ASSESSMENT OF MISTLETOE - HOST INTERACTIONS IN A HIGHLAND

SAVANNA IN WINDHOEK, NAMIBIA

A THESIS SUBMITTED IN FULFILMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE (BY THESIS)

OF

THE UNIVERSITY OF NAMIBIA

BY

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April 2017

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ABSTRACT

Mistletoes have detrimental effects in ecosystems where they occur. Mistletoes negatively reduce the growth of the host plants and increase the chance of tree mortality. Apart from tree mortality, infected trees suffer from reduced growth and loss of vigor hence contributing to overall low productivity of hosts and resulting in changes in the structure and function of savanna communities. This study's aim was to determine and to compare mistletoe - host interactions between the Botanic Garden and the Aloe Trail in Windhoek. For the assessment of prevalence and infectivity of mistletoes on woody trees, plot based sampling techniques were used to select and demarcate fifty 20mx20m plots from both the Botanic Garden and the Aloe Trail. All woody plants in the plots were measured for height and stem diameter. Each woody plant was then examined for presence of mistletoes and the total number of mistletoes on each individual plant was determined. The host species sampled were *Senegalia mellifera* (Vahl) Seigler & Ebinger and *Boscia albitrunca* (Burch.) Gilg & Gilg-Ben. The mistletoes species studied were *Tapinanthus oleifolius* (J.C.Wendl.) Danser and *Viscum rotundifolium* L.f. The Aloe Trail had a significantly higher prevalence than the Botanic Garden (Mann-Whitney test U, Z = - 0.4562, p < 0.001) because of the better management efforts such as removing the mistletoes. Infectivity of *Senegalia erubescens and Dichrostachys cinerea* was significantly high in the Aloe Trail than the Botanic Garden (Mann Whitney U test, Z = -0.4.568, p = 0.00<0.001 and Z = -2.883, p=0.04<0.05). Mistletoes were mainly associated with *S. mellifera* and *S. erubescens* (χ^2 = 9.084, df = 3, p = 0.028<0.05). This is because, these host species have a high mistletoe-host compatibility. The Spearman's rank correlation showed that the number of mistletoes were poorly

correlated to host - tree height in the Botanic Garden and in the Aloe Trail (r = -0.44, p = 0.732>0.05 and r = 0.67, p = 0.410>0.05) indicating that variation in infestation intensity may be related to other factors such as canopy diameter availability of nutrients and water and not host size. Photosynthetic rates between mistletoe host pairs were measured using a portable LI-6400XT Portable Photosynthesis System. The Wilcoxon signed - rank test (Z = -2.061, p < 0.05) revealed that hosts have higher photosynthetic rates than mistletoes because they have higher electron transport rates. Water potential between mistletoe host pairs was measured using a Scholander pressure chamber. Hosts had a lower water potential than mistletoes (Wilcoxon signed - rank test: Z = -6.313, p<0.001) because hosts make use of water efficiency mechanisms to conserve water. Management focus should be concentrated on species such as *S. mellifera* and *S. erubescens* because they were highly associated with mistletoes in both sites.

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LIST OF ABBREVIATIONS

ATP = Adenosine triphosphate

IRGA = Infrared Gas Analyzer

N = Nitrogen

$NADP^+$ = Nicotinamide adenine dinucleotide phosphate

NBRI = National Botanic Research Institute

PAR = Photosynthetically active radiation

RuBP = Ribulose bisphosphate

ACKNOWLEDGEMENTS

DEDICATION

This thesis is dedicated to my mother Aili Amutenya who is also my namesake. Mom you are my inspiration in life, you always encouraged me to never give up; your inspiration, support and motivation kept me going and enabled me to always travel on the right path.

DECLARATION

I, Aili Amutenya, hereby declare that this thesis is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.

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Aili Amutenya

CHAPTER 1. INTRODUCTION

1.1 General introduction

Savannas are the most common vegetation type in the tropics and subtropics and are the most dominant vegetation type in Africa (Sankaran *et al*. 2005; Scholes & Walker 1993). The savannas are a tropical woodland or grassland biome characterised by widely spaced trees, large herbivores and alternate wet and dry seasons which are primarily based on rainfall and maintained by occasional fires and droughts (Crowling, Richardson & Pierce 1997; Scholes & Walker 1993). One of the many categorizations of tropical savannas is nutrient-rich, fine-leafed savannas and the nutrient-poor, broad-leafed savannas (Frost *et al*. 1986; Mistry 2000). Scholes and Archer (1997) pointed out that savannas can also be subdivided based on height, canopy cover and spatial arrangement of woody plants. Savannas consist of complex dynamics that include plant-animal and plant-plant interactions (Mistry 2000).

Mistry (2000) pointed out that rainfall is seasonal; not only does this affect plants and animals, but it is also a major limitation to the human population that lives in or around savanna areas. Plants, animals and humans are adapted to the savanna environment and today savannas support most livestock and wild herbivore biomass (Sankaran *et al*. 2005).

Plants have evolved nutritional adaptations that involve using other organisms to obtain food; some of these adaptations are one sided such as that of parasitic plants. Starr *et al*. (2013) defined parasites as organisms that benefit by living in or on other organisms at the expense of the host.

Parasitic plants feed off their hosts by absorbing water, sugars and minerals (Malcom & Jonathan 1995; Starr *et al*. 2013; Visser 1981). Fadini (2011); Martínez del Rio *et al*. (1996); Roxburgh & Nicolson (2008) defined prevalence is the number of individuals that are infected by mistletoes as a proportion of all the individual trees in an area. Infectivity is the number of individuals of a particular species that are infected by mistletoes as a proportion of all the individuals of that species (Fadini 2011; Martínez del Rio *et al*. 1996; Roxburgh & Nicolson 2008). According to Veste (2007), parasitic plants can be facultative parasites that can survive for long periods and even produce seeds without a host, while obligate parasites cannot survive without a host. Hemiparasites are parasitic plants that can produce their own sugars through photosynthesis; hemiparasites that are attached to roots of host plants are called root parasites. These types of parasites obtain water and other nutrients from the soil through their roots and some from the host plants through the haustoria (Veste 2007). Haustoria is a specialized structure that enable parasitic plants to attach and penetrate the tissues of host plant's stem or root and this creates a direct connection between the vascular systems of the two plants (Malcom & Jonathan 1995).

Hemiparasites attached to stems are variously called stem or aerial parasites and hence obtain all water and inorganic nutrients from the host (Johnson & Chionski 1993; Okubamichael, Griffiths & Ward 2011). Non - photosynthetic holoparasites are unable to produce their own sugars and hence depend heavily on the host plant for survival, e.g. dodder (Johnson & Chionski 1993; Veste 2007).

Holoparasites obtain carbon, water and other nutrients from the host and may either be root parasites or stem parasites. Veste (2007) further added that only hemiparasites can be facultative parasites.

Mistletoes are referred to as evergreen, perennial, flowering and parasitic plants that are adapted to live on aerial parts of their hosts (Glatzel & Geils 2008; Okubamichael *et al.* 2011). Mistletoes are widely distributed in all major biomes and climate types and are only absent from extremely cold regions (Veste 2007). Mistletoes belong to the order Santalales and are a taxonomically diverse group of plant parasites found in five families: Loranthaceae, Viscaceae, Misodendraceae, Eremolepidaceae and Santalaceae (Visser 1981).

Mistletoes are dioecious, with females producing berries and males that produce pollen (Aukema 2003). Mistletoes can either be dwarf- or broad-leafed (Maloney & Rizzo 2002). Dwarf mistletoes are leafless and much smaller in size with non-woody shoots. The leaves are segmented and scale-like. Adams, Frankel and Lichter (1993) pointed out that they are mostly obligate as they are specific to their hosts and infect only conifers. The seeds are mostly dispersed by the forcible discharge of seeds from the fruit and the successful seeds establish on the host trees (Adams *et al.* 1993; Maloney & Rizzo 2002; Perry 1995).

Broad-leafed mistletoes are green, woody and larger in size, often with shrubby brittle stems and thick leaves (Aukema 2003). They vary in their host specificity, some infecting only one or a few host species and others infecting a wide range of host plants (Adams *et al.* 1993). The seeds are dispersed by birds (Adams *et al.* 1993; Maloney & Rizzo 2002; Perry 1995).

Healthy trees can tolerate a few mistletoe branch infections, however, if the infection is severe, trees can be weakened, have stunted growth or dead branches, or die completely (Aukema & del Rio 2002; Glatzel & Geils 2008). Mistletoe leaves contain chlorophyll that enable the production of sugar (Aukema & del Rio 2002;

Aukema 2003). Mistletoe root system invades the internal tissues of the host tree to extract water and minerals, and to anchor it to the host.

Mistletoes develop special morphological features to survive and live in the tissues of other plants (Aukema 2003). After the mistletoe seed germinates, it grows through the bark and into the tree's water conducting tissues, where root-like structures called haustoria develop. The haustoria spread up and down the tree branch as the mistletoe grows (Okubamichael *et al*. 2011). The haustorium connects the parasite with its host and allows the transportation of water, inorganic and organic compounds into the parasite (Veste 2007; Aukema & del Rio 2002; Okubamichael *et al*. 2011). If the visible portion of the mistletoe is removed new plants may re-sprout from the haustoria (Glatzel & Geils 2008). As mistletoes obtain resources such as water and nutrients from the host plant, this negatively affects the growth of the host plant in such a way that drawing water by the mistletoes causes a more negative water potential than the vessels of their hosts.

1.2 Statement of the problem

Savannas are of great socioeconomic and biological importance. There have been extensive studies on root parasites than stem parasites because of their effect on agriculturally important plants. Mistletoes have been reported to contribute to the mortality of host trees but little is known about mistletoes ecology in the Namibian ecosystems. In addition to direct tree mortality, infected trees may have reduced growth rates and loss of vigor, reduced seed production and are susceptible to attack by pathogens and insects (Mathiasen *et al*. 2008).

Even though parasitic plants are an ecologically and economically important group, the direct use of host resources by mistletoes make them potentially damaging

to the host plants (Fadini & Lima 2012). Mortality of dominant trees associated with mistletoe infectivity may negatively impact plant community dynamics and functioning leading to a disruption of biotic interactions and modification of the community structure (Coleman, Gillman & Green 1980). As a result of the parasitism stress that the host suffers, the host might suffer severe development instability of organs that will result in reduced tree quality in savanna ecosystems and ultimately loss of biodiversity (Press & Phoenix 2005). Losses of biodiversity results in changes in ecosystem functioning which may affect nutrient cycles, soil contents, and influence environmental conditions such as water cycles, weather patterns, climate .

Future ecologists have the responsibility to provide precise and concrete scientific information on the structure and dynamics of areas which form part of savanna ecosystems. Incorporating these concepts in the design, construction and management of savannas will enable the essential conservation, management and development efforts to improve biodiversity of savannas.

von Willert (1995) investigated gas exchange and water relations of two mistletoes, *Tapinanthus oleifolius* (J.C.Wendl.) Danser and *Viscum rotundifolium* L.f. on the host, *Vachellia nebrownii* (Burtt Davy) Seigler & Ebinger, in south-eastern Namibia. Hence, this study seeks to contribute to the knowledge of savanna dynamics by investigating mistletoe - host interactions in savanna highlands in Windhoek and also help in identifying the species that are at a higher risk of excessive mistletoe infections.

1.3 Aim of the study

The aim of the study was to determine and to compare mistletoe - host

interactions between the Botanic Garden and the nearby Aloe Trail in Windhoek and hence contribute to the understanding of photosynthetic and water relations among host and parasitic plants.

1.4 Specific objectives

1. To measure and compare the prevalence and infectivity of mistletoes on woody plants in the Botanic Garden and on the nearby Aloe Trail.

2. To determine the relationship between host - tree height and the number of mistletoes on the tree.

3. To compare photosynthetic and chlorophyll traits of mistletoe - host tree pairs.

4. To measure and compare water potential between mistletoes and their host plants.

1.5 Research Hypotheses

1. The prevalence and infectivity of mistletoes on host trees in the Aloe Trail is significantly higher than that of the Botanic Garden because of better management practices.

2. There is a strong relationship between tree height and number of mistletoes because taller trees have had more time to accumulate mistletoes (Okubamichael *et al*. 2011).

3. (a). Photosynthetic rate of mistletoes is significantly lower than that of the host plant due to the slow electron transport rates contributing to a lower capacity for photosynthesis.

3. (b). The chlorophyll content of mistletoes is significantly higher than that of the host plant. Mistletoes appear to have more chloroplasts, maximizing light -

capture and making up for their slower electron transport rates (Johnson & Chionski 1993).

4. The mistletoes water potential is significantly higher than that of the host plant, and mistletoes achieve this by keeping their stomata open during the day which leads to high losses of water via transpiration allowing water and nutrients to be actively absorbed from the vessels of the host plant.

1.6 Significance of the study

The information obtained from this study will contribute to the understanding of photosynthetic and water relations among host and parasitic plants as well as savanna ecosystems dynamics. Furthermore, the results obtained will also be useful for effective planning by farmers, protected areas and forest managers. A better understanding of mistletoes interactions with other organisms would help develop better management and conservation strategies in cases were the need for controlling infestation might arise. Therefore this study will significantly contribute to the understanding of mistletoe ecology in Namibia. It can also serve as a guiding tool to future researchers, who would wish to conduct further research on mistletoes ecology elsewhere in the country.

1.6 Limitation of the study

Some areas of the Aloe Trail were inaccessible due to the high density of bush - encroaching *Acacia* (now *Senegalia*) and *Vachellia spp.*, *Dichrostachys cinerea* (L.) Wight & Arn. subsp. *africana* Brenan & Brummitt var. *africana* and *Opuntia spp.* Time limitation did not allow for seasonal comparisons of data and was only able to use data from one season which is the dry season. The delayed rainy season delayed the measurement of some components (e.g. water potential (Ψ), photosynthesis, chlorophyll content and stomata quantity) because most host trees

are deciduous, which precluded measurement of these parameters before leaf flush. There was a limitation to which plants to select for the photosynthesis measurements as some leaves were too small to fit in the leaf chamber of the Infra-Red Gas Analyzer (IRGA) and experienced difficulties with getting the leaves in the right position in the light chamber for measurement.

