9%	**64.1%**	41.9%	86.3%	**64.1%**	61.9%	66.3%	**64.1%**
	% of Total	29.1%	35.0%	**64.1%**	30.6%	33.4%	**64.1%**	20.9%	43.1%	**64.1%**	30.9%	33.1%	**64.1%**
Stress	Count	23	15	**38**	26	12	**38**	16	22	**38**	24	14	**38**
	% within Disturbance factors	60.5%	39.5%	**100.0%**	68.4%	31.6%	**100.0%**	42.1%	57.9%	**100.0%**	63.2%	36.8%	**100.0%**
	% within Factor	14.4%	9.4%	**11.9%**	16.3%	7.5%	**11.9%**	10.0%	13.8%	**11.9%**	15.0%	8.8%	**11.9%**
	% of Total	7.2%	4.7%	**11.9%**	8.1%	3.8%	**11.9%**	5.0%	6.9%	**11.9%**	7.5%	4.4%	**11.9%**
Insect bite	Count	12	7	**19**	11	8	**19**	19	0	**19**	9	10	**19**
	% within Disturbance factors	63.2%	36.8%	**100.0%**	57.9%	42.1%	**100.0%**	100.0%	.0%	**100.0%**	47.4%	52.6%	**100.0%**
	% within Factor	7.5%	4.4%	**5.9%**	6.9%	5.0%	**5.9%**	11.9%	.0%	**5.9%**	5.6%	6.3%	**5.9%**
	% of Total	3.8%	2.2%	**5.9%**	3.4%	2.5%	**5.9%**	5.9%	.0%	**5.9%**	2.8%	3.1%	**5.9%**
Rodent attack	Count	32	26	**58**	25	33	**58**	58	0	**58**	28	30	**58**
	% within Disturbance factors	55.2%	44.8%	**100.0%**	43.1%	56.9%	**100.0%**	100.0%	.0%	**100.0%**	48.3%	51.7%	**100.0%**
	% within Factor	20.0%	16.3%	**18.1%**	15.6%	20.6%	**18.1%**	36.3%	.0%	**18.1%**	17.5%	18.8%	**18.1%**
	% of Total	10.0%	8.1%	**18.1%**	7.8%	10.3%	**18.1%**	18.1%	.0%	**18.1%**	8.8%	9.4%	**18.1%**
Total	**Count**	**160**	**160**	**320**	**160**	**160**	**320**	**160**	**160**	**320**	**160**	**160**	**320**
	% within Disturbance factors	**50.0%**	**50.0%**	**100.0%**	**50.0%**	**50.0%**	**100.0%**	**50.0%**	**50.0%**	**100.0%**	**50.0%**	**50.0%**	**100.0%**
	% within Factor	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**	**100.0%**
	% of Total	**50.0%**	**50.0%**	**100.0%**	**50.0%**	**50.0%**	**100.0%**	**50.0%**	**50.0%**	**100.0%**	**50.0%**	**50.0%**	**100.0%**

Appendix 12: Pearson's chi-square statistics shows the significant association between factors (shade, water, protection and age at transplant) and factors causing deaths.

Factors	**Chi-Square Tests**	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Shade	Pearson Chi-Square	5.382	3	0.146	0.143		
Water	Pearson Chi-Square	7.130	3	0.068	0.068		
Protection	Pearson Chi-Square	102.538	3	0.000	0.000		
Age	Pearson Chi-Square	2.992	3	0.393	0.411		
Shade	Likelihood Ratio	5.414	3	0.144	0.149		
Water	Likelihood Ratio	7.259	3	0.064	0.069		
Protection	Likelihood Ratio	132.803	3	0.000	0.000		
Age	Likelihood Ratio	3.024	3	0.388	0.413		
Shade	Fisher's Exact Test	5.330			0.146		
Water	Fisher's Exact Test	7.120			0.067		
Protection	Fisher's Exact Test	124.757			0.000		
Age	Fisher's Exact Test	2.997			0.406		
Shade	Linear-by-Linear Association	2.932	1	0.087	0.096	0.048	0.009
Water	Linear-by-Linear Association	0.036	1	0.849	0.887	0.443	0.037
Protection	Linear-by-Linear Association	95.994	1	0.000	0.000	0.000	0.000
Age	Linear-by-Linear Association	0.009	1	0.924	0.962	0.481	0.038
	N of Valid Cases	320					

Appendix 13: The Odds ratio comparing the effect size of stress as a result influence by factors.