CHAPTER 2. LITERATURE REVIEW

2.1 Effect of mistletoes on savannas

Mistletoes may have major effects on host plant growth and reproduction resulting in changes in the structure and function of savanna communities (Press & Phoenix 2005). These effects may lead to changes in competitive balances between host and non - host species and hence affect community structure, vegetation zonation and population dynamics (Adams *et al*. 1993; Press & Phoenix 2005).

Mistletoe infestations weaken trees by a reduction in vigor and stunted growth which may eventually lead to death of the host plant (Aukema 2003; Press & Phoenix 2005). Aukema and del Rio (2002) indicated that tree injury varies according to the type of mistletoe and tree species involved. Adams *et al*. (1993) reported that tree mortality in areas with extensive infection is often three to four times higher than in uninfected areas and that tree growth usually declines when more than half the crown is parasitized. When infected by mistletoes, smaller trees decline in growth and die more quickly than larger ones because they are not well developed to withstand heavy infestations.

The branches of trees are more likely to break when infested with mistletoes, which becomes problematic when other stresses such as drought or disease are involved (Perry 1995). With the current low rainfall totals received over the past years in Namibia, the possibility of drought on the farms is very high. Calder and Bernhardt (1993) stated that moderately infected trees showed approximately 66% mortality after a severe drought period while trees without any infections showed a 3% mortality rate.

Fire is common in savanna ecosystems (Frost *et al*. 1986). Dead and dying limbs of trees are more vulnerable to fire as they catch fire easily and make it spread quickly to other parts of the plant (Perry 1995).

2.2 Prevalence and infectivity of mistletoes

Infection is the invasion and multiplication of mistletoes into the host and abundance is the quantity or amount of something present in a particular population (Fadini 2011; Roxburgh & Nicolson 2008). The prevalence and infectivity of mistletoes differ among host species (Aukema & del Rio 2002). Mistletoe presence and abundance in a given area can be influenced by the availability of suitable host trees for colonization, the distribution of suitable host *species*, the degree of host specificity, habitat fragmentation, fire, herbivory, canopy cover, previous infection of a tree and parasite-host chemical interactions (Okubamichael *et al.* 2011; Aukema & del Rio 2002).

The differential use of hosts by mistletoes within a site has been explained by two processes. Firstly, mistletoe seedlings may establish more successfully on some host species than on others. Secondly, adult mistletoes may differ in persistence among host species (Aukema 2003; Zuria *et al*. 2014). Mistletoes have been found to infect closely related hosts and to infect the most abundant host species hence tree species that are related to the most abundant host species may be more likely to act as hosts than non - related trees (Roxburgh & Nicolson 2005).

2.3 Effect of tree size on incidence of parasitism of mistletoes

Patterns of infection and prevalence are related to two characteristics of tree species: abundance and height. The size of a tree has a significant effect on the mistletoes that may establish on a particular tree and mistletoes parasitism increases

with the size of the plant. Large and taller trees have a higher incidence of parasitism (Aukema & del Rio 2002; Okubamichael *et al*. 2011). Taller trees are assumed to provide more moisture and mistletoes prefer them because of the extra moisture that is associated with the tall trees (Aukema & del Rio 2002).

A more than proportional increase in mistletoe infestation with size arises due to the fact that older trees have had more time to accumulate mistletoes and that previously infected trees are more likely to receive seeds and become infected (Okubamichael *et al*. 2011). Seeds may fall from the mistletoe in the upper part of the tree creating new infestations on the lower branches leading to more infectivity on that particular host tree (Glatzel & Geils 2008; Perry 1995). In addition, mistletoes survive better at high light intensities and bigger trees provide more moisture than smaller trees (Aukema & del Rio 2002). The frequency of infestation for each host species is the result of differences in the balance between colonization and extinction (Aukema & del Rio 2002; Aukema 2003); infection increases when the rate of colonization exceeds the rate of extinction.

In most host-parasite relationships the parasite might develop mechanisms that help in successfully penetrating the host and the host also develops some sort of resistance to the infection of the parasite (Thompson 1994). In mistletoes, the haustorium encounters different resistance pressures by potential host trees, when some host species are resistant at different phases of haustorium penetration. A tree with a large bark is assumed to have high incidence of parasitism because large barks are mostly (but not always) associated with tall trees. The bark of many non-host plant species is resistant to haustorial penetration by mistletoes (Yan 1993). Hence, mistletoe infection may be blocked before establishment can occur which can explain why some plants are more susceptible to infection than others (Thompson 1994).

This blockage occurs as a result of chemicals that are released by the host that prevents the haustoria from developing and hence penetrating the host's tissues; some haustoria manage to develop but they fail to penetrate the host. In such cases were mistletoe infection is blocked before establishment the concept of bark size being linked to high incidence of parasitism does not hold true.

2.4 Effect of avian dispersers on the distribution of mistletoes

Mistletoes infect trees in patterns related to bird behaviour and territoriality. Infections tend to be concentrated in open, tall, open crowned trees (Calder & Bernhardt 1993). Insects, birds and wind act as pollination agents of mistletoes (Watson 2001); female mistletoe plants produce berries that are attractive to birds. Many mistletoe species depend on birds to disperse their seeds but dwarf mistletoes have seeds that are dispersed by wind, marsupials or explosively (Okubamichael *et al*. 2011). Birds and host trees act at different stages in the mistletoe life cycle. The Loranthaceae are mainly bird pollinated while the Viscaceae are wind and insect pollinated (Aukema 2003). Birds feed on and digest the pulp of the berries, break the physical dormancy of the seed, and initiate germination of mistletoe seeds by removing the fruit cover which would otherwise inhibit germination (Okubamichael *et al*. 2011).

Birds excrete ingested seeds and expose the sticky viscin, enabling seeds to firmly attach to branches of host trees on which they land (Aukema 2003). Birds are the primary dispersers of mistletoe seeds (Okubamichael *et al*. 2011; Roxburgh & Nicolson 2005). Mistletoes are a foraging substrate for insectivorous birds since many insects are associated with mistletoes as both pollinators and herbivores; mistletoes also provide nesting and roosting sites for birds (Zuria, Castellanos & Gates 2014). Mistletoe fruits act as a keystone food source for bird dispersers

because of their availability during the winter season when other sources of food in the ecosystem are scarce (Okubamichael *et al*. 2011). A dense buildup of mistletoes often occurs within an infested tree because birds are attracted to the berries and spend a lot of time feeding on them (Aukema & del Rio 2002; Aukema 2003).

Birds may perch and defecate mistletoe seeds more frequently on some hosts than on others; for example, if dispersers prefer a particular tree species for perching, feeding or nesting, it is likely to receive more mistletoe seeds than other tree species. Seed-dispersing birds are known to have preferences for perching and feeding in taller than shorter trees on the Silverbell Mountains in Arizona, USA (Aukema & Martinez del Rio 2002). Therefore, taller tree species may be more likely than shorter species to act as hosts; however, if mistletoes are incompatible with a potential host tree species on whose branches mistletoe seeds are deposited, the seeds may not be able to establish on that tree. Deposition of mistletoe seeds on an already parasitized tree could lead to more reinfections of the same tree (Aukema & Martinez del Rio 2002; Roxburgh & Nicolson 2005).

2.5 Herbivorous small mammals as dispersers of mistletoes

Herbivorous small mammals such the Bushveld Elephant-shrew (*Elephantulus intufi*), Mutimammate Mouse (*Mastomys coucha*), Natal Mutimammate Mouse (*M. natalensis*) and Namaqua Rock Mouse (*Aethomys namaquensis*) feed on and digest the pulp of mistletoe berries, and excrete the seeds. These small mammals serve as secondary dispersers of mistletoe seeds. Small mammals disperse the seeds by excretion as they move from one area to another. Mistletoes are unique in the sense that they retain their evergreen fleshy leaves and stems and bear fruits even during the dry winter months and this is advantageous for the herbivorous small mammals in such a way that they will always have food during

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the cold winter months (Okubamichael *et al*. 2011; Roxburgh & Nicolson 2005). Mistletoe fruits may act as a keystone food source for small mammals, especially during the winter season when other sources of food in the ecosystem are scarce. Small mammals may prefer to forage under shrubby hosts because they appear to be thick and bushy and provide a safe haven for the small mammals. The habitat under infected hosts is favorable for survival due to the supply of seeds and fruits falling from the upper branches to the ground, which also shields them from predators. Small mammals foraging by burrowing at bases of host tree trunks may end up damaging roots of host trees. This predisposes the host tree to water and nutrient stress that is caused by the mistletoe.

2.6 Effect of mistletoes on host plant growth and survival

2.6.1 Photosynthesis

Photosynthesis sustains the living world. Photosynthesis is the only significant solar energy storage process on earth and is a source of all food and most energy resources (Johnson & Choinski 1993; Blankenship 2010).

The primary difference between plants and animals is that plants are able to manufacture their own food through photosynthesis (Ridge 1999). During photosynthesis, carbon dioxide from the air and water from the soil react with the sun's energy to form photosynthetic products (carbohydrates, and proteins) and release oxygen as a byproduct (Hopkins & Hüner 2008; Lawlor 1987; Long, Forage & Garcia 1996; Starr *et al*. 2013; Taiz & Zeiger 2010). Photosynthesis consists of three separate processes: 1. light reactions, which convert light energy into chemical energy (adenosine triphosphate - ATP) and transfer electrons from water to nicotinamide adenine dinucleotide phosphate ($NADP^+$), forming NADPH; 2. dark

reactions, which use this chemical energy (ATP and NADPH) to reduce CO_2 to carbohydrates; and 3. diffusion, in which stomata open to allow CO_2 to diffuse into leaves from the surrounding air (Lambers *et al*. 2008; Lawlor 1987; Taiz & Zeiger 2010). The photosynthetic process is dependent on the supply of water, light and carbon dioxide: if any one of these factors is lacking then it can limit photosynthesis regardless of the availability of the other factors (Lambers, Chapin & Pons 2008; Ridge 1999).

Mistletoes deprive their hosts by diverting photosynthates or water and nutrients to their tissues at the expense of hosts nutritional and water needs (Adams *et al*. 1993). Most mistletoes have lower rates of photosynthesis, saturate at lower electron transport rates and at lower light levels than their hosts when host and mistletoe photosynthesis is compared at similar light levels (Strong, Bannister & Burritt 2000). However, Johnson and Choinski (1993) found no significant difference in photosynthetic rates between parasitic plants and their hosts in their study on photosynthesis in the mistletoe its host tree in Harare, Zimbabwe. Under the same environmental conditions, slower electron transport rates in mistletoes indicate a lower capacity for photosynthesis and may be the reason why mistletoes may have lower photosynthetic rates than their hosts.

2.6.2 Effect on chlorophyll

The chlorophyll fluorescence of a leaf can be used as a measure of photosynthetic capability (Strong *et al*. 2000). Chloroplasts are photosynthetic structures in leaves and other green tissues that contain chlorophyll which is a green plant pigment that captures the energy in light and transforms the energy into sugars (Blankenship 2010). The relationship between photosynthesis and chlorophyll content has been rarely examined in mistletoe biology (Johnson & Chionski 1993).

For example, Seel, Cooper and Press (1993) found that chlorophyll content limited light saturated rates of photosynthesis in a study that was done on the root hemiparasite *Rhinanthus minor* L.

Pigments such as chlorophyll are good absorbers of light. Organisms have different pigments, but there are only two general types used in green plant photosynthesis: carotenoids and chlorophylls. Hopkins & Hüner (2008) and Starr *et al*. (2013) pointed out that two main kinds of chlorophyll in plants; chlorophylls *a* and *b*, absorb violet-blue and red light. Chlorophyll *a* is the main photosynthetic pigment and is the only pigment that can act directly to convert light energy to chemical energy, chlorophyll *b* on the other hand acts as an accessory light - absorbing pigment, hence it complements and adds to the light absorption of chlorophyll *a*. Chlorophyll *b* can absorb photons while chlorophyll *a* cannot. Chlorophyll *b* therefore greatly increases the proportion of the photons in sunlight that plants can harvest. Carotenoids are an important group of accessory pigments that assist in photosynthesis by capturing energy from light of wavelengths that are not efficiently absorbed by either chlorophylls (Hopkins & Hüner 2008; Starr *et al*. 2013). Johnson and Choinski (1993) concluded that hosts have significantly greater chlorophyll contents than mistletoes on a fresh weight basis. A study done on *Tapinanthus vittatus* (Engl.) Danser parasitizing *Diplorhynchus condylocarpon* (Müll.Arg.) Pichon supported the literature by showing that *T. vittatus* had a lower total chlorophyll content than its host on a fresh weight basis (Strong *et al*. 2000).

2.6.3 Effect on water potential

Water is the most important constituent of most organisms and is required in abundant quantities. Plants need to balance water uptake and water loss in order to survive. Water potential (Ψ) is the physical property predicting the direction to which

water will flow governed by solute concentration and applied pressure (Campbell & Reece 2008; Glatzel & Geils 2008; Ridge 1999).Ψ is the most widely used indicator of plant water status because it is the major determinant for water movement through the plant; the more negative the Ψ value, the more dehydrated the plant is (Pérez-Harguindeguy *et al*. 2013).

Ψ represents all the water pressure in a given system; it is the sum of osmotic potential (Ψ_{Π}), matrix potential (Ψm), hydrostatic pressure ($\Psi \rho$) and gravitational potential (Ψg) (Chavarria & dos Santos 2012; Taiz & Zeiger 2010). Ψ_{Π} is the chemical potential of water in a solution due to the presence of dissolved substances, it is always negative because the water moves from an area of lower concentration of solutes to an area of higher concentration. $\Psi \rho$ is the physical pressure that water exerts on a given system and it can either be positive or negative: it is positive when a root cortex cell or a leaf mesophyll is observed to be turgid and it is negative when in a transpiring plant a xylem vessel is exposed to a stressful condition (Taiz & Zeiger 2010). Although $\Psi \rho$ is often ignored, it is important in water potential studies of tree species due to the fact that plant height exerts a great influence on water flow (Chavarria & dos Santos 2012).

Ψ determines the direction of movement of water. Water moves from an area of its high concentration to an area of low concentration (Ridge 1999; Larcher 2001). The water flows from the roots to the shoot of the plant through the xylem. According to Starr *et al*. (2013) and Taiz & Zeiger (2010) the upward movement of water is explained by the mechanism called the cohesion - tension theory, which states that the water that evaporates from the leaves creates a flexible strength in the xylem. In the xylem the hydrogen bonds provide a continuous intermolecular attraction (cohesion) between the water molecules from the leaf to the root. Hence,

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the water column in the xylem lumen is driven out of a region with a higher Ψ such as the root and the stem to a region with a lower water potential such as the leaves. In a plant, the water moves continuously from the xylem bundles to the intercellular spaces in the leaves, where the Ψ is lower (Figure 2.1) (Ridge 1999; Starr *et al*. 2013; Taiz & Zeiger 2010).