Odds ratio for factor	**Shade**	**Water**	**protection**	**Age**
Stress				
factor absent				
Odds factor absent = number alive in the absence of factor	93	98	67	99
number dead from stress in the absence of factor	23	26	16	24
Odds factor absent =		62 / 98	93 / 67	
Odds factor absent = alive	4.04	3.77	4.19	4.13
factor present				
Odds factor present = number alive in the presence of factor	112	107	138	106
number dead from stress in the presence of factor	15	12	22	14
Odds present =	48 / 112	53 / 107	22 / 138	
Odds factor present =	7.47	8.92	6.27	7.57
Odds ratio factor				
Odds ratio = Odds factor absent	4.04	3.77	4.19	4.13
Odds factor present	7.47	8.92	6.27	7.57
Odds ratio =	0.72 / 0.43	0.63 / 0.50	1.39 / 0.16	
Odds ratio =	0.54	0.42	0.67	0.54

Appendix 14: The Odds ratio shows calculation and the effect size of stress resulting from the influence factors.

Odds ratio for factor	**Shade**	**Water**	**protection**	**Age**
Insects				
factor absent				
Odds factor absent = number alive in the absence of factor	93	98	67	99
number dead from insects in the absence of factor	12	11	19	9
Odds factor absent =		62 / 98	93 / 67	
Odds factor absent =	7.75	8.91	3.53	11.00
factor present				
Odds factor present = number alive in the presence of factor	112	107	138	106
number dead from insects in the presence of factor	7	8	0.1	10
Odds present =	48 / 112	53 / 107	22 / 138	
Odds factor present =	16.00	13.38	1380.00	10.60
Odds ratio factor				
Odds ratio = Odds factor absent	7.75	8.91	3.53	11.00
Odds factor present	16.00	13.38	1380.00	10.60
Odds ratio =	0.72 / 0.43	0.63 / 0.50	1.39 / 0.16	
Odds ratio =	0.48	0.67	0.00	1.04

Appendix 15: The Odds ratio shows calculation and the effect size of rodents resulting from the influence factors.

Odds ratio for factor	**Shade**	**Water**	**protection**	**Age**
Rodents				
factor absent				
Odds factor absent = number alive in the absence of factor	93	98	67	99
number dead from rodents in the absence of factor	32	25	58	28
Odds factor absent =		62 / 98	93 / 67	
Odds factor absent =	2.91	3.92	1.16	3.54
factor present				
Odds factor present = number alive in the presence of factor	112	107	138	106
number dead from rodents in the presence of factor	26	33	0.1	30
Odds present =	48 / 112	53 / 107	22 / 138	
Odds factor present =	4.31	3.24	1380.00	3.53
Odds ratio factor				
Odds ratio = Odds factor absent	2.91	3.92	1.16	3.54
Odds factor present	4.31	3.24	1380.00	3.53
Odds ratio =	0.72 / 0.43	0.63 / 0.50	1.39 / 0.16	
Odds ratio =	0.67	1.21	0.00	1.00

Appendix 16: Loglinear analyses show the observed and expected dead and vitality of plants as a result of the interactions between factors.