The transpiration pull is the most important cause of xylem sap flow and the loss of water by transpiration increases the pressure of water in the air but decreases water potential on the transpiring surface within the leaf (Liu *et al*. 2012), (Figure 2.1). The more negative water potential on the transpiring surface pulls the water to the transpiring surface from the xylem vessels (Liu *et al*. 2012). Ψ can be studied at many levels but for the purposes of this study, I will focus on stem water potential.
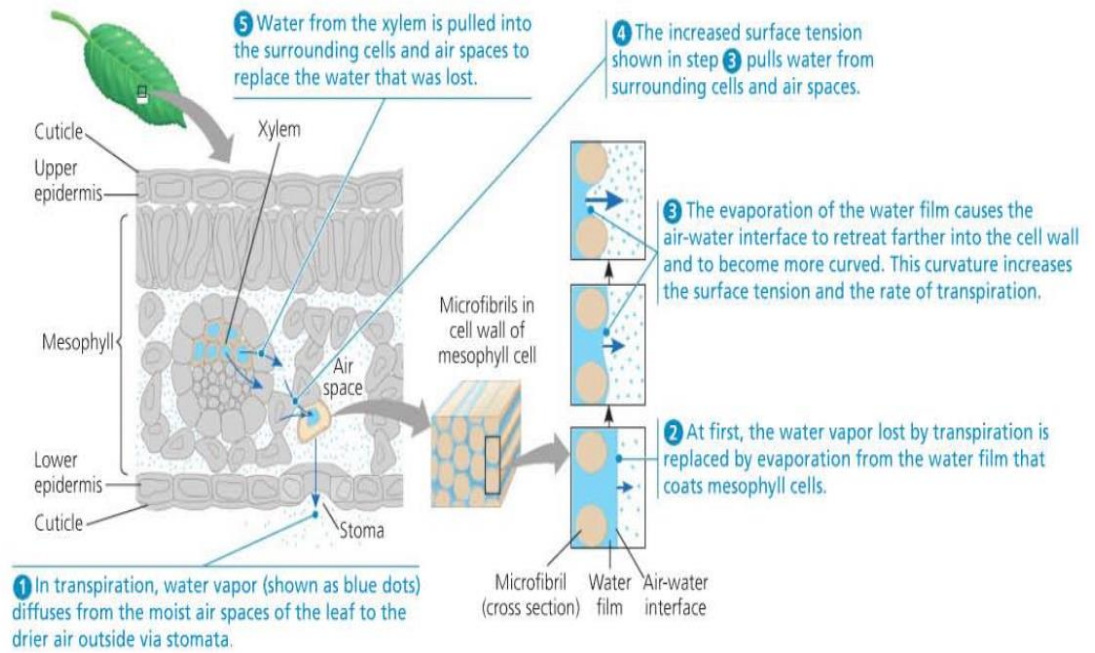
Figure 2.1 Diagram showing the water pathway within the leaf

Source: Campbell & Reece (2008, p. 776)

2. 6. 3. 1 Factors that influence water potential of plants

Although there has been a lot of studies on plant Ψ, a lot of emphasis is on irrigated plants and little attention has been focused on water potential for ecological studies. According to Hopkins and Hüner (2008) and Taiz and Zeiger (2010) Ψ is affected by factors such as soil, root and stem structure, leaf structure and atmospheric conditions.

Poor root health causes stem Ψ to be more negative even though the process of root water uptake is not well understood (Taiz & Zeiger 2010). Any factor that influences root health, such as physical damage, damage by pests, diseases, or poor soil aeration are more likely to reduce the ability of roots to absorb water, which results in the stem Ψ to be more negative (Taiz & Zeiger 2010).

Leaf structure includes leaf resistance and leaf conductance (Taiz & Zeiger 2010). Leaf resistance occurs mostly due to stomates opening and closing. Leaf conductance is the opposite of leaf resistance. Leaf conductance increases with increasing light levels and decreases with higher CO_2 concentrations, and higher vapor pressure deficits (Taiz & Zeiger 2010). In addition, leaf structure affects the rate of water loss from the leaf. During the daytime, fully exposed, outer canopy leaves will lose water at a faster rate than shaded inner canopy leaves. A faster rate of water loss causes a more negative Ψ.

Water-stressed plants tend to have a more negative Ψ than well hydrated plants (Taiz & Zeiger 2010). In addition, plants exposed to high light intensity tend to have more negative Ψ than plants exposed to low light intensities. Taiz and Zeiger (2010) showed that hotter and dryer conditions cause a more negative stem Ψ and that plants exposed to high temperatures tend to have more negative Ψ than plants exposed to low temperatures.

2. 6. 3. 2 Mistletoes and water potential

The growth and survival of xylem - tapping mistletoes depend upon maintaining a negative gradient in leaf Ψ across the haustorial junction between the host and the parasite (Taiz & Zeiger 2010). To do this, mistletoes maintain higher transpiration rates than their hosts by opening their stomata and experience high water loss; in most cases a higher transpiration rate enables the mistletoes to accumulate more minerals especially nitrogen (Okubamichael *et al*. 2011). Calder & Bernhardt (1993) pointed out that as internal water potentials fall and a tree becomes more water stressed, heavy mistletoe infections further stress the host. This feature, combined with the higher osmotic pressure of mistletoes than the host, results in a

mistletoe being able to obtain water even when its host is severely water stressed (Glatzel 1983).

A Ψ gradient is essential for the movement of water and nutrient fluxes from the hosts to the mistletoes; this Ψ gradient is very important for the host as it is the suction force through which they obtain water from the soil (Larcher 2001). von Willert and Popp (1995) pointed out that transpiration is affected by two major factors among others. The first being the driving force for water movement from the soil to the atmosphere, this diving force is the difference in Ψ between the soil and the atmosphere surrounding the plant; this difference creates a gradient which forces water to move toward areas with less water. Hence the drier the air around the plant the larger the driving force is for water to move through the plant and the faster the transpiration rate (Hopkins & Hüner 2008; Larcher 2001; Ridge 1999; Starr *et al.* 2013). The second being the resistances to water movement in the plant. Three major resistances include: cuticle resistance, stomata resistance and boundary layer resistance; these resistance slow water movement. The greater any individual resistance is to water movement, the slower the transpiration rate (Hopkins & Hüner 2008; Larcher 2001; Ridge 1999).

2.6.4 Effect of mistletoe on host stomata density

Stomata are natural openings in leaves and herbaceous stems. Herbaceous stems are stems that have little or no woody tissue (Campbell & Reece 2008); they allow for the exchange of gases such as water vapor, carbon dioxide, and oxygen (Figure. 2.2). They control water loss and CO_2 uptake during photosynthesis.
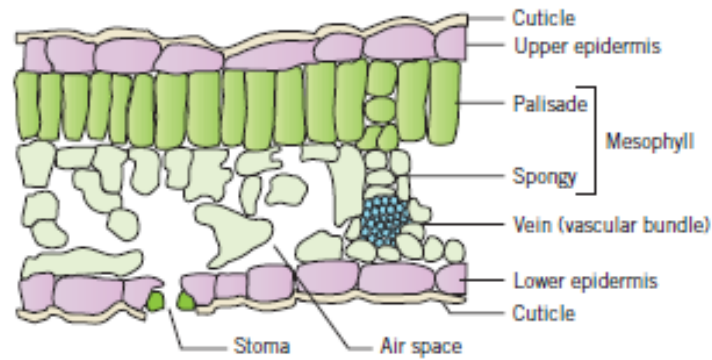
Figure 2.2 A cross-section of a leaf

Source: Hopkins & Hüner (2008, p. 20)

Stomata morphology is an important trait that determines the ability of mistletoes to passively uptake nutrients from host trees and at the same time control water loss (Okubamichael *et al.* 2011; von Willert & Popp 1995). The leaves of mistletoes have been observed to be more dark green with more chloroplasts which maximizes light capture. Mistletoes have also been observed to have larger and higher density of stomata than their hosts and are closely packed together to avoid loss of large amounts of water at the same time maximizing on the uptake of gases. Mistletoes loose more water than their host trees but can also control water loss (Perry 1995).

2.6.4.1 Environmental factors that affect stomata density

Plant growth and development is affected by the environment. Several environmental parameters affecting stomata density of leaves include sunlight, availability of water, temperature and carbon dioxide (Weyers & Meidner 1990; Young *et al*. 2004).

The sun is responsible for providing energy for all organisms on earth, photons in the form of wave energy from the sun stimulate the opening and closing of stomata. In the presence of sunlight photosynthesis occurs as a result of the balance between the opening of stomata to get CO_2, which allows water loss, and closing of stomata to reduce water loss, which also stops the entry of CO_2. To conduct photosynthesis and bring in carbon dioxide more stomata are needed to regulate gas exchange throughout the day. When a tree does not get much light there is more stomata, so that the intake of CO_2 is enough to complete photosynthesis. Furthermore, a plant that gets a lot of light has a low stomata density, because it is open more during the day and is able to bring in more carbon dioxide with less stomata and hence this causes the plant to be able to complete photosynthesis more often (Young *et al*. 2004).

Water is required by all living organisms and plants can be stressed by a lack of water as well as an excess of it. Guard cells surround the stomata and play an important role in controlling the opening and closing of the stomata (Figure 2.2). When the guard cells swell up with water, the stomata open and when the guard cells are emptied, the stomata close because there will not be enough water to create pressure in the guard cells for stomata opening hence this response helps the plant conserve water (Young *et al*. 2004).

When temperatures are increased above 30 degrees Celsius, respiration is increased, which increases the internal carbon dioxide concentration in the leaf and hence causing stomata to close temporarily. Should the temperature decrease to 0ºC, the process of photosynthesis stops and causes the stomata to close (Young *et al*. 2004). Leaves of plants that are exposed to cooler temperatures will have a lower stomata density because the stomata will not have to close as often; for leaves of

plants that are exposed to high temperatures, the atmosphere is hotter so there are more stomata but extreme hot conditions will cause the stomata to close. Stomata in lower temperatures slows down photosynthesis; therefore, at low temperatures plants have lower stomata density but wide thin leaves. At higher temperatures leaves can loose water through evaporation, speeding up the photosynthesis process, and will have a higher stomata density (Young *et al*. 2004).

In areas where the atmospheric concentration of carbon dioxide is high, the leaves of trees in that particular area tend to have less stomata. Since there is a higher concentration of CO_2 available, the leaves do not need a lot of stomata to bring in CO_2 for photosynthesis (Tognetti *et al*. 2000). Whereas, when there is a limited amount of carbon dioxide (CO_2) present in the atmosphere, there needs to be more stomata to allow the plant to take in more CO_2 to carry out photosynthesis. Therefore, in order for the intake of CO_2 there must be light present (Young *et al*. 2004).

CHAPTER 3. MATERIALS AND METHODS

3.1 Description of the study sites

3.1.1 Location and extent

The study was carried out in Windhoek City in the Khomas Region of Namibia. I used the National Botanical Garden (22º34ˈ15″S; 17º05ˈ38.6″E) of the National Botanical Research Institute (NBRI, Ministry of Agriculture, Water and Forestry) and the Aloe Trail (22º34ˈ03.8″S; 17º 05ˈ40.3″E), situated adjacent to the NBRI (Figure 3.3). The National Botanic Garden covers an area of 12 ha. It is fenced off and managed by the NBRI (Figure 3.1).

Figure 3.1 Picture showing the National Botanical Garden, Namibia

Source: A. Amutenya (2015)

The 9ha Aloe Trail, owned by the City of Windhoek, is an open *Senegalia* savanna woodland that is located on top of a mountain that overlooks the city (Figure 3.2). It is not fenced off and so is easily accessed by members of the public. Wood collection for firewood is relatively common.

Figure 3.2 A section of the Aloe Trail in Windhoek, Namibia

Source: A. Amutenya (2015)

Study Sites in Khomas Region

QGIS 2.16.1
Coordinate System :WGS84
October 2016

250 0 250 500 750 1000 m

Legend
● Place Location
  Regional Boundaries
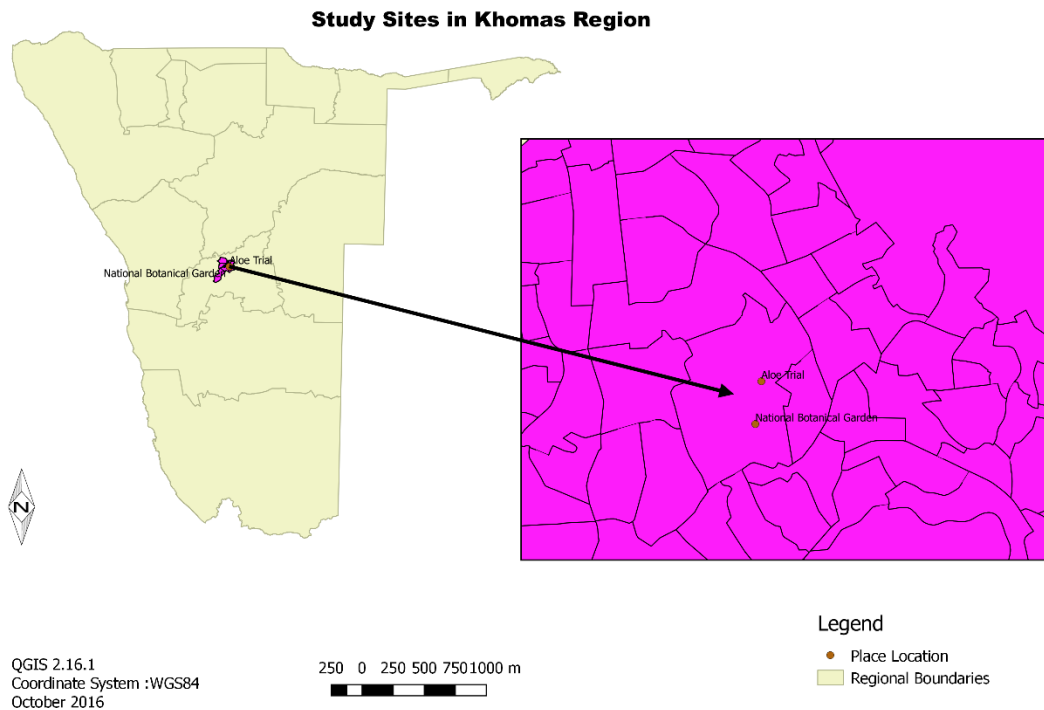
Figure 3.3 Location of the study area, indicating the two study sites: the Botanic Garden and the Aloe Trail.

3.1.2 Climate

Windhoek's climate is described as semi - arid (Mendelsohn *et al*. 2002). The city is situated at 1700 m above sea level (Urban green cc, 2011). The dry season is mainly from May to October and the wet season is mainly from November to April. Mean annual rainfall ranges from 350 - 400 mm, most of which occurs between January and March (Mendelsohn *et al*. 2002). The minimum temperatures range from 5 °C to 18°C and the maximum temperatures range from 30 °C to 32 °C (Mendelsohn *et al*. 2002; Government of the Republic of Namibia, Ministry of Works and Transport: Meteorological Services Division 2012).

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3.1.3 Flora

Both sites lie on a hilly terrain with vegetation that is described as highland savanna and dominated by trees and shrubs. The Aloe Trail appeared to be more bush encroached than the Botanical Garden. In the Botanic Garden, a lot of indigenous plant species are present including succulents and sedges, alien invasive plant species such as *Opuntia engelmanii* Salm-Dyck subsp. *lindheimeri* (Engelm.) U.Guzmán & Mandujamo and *O. imbricata* (Haw.) DC., which are regarded as some of the nasty nine most invasive plants in Namibia, are cleared but in the Aloe Trail no clearing of invasive plants is carried out. The vegetation of the area is dominated by *Acacia Senegalia* spp*., Searsia* spp*., Grewia* spp*., Vachellia* spp*.,* and *Ziziphus mucronata* Willd. subsp. *mucronata*. The Windhoek aloe, *Aloe littoralis* Baker, is also well represented in relatively dense stands. Common grasses include *Aristida* spp*., Eragrostis* spp*.* and *Sporobolus* spp*.*

3.1.4 Fauna

In both sites there are no records of large (>5kg) herbivores. The area is home to a variety of small mammals such as bushveld elephant-shrew (*E. intufi*), cape ground squirrel (*Xerus inauris*), and the natal multimammate mouse (*M. natalensis*). Many bird species, including masked weaver (*Ploceus velatus)*, lesser masked weaver (*P. intermedius)*, pale winged starling (*Onychognathus nabouroup)*, glossy starling (*Lamprotornis nitens)* and monteiro´s hornbill (*Tockus monteiri*) have been observed. Insects and reptiles have also been observed.

3.1.5 Geology, soils and the physical environment

The Botanic Garden is characterized by the paved walking trails and the Aloe Trail by gravel walking trails. The area is a highland plateau surrounded by mountainous, hilly and rocky terrain (Mendelsohn *et al.* 2002). The plateau lies

between three mountain ranges: the Eros Mountains to the north-east of the city, the Auas Mountains to the south - east and the Khomas Hochland Mountain to the west. The urban area has a Biotite Schist geology formation which can be observed on the slopes of many of the road embankments incised in and around Windhoek. The topsoil is thin and poorly developed as a result of alluvial colluvial deposition of fine sands and silts that are mixed with residual quartz pebbles (Brink 1981).

3.2 Description of the study species

Commonly known mistletoe species found in the Windhoek area are the evergreen shrubby *Tapinanthus oleifolius* and *Viscum rotundifolium*. *T. oleifolius* is epiphytic in nature and is mostly adapted to dry habitats. The flowers are red; the fruits are smooth (Figure 3.4. a), red and berrylike. The seeds are very sticky and attach easily to branches of host trees and on the bills and legs of birds. The peak flowering season of *T. oleifolius* is in late spring (November) but it continues to flower throughout the whole of summer from October to April (von Willert and Popp 1995). It blends in with the colour of leaves of the host tree hence it is mostly inconspicuous. It is more conspicuous in the dry season when host plants have shed their leaves (Visser, 1981).

V. rotundifolium, commonly known as red-berry mistletoe, is epiphytic in nature and grows in clumps (Popp, 1995). It has leathery fleshy leaves with small creamy-green flowers and orange-red fruits (Figure 3.4.b). It flowers in mid - winter and it can be found parasitizing a wide variety of hosts including other mistletoes (Mannheimer 2012; von Willert & Popp 1995).

a)   b)
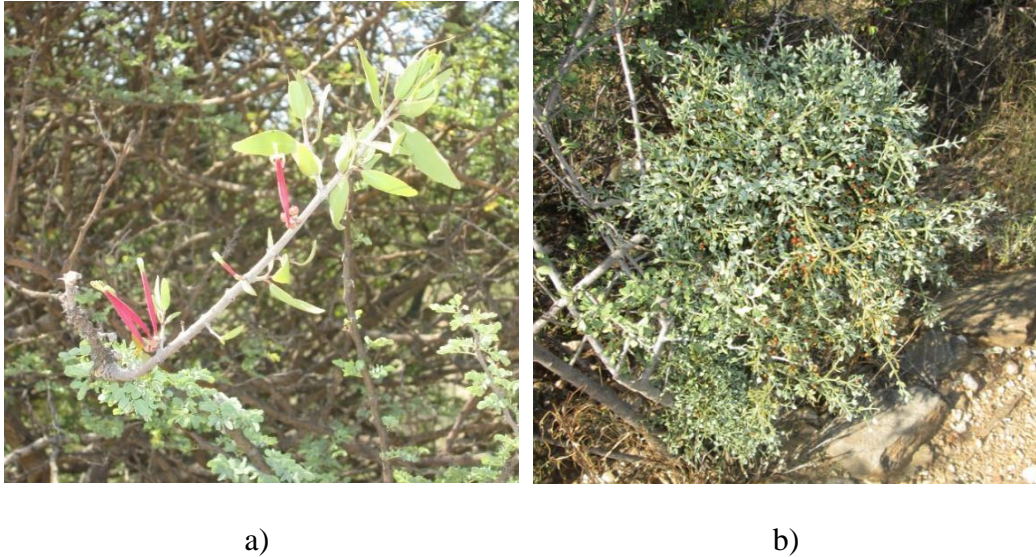
Figure 3.4 *Tapinanthus oleifolius* (with red flowers) infesting *Senegalia mellifera* (a)
and *Viscum rotundifolium* infesting *Boscia albitrunca* (b)

Source: A. Amutenya (2015)

3. 3 Data collection

3.3.1 Prevalence and infectivity of mistletoes on woody trees

For the purposes of this study, the Botanic Garden will be referred to as the Garden and the Aloe Trail will be referred to as the Trail. Fifty plots were sampled in the Garden and fifty plots were sampled in the Trail. Tape measures of 50m long were used to demarcate the plots systematically, 20mx20m plots were demarcated within the two sites. A minimum distance of 5m was maintained between the plots. Caution was taken for the plots not to be too close to the walking paths in the Garden and the Trail hence a minimum distance of 10m was maintained from the path to the nearest plot. In each plot, host tree and mistletoe were identified to species level. Height of all woody plants in each plot were measured using a ranging pole and diameter at breast height (dbh) (cm) using a tailor tape, dbh was measured at approximately 1.3m from the ground. For plants that were multi - stemmed the dbh

was measured on the largest stem. Each woody plant was then examined for presence of mistletoes. The total number of mistletoes on each individual plant was counted. Saplings of woody plants were not included during the data collection process. This was done during April to June 2015.

3.3.2 Measurement of photosynthesis

Photosynthesis was determined by measuring the rate of CO_2 uptake, with a leaf enclosed in a 6-cm^3 chamber within a closed system. The portable photosynthesis system was able to control the environmental factors that are important to photosynthesis such as CO_2, light, humidity, and temperature. Measurements of photosynthesis and transpiration were based on the differences in CO_2 and H_2O in an air stream that was flowing through a plant leaf enclosed in a cuvette of a LI-6400XT Portable Photosynthesis System (Li-Cor, Lincoln, NE, USA 2012).

Pairs of plants were used for measurement, one from the host and the other from the mistletoe. The target mistletoe - host pairs were purposively selected in the Garden. Fully expanded healthy mature shade leaves were selected for measurement. Caution was taken not to choose leaves that were too old but rather choose healthy mature leaves. Measurements were done between the 9:00 am and 3:00 pm. A total of 36 measurements were made, 24 from the host parasite pair *Boscia albitrunca* (Burch.) Gilg & Gilg-Ben. – *V. rotundifolium* (12 from the parasite and 12 from the host) and 12 measurements from the uninfected *B. albitrunca* that acted as a control.

Light response measurements were made during the dry season on three consecutive mornings between 0800 h and 1200 h so as to see how the two species react to the different light intensities and to determine at which ambient light to make

the measurements. Measurements were done sequentially at photosyntheticaly active radiation (PAR) levels from 2500 μm m^{-2} s^{-1} down to 0 μm m^{-2} s^{-1} (2500, 2150, 2000, 1700, 1500, 1300, 1000, 750, 500, 300, 150 and 0 μm m^{-2} s^{-1}), temperature at 30°C, air pressure at 83 kPa, humidity was kept at a range of 45 - 49 and internal CO_2 concentration was kept at 400 μmol mol^{-1}. From the light response measurements it was decided to make the measurements at PAR 2000 0 μm m^{-2} s^{-1} as this was the suitable PAR for Namibia's hot dry climate.

B. *albitrunca* was chosen because it was the only host (during the duration of the study) whose simple leaves could fit in the right position in the leaf chamber of (IRGA) while other species such as *Senegalia mellifera* (Vahl) Seigler & Ebinger experienced difficulties with getting the leaves in the right position in the light chamber for measurement. *V*. *rotundifolium* was chosen as the parasite for measurement because in the specific area which is the Garden *B*. *altitrunca* was parasitsed by *V*. *rotundifolium*.

The photosynthesis measurements were done at PAR level of 2000 μm m^{-2} s^{-1}, temperature at 30°C, air pressure at 83 kPa, humidity was kept at a range of 45 - 49 and internal CO_2 concentration was kept at 400 μmol mol^{-1}. The measurements were done during the months of October and November 2015. The following measurements were made: photosynthesis (*A*) (μmol CO_2 m^{-2} s^{-1}), transpiration (*E*) (mmol H_2O m^{-2} s^{-1}) and conductivity (*gs*) (mol H_2O m^{-2} s^{-1}).

3.3.3 Measurement of chlorophyll *a*, chlorophyll *b*, and carotenoid content

Leaves were purposively selected in the Garden of the NBRI, shade leaves were selected for this analysis. Shade leaves are found on lowest part of the tree crown and they appear to be growing underneath other branches; shade-leaves were

used in this study as they are larger in size often are more efficient in harvesting sunlight (Campbell & Reece 2008). Caution was taken not to choose leaves that were too old but rather choose healthy mature leaves. The mistletoe - host pairs that were selected were; *B. albitrunca - V. rotundifolium*. A total of 20 mistletoe - host pairs were selected. For each plant there were three replicates bringing the total number of measurements to 120. Leaf pairs were collected during the early morning hours to ensure that they were not water stressed. In the laboratory I worked in dim light.

From each plant sample, 0.25 g was weighed. The sample was ground using a pestle and mortar. Total pigments were extracted by adding 5ml of 80% acetone to the sample. The contents were transferred to a centrifuge tube and centrifuged at 1500g for 5 minutes. The supernatant was kept and the pellet was discarded. The supernatant was transferred to a cuvette. The absorbance was measured at 664, 647 and 441 nm which are the major absorption peaks for chlorophyll *a*, *b* and carotenoid (Yang *et al*. 1998). Chlorophyll *a*, *b* and carotenoids were measured by dual - beam spectrophotometer (Model Jasco V-550).

3.3.4 Measurement of water potential (Ψ)

The principal rationale of the Scholander pressure chamber is that as negative pressure develops inside the xylem due to transpiration, the xylem contracts due to the tension developed in the water and the cohesion of the molecules. When the stem is cut, the negative pressure is released, the xylem recovers to its original, unstressed size and the water withdraws from the cut surface. When the water appears at the cut surface an estimate of the magnitude of the tension can be obtained (Boyer 1995; Kramer & Boyer 1995).

Pairs of samples were collected for measurement, one from the host and the other from the mistletoe. The target host plants were randomly selected in the Garden. The parasite host pairs that were measured were; *S. mellifera - T. oleifolius* and *B. albitrunca - V. rotundifolium*. These mistletoe - host pairs were chosen for measurement because of the results of the 2015 pilot study which showed that *S. mellifera* and *B. albitrunca* were the woody plant species that were mostly infected by mistletoes (*T. oleifolius* and *B. albitrunca* respectively) and also recorded the highest abundance of mistletoes in the Garden. Twenty eight mistletoes - host pairs were selected, 14 measurements from the pair *S. mellifera - T. oleifolius* and 14 measurements from the pair *B. albitrunca - V. rotundifolium*. Measurements were taken in duplicates. For each pair an uninfected tree was also measured which acted as a control bringing the total measurements to 84. The leaf Ψ of mistletoe - host plant pairs were measured during pre-dawn using a Scholander pressure chamber (AOAC International, 2000). Ψ was measured immediately after the leaves were cut to ensure minimal water loss.

A freshly cut twig with the cut end protruding towards the outside was placed inside a sealed chamber and pressurised gas was added to the chamber slowly. As the pressure increased, at some point sap was forced out of the xylem and was visible at the cut end of the stem. The cut end of the stem was examined under a simple $10\times$ magnifying lens, the gas supply was cut off and the pressure inside the chamber was noted with the first observation of water exuded on the surface of the twig. The amount of pressure that it takes to cause water to appear at the cut surface of the petiole showed how much tension the leaf was experiencing on its water. The Ψ then equals the water potential in the stem where the twig is attached (Martínez *et al.* 2013).

Prior to the actual Ψ measurements, one host mistletoe pair (*B. albitrunca - T. oleifolius*) and a control (uninfected *B. albitrunca*) was selected and measurements were done every hour for 24 hours so as to compare the readings during the night and day and also to determine the optimal time for the actual measurements. From these measurements it was decided that it was best to do the measurements during predawn just before sunrise. When measurements are done during pre-dawn; Ψ changes slowly, Ψ is at its minimum and the plant is observed to be in or close to equilibrium with the soil moisture as opposed to during the day when the leaf Ψ declines below the soil water potential due to transpiration (Pérez-Harguindeguy *et al*. 2013). These measurements were done during the months of March and April 2016.