				Observed		Expected			
Shade	Water	Protection	Vitality	Count(a)	%	Count	%	Residuals	Std. Residuals
Absent	Absent	Absent	dead	29.500	9.2%	29.500	9.2%	.000	.000
			Little chance of recovery	4.500	1.4%	4.500	1.4%	.000	.000
			Good chance of recovery	6.500	2.0%	6.500	2.0%	.000	.000
			Good vigor and no growth	1.500	.5%	1.500	.5%	.000	.000
			Actively growing	.500	.2%	.500	.2%	.000	.000
		Present	dead	8.500	2.7%	8.500	2.7%	.000	.000
			Little chance of recovery	2.500	.8%	2.500	.8%	.000	.000
			Good chance of recovery	15.500	4.8%	15.500	4.8%	.000	.000
			Good vigor and no growth	15.500	4.8%	15.500	4.8%	.000	.000
			Actively growing	.500	.2%	.500	.2%	.000	.000
	Present	Absent	dead	24.500	7.7%	24.500	7.7%	.000	.000
			Little chance of recovery	1.500	.5%	1.500	.5%	.000	.000
			Good chance of recovery	5.500	1.7%	5.500	1.7%	.000	.000
			Good vigor and no growth	9.500	3.0%	9.500	3.0%	.000	.000
			Actively growing	1.500	.5%	1.500	.5%	.000	.000
		Present	dead	6.500	2.0%	6.500	2.0%	.000	.000
			Little chance of recovery	3.500	1.1%	3.500	1.1%	.000	.000
			Good chance of recovery	8.500	2.7%	8.500	2.7%	.000	.000
			Good vigor and no growth	19.500	6.1%	19.500	6.1%	.000	.000
			Actively growing	4.500	1.4%	4.500	1.4%	.000	.000
Present	Absent	Absent	dead	21.500	6.7%	21.500	6.7%	.000	.000
			Little chance of recovery	2.500	.8%	2.500	.8%	.000	.000
			Good chance of recovery	3.500	1.1%	3.500	1.1%	.000	.000

		Good vigor and no growth	13.500	4.2%	13.500	4.2%	.000	.000
		Actively growing	1.500	.5%	1.500	.5%	.000	.000
	Present	dead	4.500	1.4%	4.500	1.4%	.000	.000
		Little chance of recovery	3.500	1.1%	3.500	1.1%	.000	.000
		Good chance of recovery	7.500	2.3%	7.500	2.3%	.000	.000
		Good vigor and no growth	10.500	3.3%	10.500	3.3%	.000	.000
		Actively growing	16.500	5.2%	16.500	5.2%	.000	.000
Present	Absent	dead	19.500	6.1%	19.500	6.1%	.000	.000
		Little chance of recovery	1.500	.5%	1.500	.5%	.000	.000
		Good chance of recovery	4.500	1.4%	4.500	1.4%	.000	.000
		Good vigor and no growth	8.500	2.7%	8.500	2.7%	.000	.000
		Actively growing	8.500	2.7%	8.500	2.7%	.000	.000
	Present	dead	4.500	1.4%	4.500	1.4%	.000	.000
		Little chance of recovery	.500	.2%	.500	.2%	.000	.000
		Good chance of recovery	2.500	.8%	2.500	.8%	.000	.000
		Good vigor and no growth	2.500	.8%	2.500	.8%	.000	.000
		Actively growing	32.500	10.2%	32.500	10.2%	.000	.000

Appendix 17: The likelihood ratio shows the Goodness-of-Fit model for vitality data.

	Chi-Square	df	Sig.
Likelihood Ratio	.000	0	INF
Pearson	.000	0	INF

Appendix 18: The loglinear analysis shows the K-Way and Higher-Order effects for interaction of factors.

			Likelihood Ratio		Pearson		
	K	df	Chi-Square	Sig.	Chi-Square	Sig.	Number of Iterations
K-way and Higher Order Effects(a)	1	79	365.654	.000	393.500	.000	0
	2	71	275.369	.000	281.411	.000	2
	3	49	59.048	.154	52.826	.329	8
	4	21	25.897	.210	23.224	.332	6
	5	4	.644	.958	.349	.986	13
K-way Effects(b)	1	8	90.285	.000	112.089	.000	0
	2	22	216.321	.000	228.585	.000	0
	3	28	33.151	.230	29.602	.382	0
	4	17	25.253	.089	22.875	.153	0
	5	4	.644	.958	.349	.986	0

Appendix 19: The cross tabulation shows the presence and absence of factors (shade, water, protection and age at transplant) and their effect on vitality of seedlings