3.3.5 Determining stomata quantity

3.3.5.1 Obtaining stomata impressions

The target mistletoe - host pairs were randomly selected in the Garden of the NBRI. The mistletoe - host pairs that were selected were; *S. mellifera - T. oleifolius*. Shade leaves were used for this analysis. A total of 14 mistletoe - host pairs were selected; three leaves were collected from each plant. The lower (abaxial) surface of the leaves that were collected was coated with a thick coat of clear nail polish. The nail polish was allowed to dry for a few minutes. Once completely dry, a clear tape was used to stick the leaf area containing the dry nail polish. The nail polish was gently removed from the leaf. A cloudy impression of the leaf surface was then stuck to the piece of tape (this is called the leaf impression). The leaf impression was then tapped to a clean microscope slide, scissors were used to cut off excess tape and the slides were labelled accordingly.

3.3.5.2 Determining the number of stomata

Before any counts were done, the diameter of field of view for the specific microscope in use was determined. A compound microscope was used to count the number of stomata per field of view used. This was repeated for two more fields for each leaf impression.

3.4 Data analysis

3.4.1 Mistletoe prevalence and infectivity on woody plants

The data were analyzed using SPSS v. 23. For the purposes of this study, prevalence was calculated as the number of infected trees as a proportion of the total number of trees and infectivity as the number of infected individuals of a species as a proportion of the total individuals of that species.

A Shapiro - Wilk test (Zar 1999) was used to determine whether the data on prevalence, infectivity, tree height, tree diameter and number of mistletoes per woody plant followed a normal distribution. Prevalence and infectivity data were not normally distributed (df=100, $p<0.001$ in both cases), and were compared between the two sites using the Mann-Whitney U test. The Mann-Whitney U test was used again to determine whether there was any significant difference in infectivity of the different species between the two sites. For the infectivity analysis species that were not infected in both sites were removed from the analysis. A Chi-Square test was further used to test if prevalence of any mistletoes were associated with any host.

3.4.2 Host - tree size and the number of mistletoes

Tree height, tree diameter and number of mistletoes per woody plant data were not normally distributed (df=232, $p<0.001$) and hence the Spearman's

correlation test was used to determine the relationship between tree height and the number of mistletoes as well as between tree diameter and the number of mistletoes at the two sites (Sokal & Rohlf, 2012).

3.4.3 Photosynthetic traits, chlorophyll and carotenoid content, water potential and number of stomata of mistletoe - host tree pairs

A Shapiro - Wilk test (Zar 1999) was used to determine whether the data on photosynthesis, transpiration, chlorophyll and carotenoid content, water potential and number of stomata followed a normal distribution. Photosynthesis data were not normally distributed (df=24, p<0.001) and were compared between the host and the parasite using the Wilcoxon signed - rank test. Transpiration data were normally distributed (df=24, p>0.05) and were compared between the host and the parasite using a paired t - test.

Chlorophyll and carotenoid content data were not normally distributed (df=40, p<0.05) and thus were compared between the host and the parasite using a Wilcoxon signed - rank test.

Chlorophyll a and b, and carotenoid were determined on a fresh weight basis (μg chl/g) using a modification of Yang et al. (1998):

Chlorophyll a (μg/ml) = 12.25 x A_{664} – 2.55 x A_{647}

Chlorophyll b (μg/ml) = 20.31 x A_{647} – 4.91 x A_{664}

Where A is absorbance at the wavelength specified

Total chlorophyll = chlorophyll a + chlorophyll b.

Car (μg/ml) = 4.69 x A_{441} – 0.267 x (chl $a+b$),

where Car is carotenoids.

Photosynthesis and chlorophyll data were not normally distributed (df=24, p<0.001) and hence a linear regression analysis was used to determine the relationship between photosynthetic rate and chlorophyll content.

Water potential data were not normally distributed (df=52, p< 0.05) and were compared between the host and the parasite using the Wilcoxon signed - rank test.

The number of stomata was determined per microscopic field of view as: average number of counts/400x microscopic field. Area of field of view = πr^2.

Number of stomata mm^{-2} = average number of counts/400x microscopic field/ area of field of view.

Stomata data were not normally distributed (df=28, p<0.05) and were compared between the host and the parasite using a Wilcoxon signed - rank test.

CHAPTER 4. RESULTS

4.1 Vegetation structure of the Botanic Garden and the Aloe Trail

The Trail had more mistletoe - infected trees and more mistletoes than the Garden (Mann Whitney U test, $Z = -0.4562$, p<0.001, Whitney U test, $Z = -0.527$, p<0.001), (Table 4.1).

Table 4.1 Patterns of mistletoes infection data collected from the Botanic Garden and the Aloe Trail.

Site	Total no. of trees sampled	Proportion of infected trees (%)	No. of mistletoes	No. of woody species	No. of mistletoes species
Botanical Garden	1445	4.7	149	23	2
Aloe Trail	1133	14.8	545	17	1

There were no mistletoe-infected *Senegalia hereroensis* (Engl.) Kyal. & Boatwr. and *Catophracteshi78 alexandrii* D.Don in the Garden, and no mistletoe-infected *B. albitrunca* in the Trail. In the Trail; *T. oleifolius* was found to be parasitizing a wide range of hosts while *V. rotundifolium* was only found to parasitize *B. albitrunca* (Table 4.2).

Table 4.2 Tree species that were infected by mistletoes in the Botanic Garden and the Aloe Trail. A tick (✓) represents presence of species in an area and a cross (×) represents absence in an area.

Host species	Botanical Garden	Aloe Trail	Mistletoes species
Senegalia erubescens	✓	✓	*Tapinanthus oleifolius*
Senegalia hereroensis	×	✓	*T. oleifolius*
Senegalia mellifera	✓	✓	*T. oleifolius*
Vachellia reficiens	✓	✓	*T. oleifolius*
Boscia albitrunca	✓	×	*Viscum rotundifolium*
Catophractes alexandrii	×	✓	*T. oleifolius*
Dichrostachys cinerea	✓	✓	*T. oleifolius*

In the Garden, mistletoes were significantly associated with *Senegalia mellifera* and *Senegalia erubescens* the species (χ^2 = 9.084, df = 3, p = 0.028<0.05). In the Trail, mistletoes were significantly associated to *S. mellifera* and *Dichrostachys cinerea* (χ^2 = 13.469, df = 3, p = 0.004<0.05), (Figure 4.1 and 4.2).
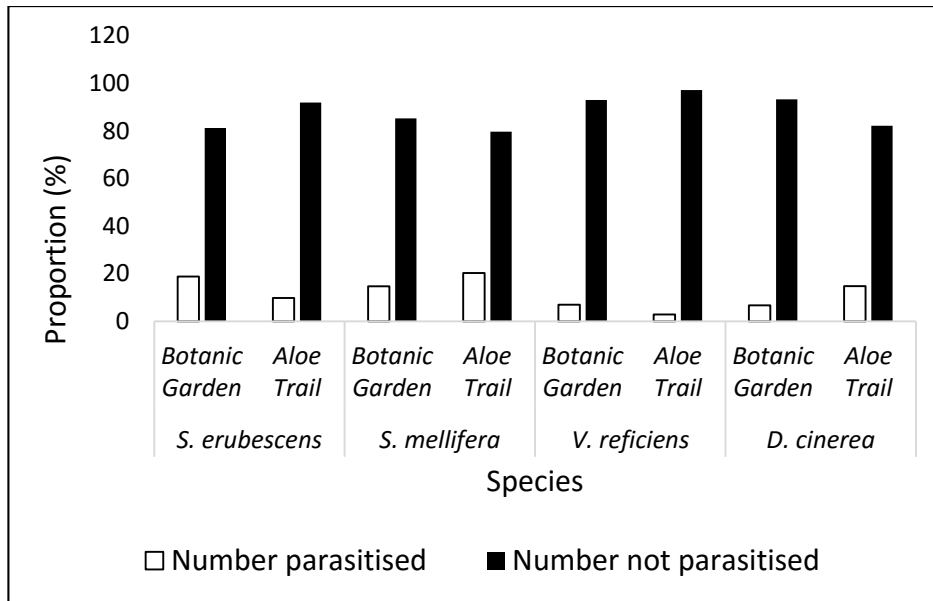
Figure 4.1 The proportion (%) of infected and uninfected woody trees in the Botanic Garden and in the Aloe Trail.
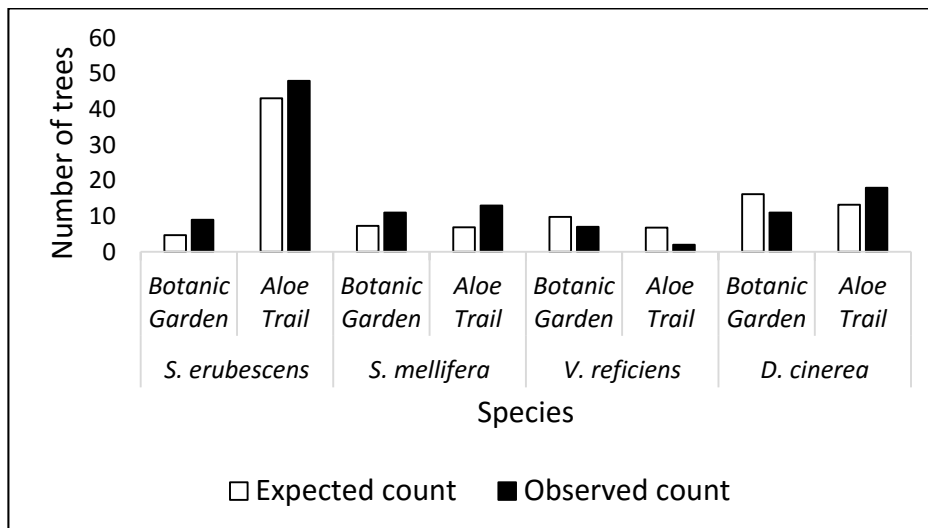
Figure 4. 2 The expected and observed count of infected individuals in the Botanic Garden and in the Aloe Trail.

4.2 Prevalence of mistletoes on woody plants

The Trail had a significantly high median prevalence of 13% compared to the Garden with 6% (Mann Whitney U test, $Z = -0.4562$, p<0.001, Figure 4.3). The lines in the

box represent the median value, the whisker below and above the box represent the values below the median (minimum values) and above the median (maximum values) respectively.
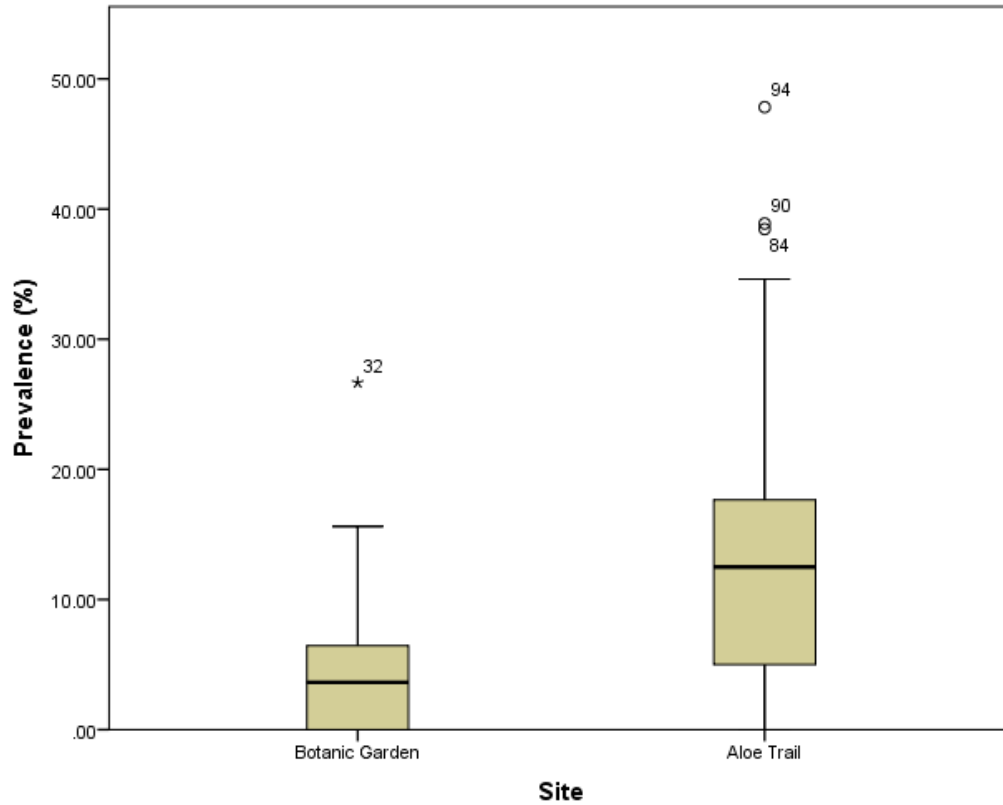
Figure 4.3 The prevalence (%) of mistletoes in the Botanic Garden and in the Aloe Trail.

4.3 Infectivity of mistletoes on woody plants

The Trial had a significantly high median infectivity of 22% for *S. erubescens* compared to 0.1% of the Garden (Mann Whitney U test, Z = -0.4.568, p<0.001), (Figure 4.4). The Trial had a significantly high median infectivity of 0.4 % *D. cinerea* compared to the 0.1 % of the Garden (Mann Whitney U test, Z = -2.883, p = 0.04<0.05), (Figure 4.5). The infectivity of *Senegalia mellifera* and *Vachellia reficiens* in the Trial were not significantly different from that of the Tail (Table 4.3).

Table 4.3 The infectivity of the different species between the Botanic Garden and the Aloe Trail.

Species	Infectivity
Senegalia erubescens	Mann Whitney U test, Z = -0.4.568, p<0.001
Senegalia mellifera	Mann Whitney U test, Z = -0.656, p = 0.512 > 0.05
Vachellia reficiens	Mann Whitney U test, Z = -0.421, p = 0.674 > 0.05
Dichrostachys cinerea	Mann Whitney U test, Z = -2.883, p=0.04<0.05
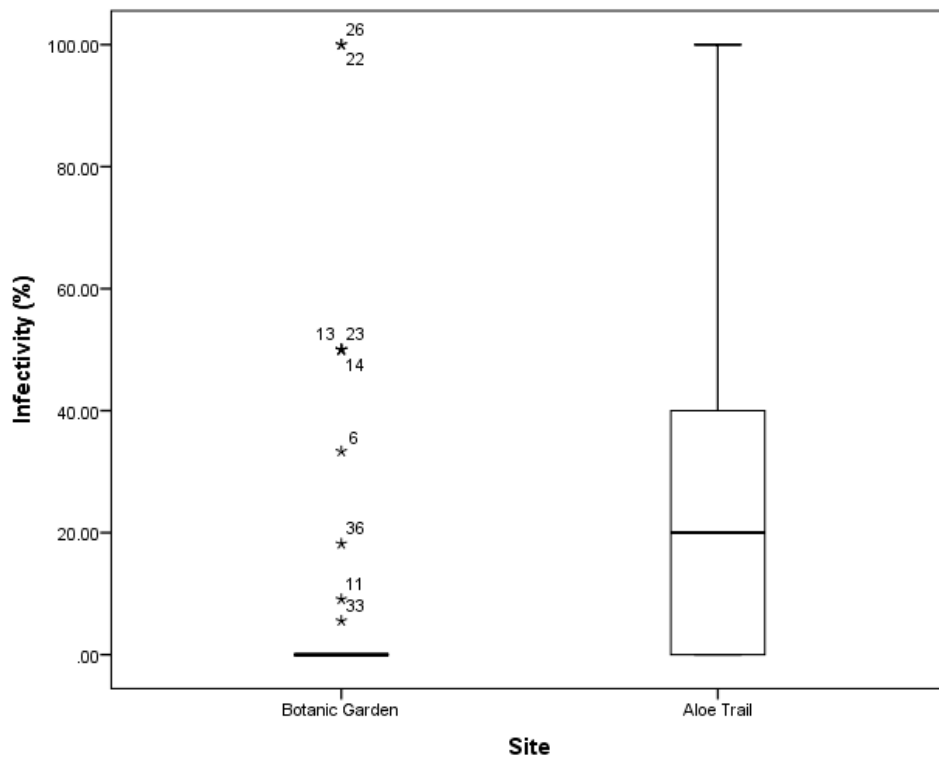
Figure 4.4 Infectivity (%) of *Senegalia erubescens* between the Botanic Garden and the Aloe Trail.
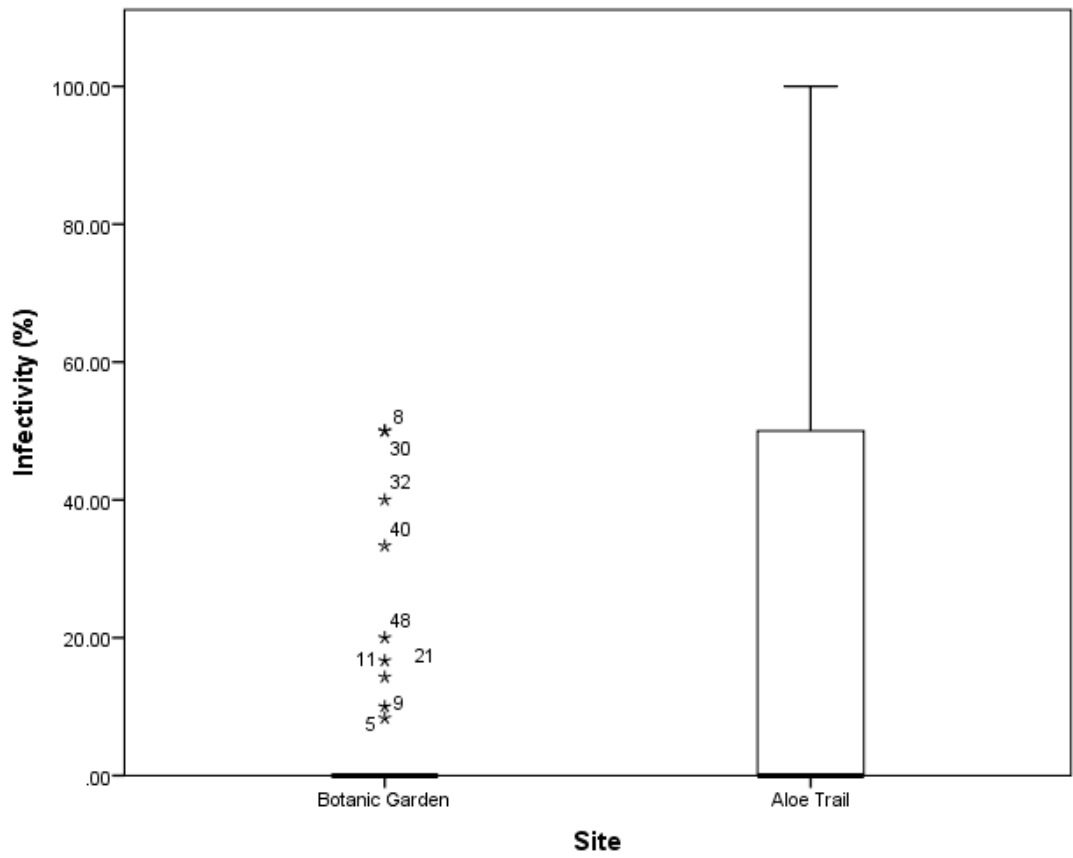
Figure 4.5 Infectivity (%) of *Dichrostachys cinerea* between the Botanic Garden and the Aloe Trail.

4.4 The relationship between tree size and number of mistletoes

Table 4.4 The correlation results between the Botanic Garden and the Aloe Trail.

Correlation	Botanic Garden	Aloe Trail
Tree height (m) vs number of mistletoes	r=-0.44,n=62, p=0.732>0.05	R=0.67,=151, p=0.410>0.05)
Tree diameter (mm) vs number of mistletoes	r=0.12,n=62, p=0.929>0.05	r=-0.131,n=151, p=0.109>0.05

4.4.1 The relationship between host - tree size and the number of mistletoes on woody trees at the Botanic Garden

There was no linear relationship between tree diameter and the number of mistletoes ($r = 0.12$, n = 62, p = 0.929>0.05), indicating that the number of mistletoes did not increase with tree diameter (Figure 4.6). r indicates the spearman's correlation coefficient value. There was no linear relationship between tree height and number of mistletoes ($r = -0.44$, n=62, p = 0.732>0.05), indicating that the number of mistletoes did not increase with tree height (Figure 4.7).
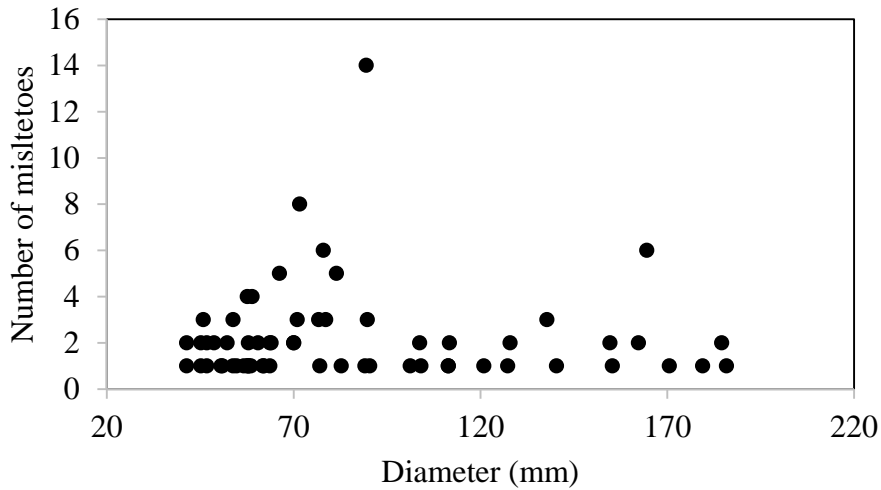
Figure 4.6 The relationship between tree stem diameter and number of mistletoe parasites on trees in the Botanic Garden.
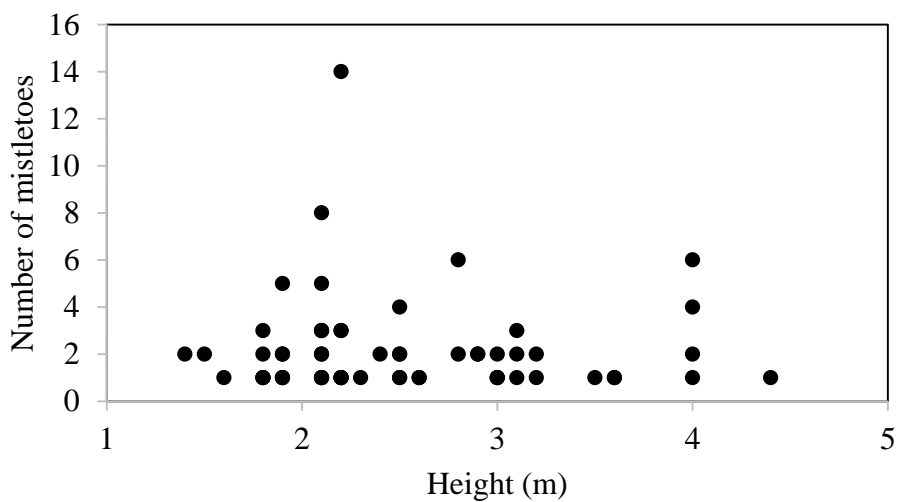
Figure 4.7 The relationship between tree height diameter and number of mistletoe parasites on trees in the Botanic Garden.

4.4.2 The relationship between host - tree size and the number of mistletoes on woody trees at the Aloe Trail

There was no linear relationship between tree diameter and the number of mistletoes (r = -0.131, n = 151, p = 0.109>0.05), indicating that the number of mistletoes did

not increase with tree diameter. There was no linear relationship between tree height and number of mistletoes ($r = 0.67$, n = 151, p = 0.410>0.05), indicating that the number of mistletoes did not increase with tree height (Figure 4.8 and 4.9).
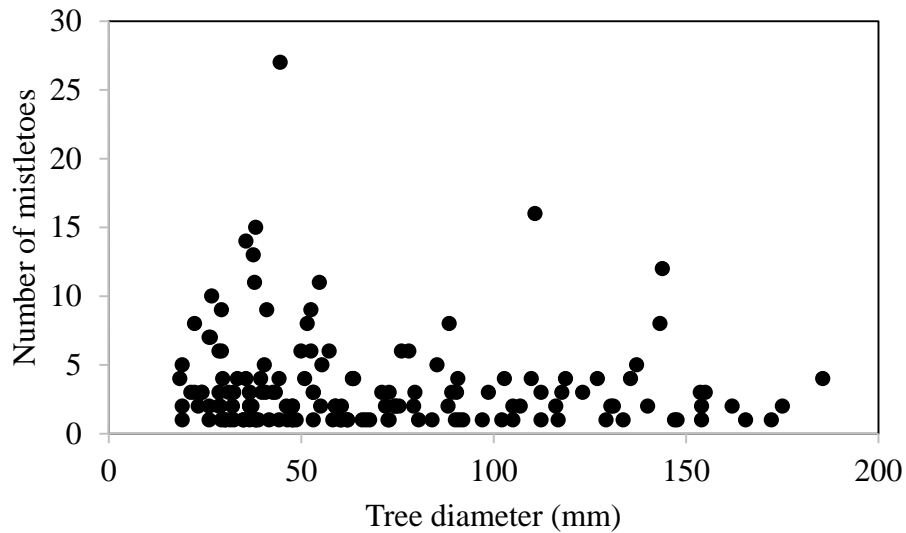
Figure 4.8 The relationship between tree stem diameter and number of mistletoe parasites on trees in the Aloe Trail.
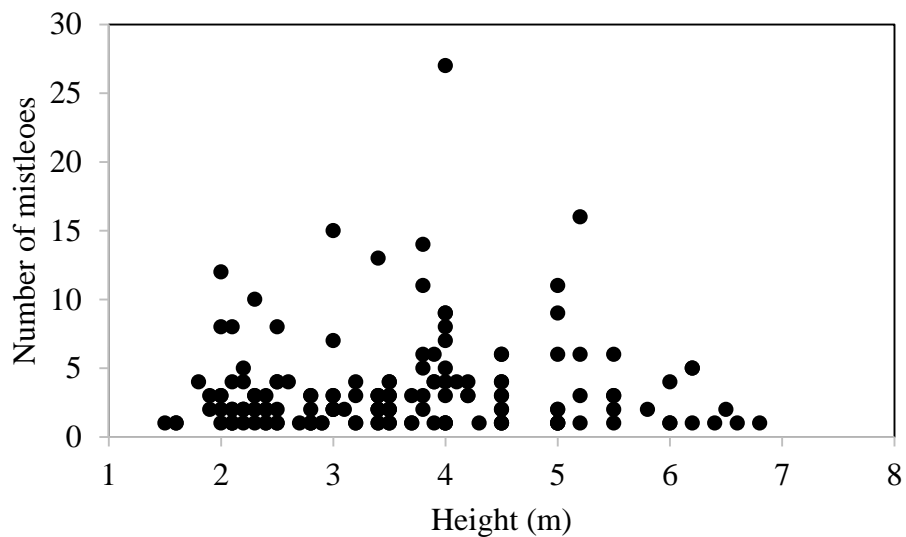
Figure 4.9 The relationship between tree height and number of mistletoe parasites on trees in the Aloe Trail.