		Shade		Total	Water		Total	Protection		Total	Age at transplant		Total
VITALITY		**Absent**	**Present**		**Absent**	**Present**		**Absent**	**Present**		**Three months at transplant**	**One year at transplant**	
Dead	Count	67	48	**115**	62	53	**115**	93	22	**115**	61	54	**115**
	% within Vitality	58.3	41.7	**100.0**	53.9	46.1	**100.0**	80.9	19.1	**100.0**	53.0	47.0	**100.0**
	% within Factor	41.9	30.0	**35.9**	38.8	33.1	**35.9**	58.1	13.8	**35.9**	38.1	33.8	**35.9**
	% of Total	20.9	15.0	**35.9**	19.4	16.6	**35.9**	29.1	6.9	**35.9**	19.1	16.9	**35.9**
Little chance of recovery	Count	10	6	**16**	11	5	**16**	8	8	**16**	10	6	**16**
	% within Vitality	62.5	37.5	**100.0**	68.8	31.3	**100.0**	50.0	50.0	**100.0**	62.5	37.5	**100.0**
	% within Factor	6.3	3.8	**5.0**	6.9	3.1	**5.0**	5.0	5.0	**5.0**	6.3	3.8	**5.0**
	% of Total	3.1	1.9	**5.0**	3.4	1.6	**5.0**	2.5	2.5	**5.0**	3.1	1.9	**5.0**
Good chance of recovery	Count	34	16	**50**	31	19	**50**	18	32	**50**	39	11	**50**
	% within Vitality	68.0	32.0	**100.0**	62.0	38.0	**100.0**	36.0	64.0	**100.0**	78.0	22.0	**100.0**
	% within Factor	21.3	10.0	**15.6**	19.4	11.9	**15.6**	11.3	20.0	**15.6**	24.4	6.9	**15.6**
	% of Total	10.6	5.0	**15.6**	9.7	5.9	**15.6**	5.6	10.0	**15.6**	12.2	3.4	**15.6**
Good vigour and no growth	Count	44	33	**77**	39	38	**77**	31	46	**77**	27	50	**77**
	% within Vitality	57.1	42.9	**100.0**	50.6	49.4	**100.0**	40.3	59.7	**100.0**	35.1	64.9	**100.0**
	% within Factor	27.5	20.6	**24.1**	24.4	23.8	**24.1**	19.4	28.8	**24.1**	16.9	31.3	**24.1**
	% of Total	13.8	10.3	**24.1**	12.2	11.9	**24.1**	9.7	14.4	**24.1**	8.4	15.6	**24.1**
Actively growing	Count	5	57	**62**	17	45	**62**	10	52	**62**	23	39	**62**
	% within Vitality	8.1	91.9	**100.0**	27.4	72.6	**100.0**	16.1	83.9	**100.0**	37.1	62.9	**100.0**
	% within Factor	3.1	35.6	**19.4**	10.6	28.1	**19.4**	6.3	32.5	**19.4**	14.4	24.4	**19.4**
	% of Total	1.6	17.8	**19.4**	5.3	14.1	**19.4**	3.1	16.3	**19.4**	7.2	12.2	**19.4**
T0TAL	**Count**	**160**	**160**	**320**	**160**	**160**	**320**	**160**	**160**	**320**	**160**	**160**	**320**
	% within Vitality	**50.0**	**50.0**	**100.0**	**50.0**	**50.0**	**100.0**	**50.0**	**50.0**	**100.0**	**50.0**	**50.0**	**100.0**
	% within Factor	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
	% of Total	**50.0**	**50.0**	**100.0**	**50.0**	**50.0**	**100.0**	**50.0**	**50.0**	**100.0**	**50.0**	**50.0**	**100.0**

Appendix 20: Pearson chi-square statistics shows the significance of associations between the presence and absence factors (shade, water, protection and age at transplant) on plant vitality.

Factors		Chi-Square Tests vitality					
		Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Shade	Pearson Chi-Square	55.803	4	0.000	0.000		
Water	Pearson Chi-Square	18.492	4	0.001	0.001		
Protection	Pearson Chi-Square	79.128	4	0.000	0.000		
Age	Pearson Chi-Square	28.105	4	0.000	0.000		
Shade	Likelihood Ratio	63.557	4	0.000	0.000		
Water	Likelihood Ratio	19.047	4	0.001	0.001		
Protection	Likelihood Ratio	85.239	4	0.000	0.000		
Age	Likelihood Ratio	29.213	4	0.000	0.000		
Shade	Fisher's Exact Test	62.179			0.000		
Water	Fisher's Exact Test	18.670			0.001		
Protection	Fisher's Exact Test	84.098			0.000		
Age	Fisher's Exact Test	28.675			0.000		
Shade	Linear-by-Linear Association	22.862	1	0.000	0.000	0.000	0.000
Water	Linear-by-Linear Association	7.829	1	0.005	0.006	0.003	0.001
Protection	Linear-by-Linear Association	72.860	1	0.000	0.000	0.000	0.000
Age	Linear-by-Linear Association	6.685	1	0.010	0.011	0.005	0.001
	N of Valid Cases	320					

Appendix 21: The Odds ratio shows calculation and the effect size of plant vitality (little chance of survival) resulting from the influence of factors.