4.5 Photosynthetic rates of mistletoe - host tree pairs at the Garden

Boscia albitrunca had a significantly high median photosynthetic rate of 2.6 μmol CO_2 m^{-2} s^{-1} compared to *V. rotundifolium* with 1.4 μmol CO_2 m^{-2} s^{-1} (Wilcoxon

signed - rank test, $Z = -2.061$, p< 0.05; Figure 4.10). *B. albitrunca* displayed a positive skewed photosynthetic rate (the mean is greater than the median), the top whisker is much longer than the bottom whisker indicating that more values were concentrated on the upper scale than on the lower scale. *V. rotundifolium* displayed a negative skewed photosynthetic rate, the bottom whisker was much longer, indicating that more values were concentrated on the lower scale however, the top whisker is not visible because the upper quartile is equal to the maximum observed photosynthetic rate value which is 1.4.
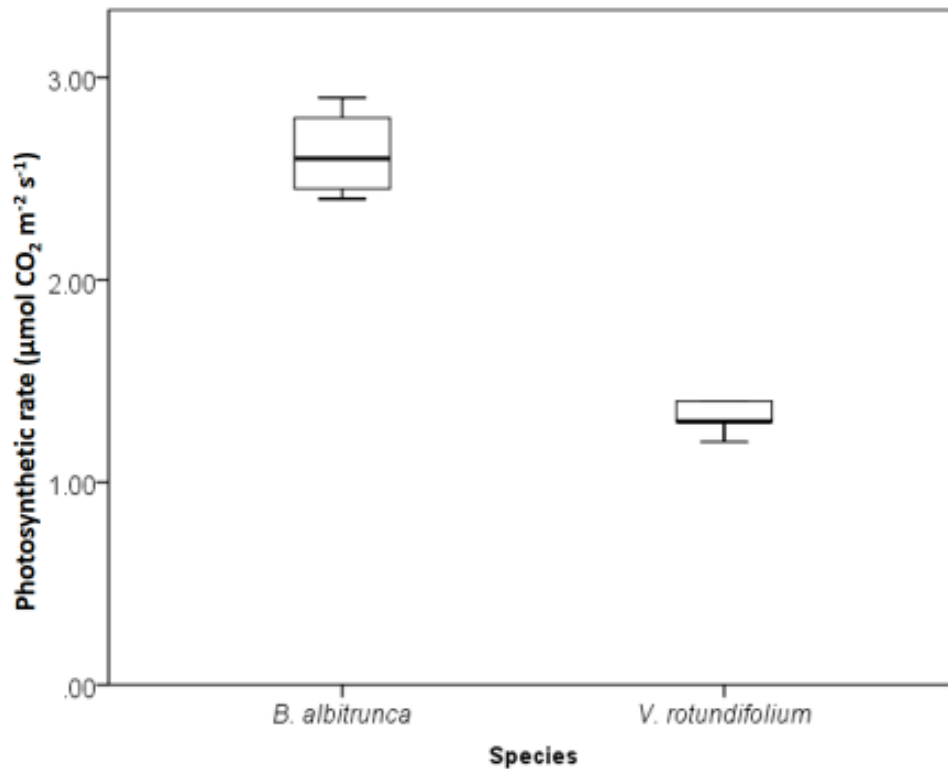
Figure 4.10 Median photosynthetic rate (μmol CO_2 m^{-2} s^{-1}) of the host *B. albitrunca* and the mistletoe *V. rotundifolium* at the Botanic Garden.

The uninfected *B. albitrunca* had a significantly high photosynthetic rate of 5.6 μmol CO_2 m^{-2} s^{-1} compared to the infected *B. albitrunca* with 2.8 μmol CO_2 m^{-2} s^{-1} (Wilcoxon signed - rank test, $Z = -4.29$, $P < 0.001$; Figure 4.11). The uninfected *B.*

albitrunca and infected *B. albitrunca* both displayed a symmetrical photosynthetic rate which implies that the values are equally spread from the median to the lower and upper scale.
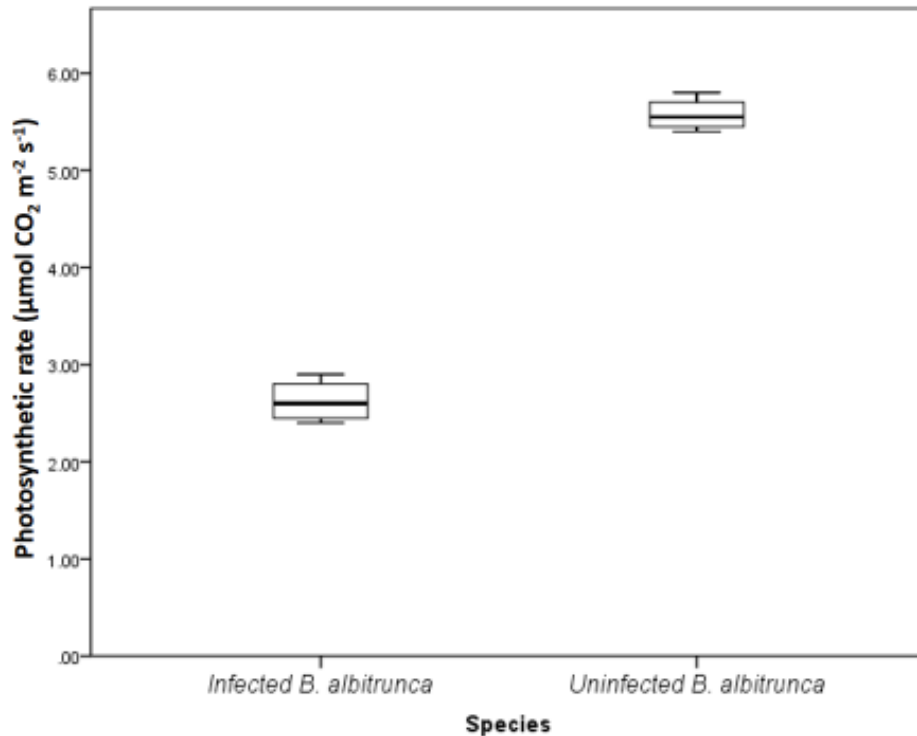
Figure 4.11 Median photosynthetic rate (μmol CO_2 m^{-2} s^{-1}) of the mistletoe-infected *B. albitrunca* and an uninfected *B. albitrunca* at the Botanic Garden.

4.6 Transpiration rates of mistletoe - host tree pairs at the Garden

V. rotundifolium had a significantly higher transpiration rate than *B. albitrunca* (paired T - test, $t = -9.626$, df=23 $p<0.001$; Figure 4.12). The bars represent the standard error of the mean; there was more variation in the transpiration rate of *V. rotundifolium* treatments (SE = 0.04 mmol H_2O m^{-2} s^{-1}), whereas *B. albitrunca* displayed little variation in the transpiration rate treatments (SE = 0.02 mmol H_2O m^{-2} s^{-1}).
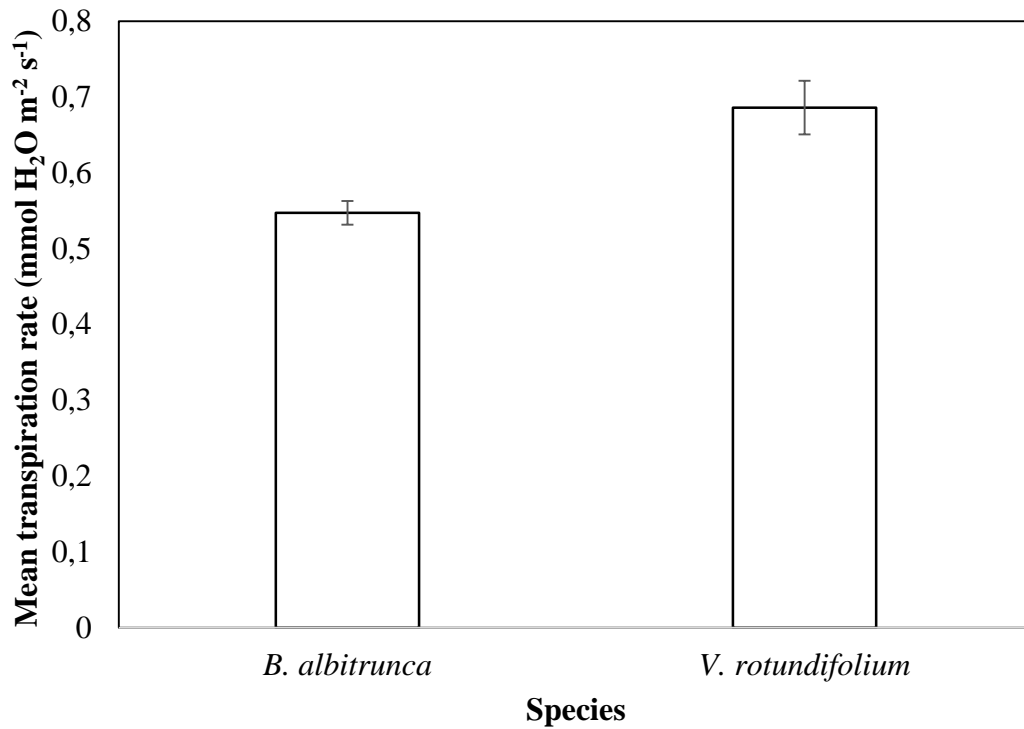
Figure 4.12 Mean transpiration rate (mmol H_2O m^{-2} s^{-1}) of host (infected *B. albitrunca*) in the Botanic Garden.

Infected *B. albitrunca* had a significantly higher transpiration rate than the uninfected *B. albitrunca* (paired T - test, t = -8.583, df =23, $p<0.001$, Figure 4.13). The bars represent the standard error of the mean; the small standard error bars indicate that there is very little variation in the transpiration rate treatments of both infected *B. albitrunca* (SE = 0.02 mmol H_2O m^{-2} s^{-1}) and uninfected *B. albitrunca* (SE = 0.02 mmol H_2O m^{-2} s^{-1}).
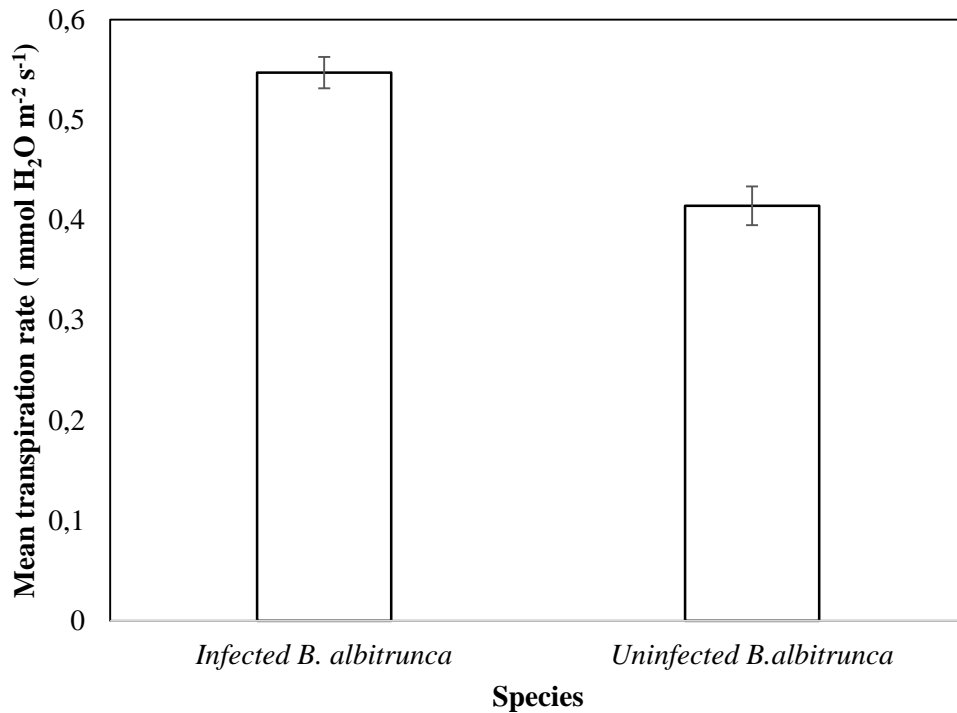
Figure 4.13 Mean (±SE) transpiration rates (mmol H_2O m^{-2} s^{-1}) of host (infected *B. albitrunca*) and the control (uninfected *B. albitrunca* in the Botanic Garden.

4.7 Chlorophyll and carotenoid contents of mistletoes and host plants at the Garden

B. albitrunca had a significantly high median chlorophyll content of 25 *μg g*-1 compared to *V. rotundifolium* with 12 *μg g*-1 (Wilcoxon signed - rank test, $Z = -5.511$, $p<0.001$; Figure 4.14). *B. albitrunca* displayed symmetry in chlorophyll content which implies that the values are equally spread from the median to the lower and upper scale. *V. rotundifolium* displayed a positively skewed chlorophyll content indicating that more values are concentrated on the upper scale than on the lower scale. It was observed that two data points showed a chlorophyll content that was out of the range; these values are outliers as they appear above the boxes.
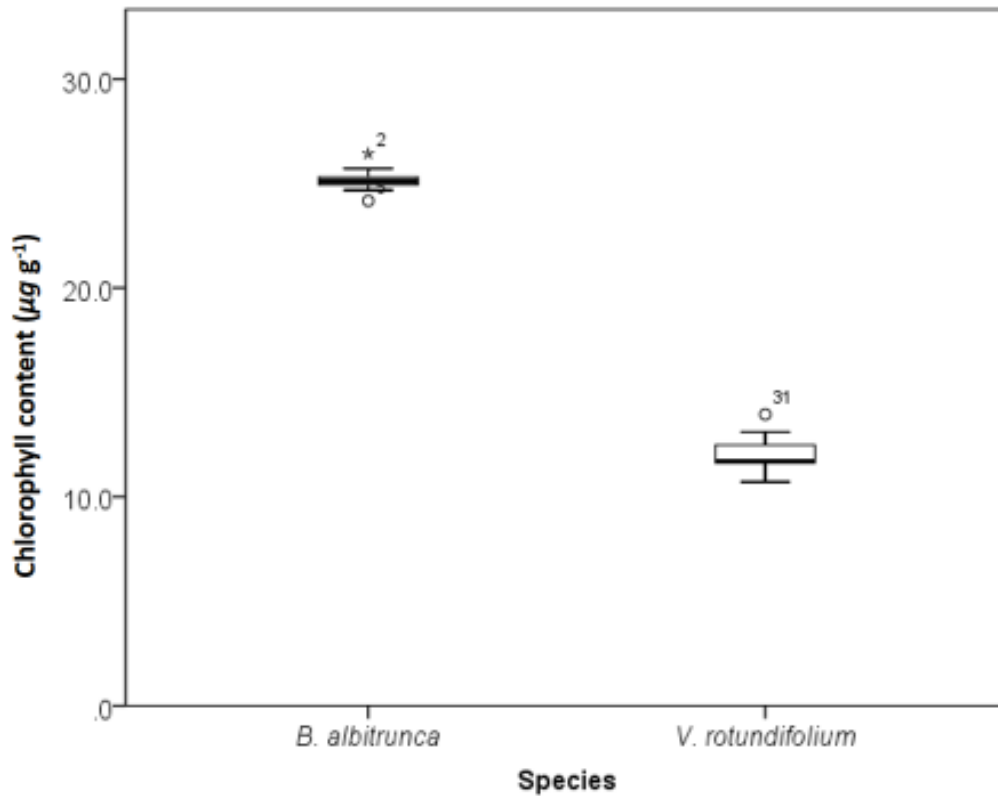
Figure 4.14 The median chlorophyll contents (μg g^{-1}) of the host (*B. albitrunca*) and the parasite (*V. rotundifolium*) in the Botanic Garden.