Odds ratio for factor	Shade	Water	protection	Age
Little chance of recovery				
factor absent				
Odds factor absent = number dead in the absence of factor	67	62	93	61
number little chance of recovery in the absence of factor	10	11	8	10
Odds factor absent =		62 / 98	93 / 67	
Odds factor absent =	6.70	5.64	11.63	6.10
factor present				
Odds factor present = number died in the presence of factor	48	53	22	54
number little chance of recovery in the presence of factor	6	5	8	6
Odds present =	48 / 112	53 / 107	22 / 138	
Odds factor present =	8.00	10.60	2.75	9.00
Odds ratio factor				
Odds ratio = Odds factor absent	6.70	5.64	11.63	6.10
Odds factor present	8.00	10.60	2.75	9.00
Odds ratio =	0.72 / 0.43	0.63 / 0.50	1.39 / 0.16	
Odds ratio =	0.84	0.53	4.23	0.68

Appendix 22: The Odds ratio shows calculation and the effect size of plant vitality (good chance of survival) resulting from the of influence factors.

Odds ratio for factor	**Shade**	**Water**	**protection**	**Age**
good chance of recovery				
factor absent				
Odds factor absent = number dead in the absence of factor	67	62	93	61
number good chance of recovery in the absence of factor	34	31	18	19
Odds factor absent =		62 / 98	93 / 67	
Odds factor absent =	1.97	2.00	5.17	3.21
factor present				
Odds factor present = number undisturbed in the presence of factor	48	53	22	54
number good chance of recovery in the presence of factor	16	19	32	11
Odds present =	48 / 112	53 / 107	22 / 138	
Odds factor present =	3.00	2.79	0.69	4.91
Odds ratio factor				
Odds ratio = Odds factor absent	1.97	2.00	5.17	3.21
Odds factor present	3.00	2.79	0.69	4.91
Odds ratio =	0.72 / 0.43	0.63 / 0.50	1.39 / 0.16	
Odds ratio =	0.66	0.72	7.52	0.65

Appendix 23: The Odds ratio shows calculation and the effect size of plant vitality (good vigour) resulting from the influence of factors.

Odds ratio for factor	**Shade**	**Water**	**protection**	**Age**
Good vigour				
factor absent				
Odds factor absent = number dead in the absence of factor	67	62	93	61
number Good vigour in the absence of factor	44	39	31	27
Odds factor absent =		62 / 98	93 / 67	
Odds factor absent =	1.52	1.59	3.00	2.26
factor present				
Odds factor present = number undisturbed in the presence of factor	48	53	22	54
number Good vigour in the presence of factor	33	38	46	50
Odds present =	48 / 112	53 / 107	22 / 138	
Odds factor present =	1.45	1.39	0.48	1.08
Odds ratio factor				
Odds ratio = Odds factor absent	1.52	1.59	3.00	2.26
Odds factor present	1.45	1.39	0.48	1.08
Odds ratio =	0.72 / 0.43	0.63 / 0.50	1.39 / 0.16	
Odds ratio =	1.05	1.14	6.27	2.09

Appendix 24: The Odds ratio shows calculation and the effect size of plant vitality (actively growing) resulting from the influence of factors.

Odds ratio for factor actively growing	**Shade**	**Water**	**protection**	**Age**
factor absent				
Odds factor absent = number dead in the absence of factor	67	62	93	61
number actively growing in the absence of factor	5	17	10	23
Odds factor absent =		62 / 98	93 / 67	
Odds factor absent =	13.40	3.65	9.30	2.65
factor present				
Odds factor present = number dead in the presence of factor	48	53	22	54
number actively growing in the presence of factor	57	45	52	39
Odds present =	48 / 112	53 / 107	22 / 138	
Odds factor present =	0.84	1.18	0.42	1.38
Odds ratio factor				
Odds ratio = Odds factor absent	13.40	3.65	9.30	2.65
Odds factor present	0.84	1.18	0.42	1.38
Odds ratio =	0.72 / 0.43	0.63 / 0.50	1.39 / 0.16	
Odds ratio =	15.91	3.10	21.98	1.92

Appendix 25: The K-S test and Shapiro-Wilk test of Normality for growth in height and diameter over factors (shade, water, protection and age at transplant).

Tests of Normality

	Shade	*Kolmogorov-Smirnov*			*Shapiro-Wilk*			***Age***	*Kolmogorov-Smirnov*			*Shapiro-Wilk*		
		Statistic	df	Sig.	Statistic	Df	Sig.		Statistic	df	Sig.	Statistic	df	Sig.
Height	Absent	0.233	93	0.000	0.865	93	0.000	Three months	0.221	100	0.000	0.914	100	0.000
	Present	0.165	113	0.000	0.937	113	0.000	One year	0.214	106	0.000	0.908	106	0.000
Diameter	Absent	0.179	93	0.000	0.927	93	0.000	Three months	0.179	100	0.000	0.936	100	0.000
	Present	0.229	113	0.000	0.904	113	0.000	One year	0.190	106	0.000	0.908	106	0.000
	Protection	*Kolmogorov-Smirnov*			*Shapiro-Wilk*			***Water***	*Kolmogorov-Smirnov*			*Shapiro-Wilk*		
		Statistic	df	Sig.	Statistic	Df	Sig.		Statistic	Df	Sig.	Statistic	Df	Sig.
Height	Absent	0.216	68	0.000	0.911	68	0.000	Absent	0.226	98	0.000	0.870	98	0.000
	Present	0.213	138	0.000	0.915	138	0.000	Present	0.163	108	0.000	0.947	108	0.000
Diameter	Absent	0.183	68	0.000	0.929	68	0.001	Absent	0.175	98	0.000	0.919	98	0.000
	Present	0.192	138	0.000	0.930	138	0.000	Present	0.214	108	0.000	0.930	108	0.000

Appendix 26: A summary of the Kruskal-Wallis ranked data on factor and their effect on variables, growth in height.

Shade			**Water**			**Protection**			**Age at transplant**		
Height	*N*	*Mean Rank*		*N*	*Mean Rank*		*N*	*Mean Rank*		*N*	*Mean Rank*
Absent	93	79.02	Absent	98	90.08	Absent	68	92.93	Three months	100	90.34
Present	113	123.65	Present	108	115.68	Present	138	108.71	One year	106	115.92
Total	**206**		**Total**	**206**		**Total**	**206**		**Total**	**206**	
Diameter											
Absent	93	74.74	Absent	98	84.28	Absent	68	96.60	Three months	100	89.22
Present	113	127.17	Present	108	120.94	Present	138	106.90	One year	106	116.97
Total	**206**		**Total**	**206**		**Total**	**206**		**Total**	**206**	

Appendix 27: The Kruskal-Wallis test *H*, show the significance of the relationships between the presence and absence of factors and plant growth in height.

			Shade	Water	Protection	Age at transplant
			Height	Height	Height	Height
Chi-Square			30.923	10.248	3.448	10.240
Df			1	1	1	1
Asymp. Sig.			0.000	0.001	0.063	0.001
Monte Carlo Sig.	Sig.		0.000	0.001	0.064	0.001
	99% Confidence Interval	Lower Bound	0.000	0.000	0.058	0.000
		Upper Bound	0.000	0.002	0.070	0.002

Appendix 28: The Kruskal-Wallis test *H*, show the significance of the relationships between the presence and absence of factors and plant growth in diameter.

			Shade	Water	Protection	Age at transplant
			Diameter	Diameter	Diameter	Diameter
Chi-Square			42.101	20.736	1.452	11.895
Df			1	1	1	1
Asymp. Sig.			0.000	0.000	0.228	0.001
Monte Carlo Sig.	Sig.		0.000	0.000	0.232	0.000
	99% Confidence Interval	Lower Bound	0.000	0.000	0.221	0.000
		Upper Bound	0.000	0.001	0.243	0.001