Boscia albitrunca had a significantly high median carotenoid content of 4.9 *µg g*-1 compared to *V. rotundifolium* with 2.5 *µg g*-1 (Wilcoxon signed - rank test, Z = -5.511, p<0.001; Figure 4.15). *B. albitrunca* displayed a positively skewed chlorophyll content indicating that more values are concentrated on the upper scale than on the lower scale. *V. rotundifolium* displayed symmetry in the number of stomata which implies that the values are equally spread from the median to the lower and upper scale.
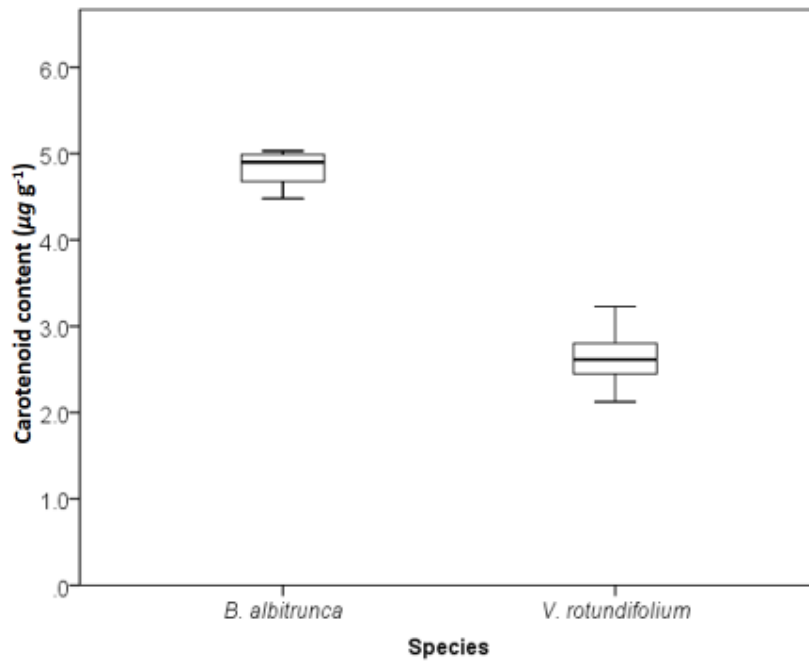
Figure 4.15 The median carotenoid content (μg g^{-1}) of the host (*B. albitrunca*) and the parasite (*V. rotundifolium*) at the Botanic Garden.

4.8 Effect of chlorophyll on the photosynthesis

There was a no significant relationship between photosynthetic rate and chlorophyll content in the host and the parasite (r^2 = 0.235, n = 12, p = 0.110 >0.05 and r^2 = 0.083, n = 12, p = 0.363> 0.05), indicating that chlorophyll content did not affect photosynthetic rate in both the host and the parasite in this study (Figures 4.16 and 4.17).
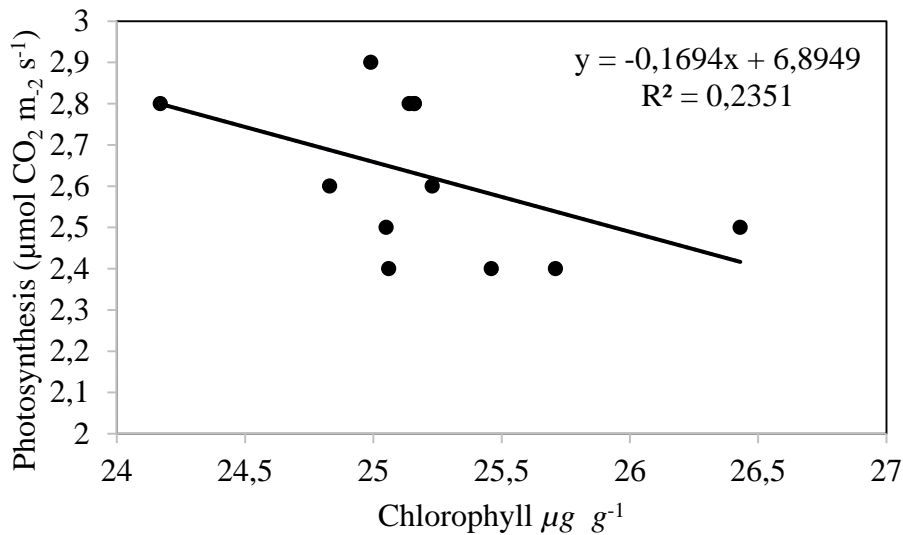
Figure 4.16 The relationship photosynthetic rate (μmol CO_2 m^{-2} s^{-1}) and chlorophyll content (μg g^{-1}) in the host in the Botanic Garden.
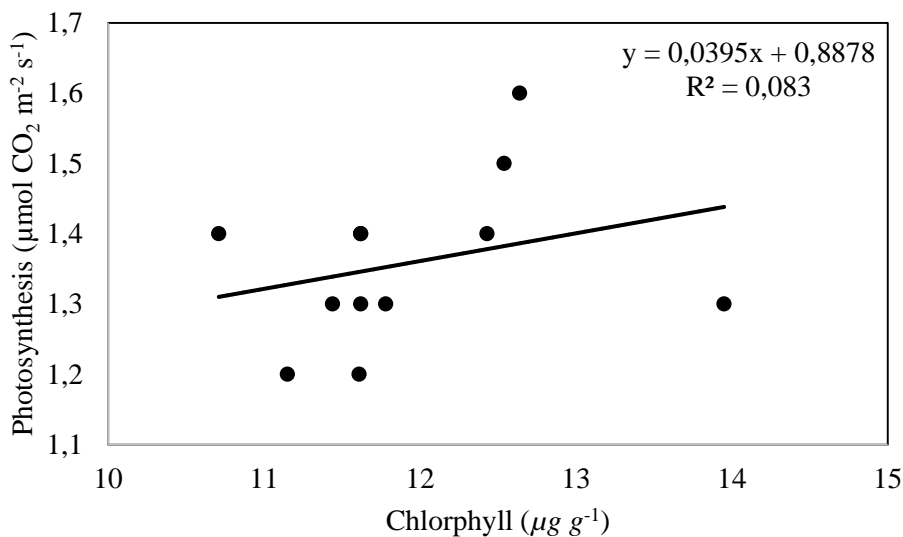
Figure 4.17 The relationship photosynthetic rate (μmol CO_2 m^{-2} s^{-1}) and chlorophyll content (μg g^{-1}) in the parasite in the Botanic Garden.

4.9 Time series curve of water potential of mistletoe - host pair and control at the Garden

After sunrise (07h00) the Ψ increased steeply until noon (12h00 hr), after which it decreased until late night hours (22:00 hr). After midnight (00:00 am) the Ψ

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stabilised and just after sunrise (07h00) it started increasing again (Figure 4.18). Even though the different species display different Ψ figures during the course off the day, the patterns were similar for all similar to each other.
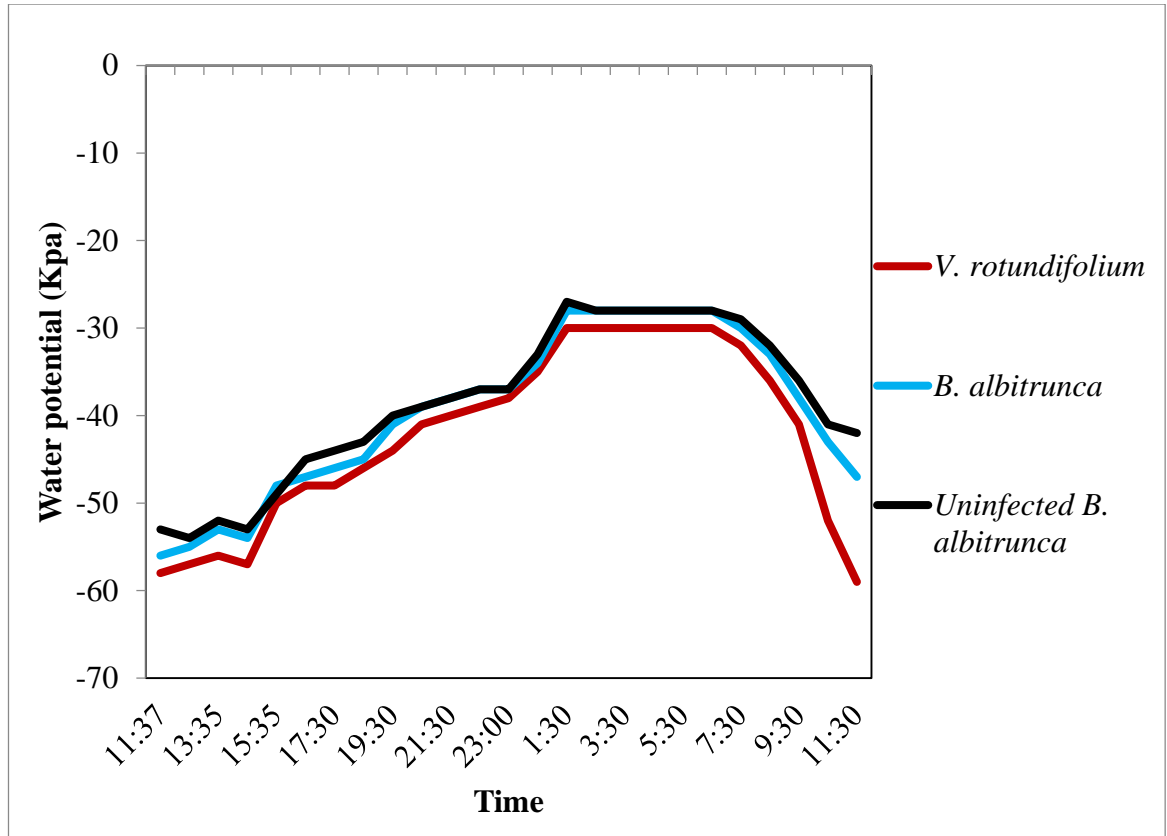
Figure 4.18 Time series graph of water potential (kPa) for the host (infected *B. albitrunca*, parasite (*T. oleifolius*) and control (uninfected *B. albitrunca*) in the Botanic Garden.

4.10 Water potential of mistletoes and host plants at the Garden

Tapinanthus oleifolius had a significantly more negative Ψ of -38 kPa compared to *S. mellifera* with -25 kPa (Wilcoxon signed - rank test, $Z = -6.313$, p<0.001, Figure 4.19). *T. oleifolius* displayed a negative skewed Ψ, the bottom whisker was much longer than the top whisker indicating that more values were concentrated on the lower scale than on the upper scale top whisker is not visible because the upper

quartile is equal to the maximum observed Ψ value which is -3 kPa. For *S. mellifera* the median and the upper quartile is equal to maximum observed Ψ value which is -26 kPa.
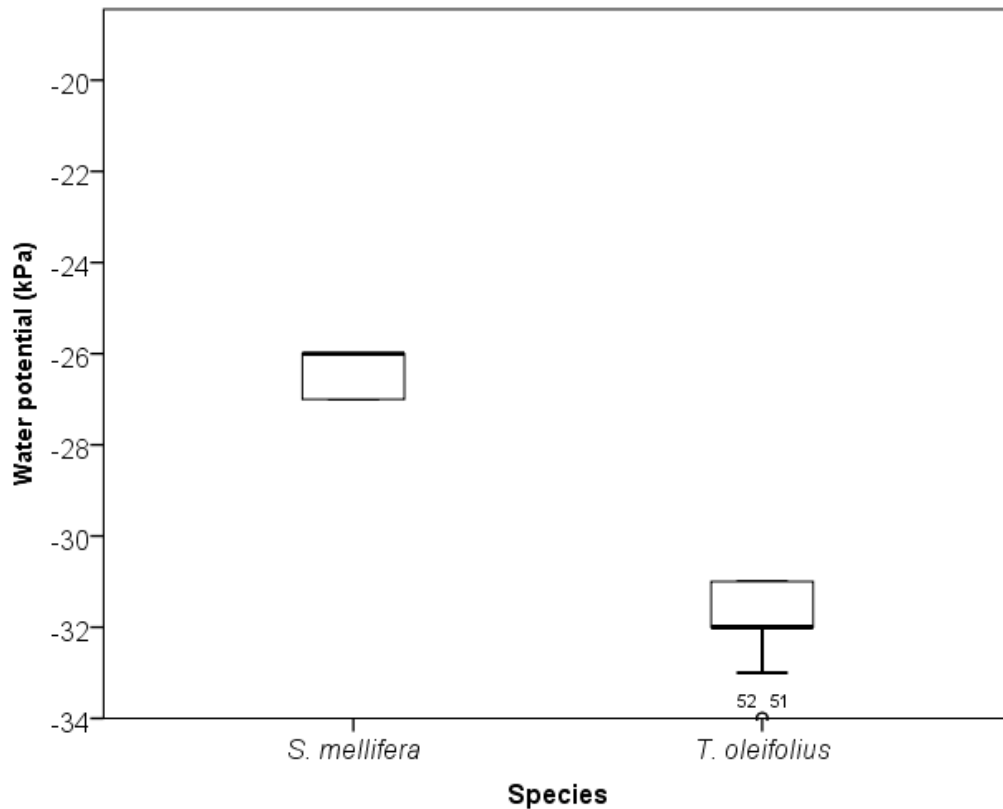
Figure 4.19 The median water potential (kPa) for the host (*S. mellifera*) and the parasite (*T. oleifolius*) in the Botanic Garden.

Infected *S. mellifera* had a significantly more negative Ψ of -27 kPa compared to uninfected *S. mellifera* with -25 kPa (Wilcoxon signed - rank test, Z = -6.508, p<0.001; Figure 4.20). For both species (Infected *S. mellifera* and uninfected *S. mellifera*) the upper and bottom whiskers are not visible indicating that the upper quartiles are equal to the maximum observed Ψ values (a = -26, b = -25) and the lower quartiles are equal to the minimum observed water potential values (a = -27, b

= -26), (where a represents infected *S. mellifera* and b represents uninfected *S. mellifera*).
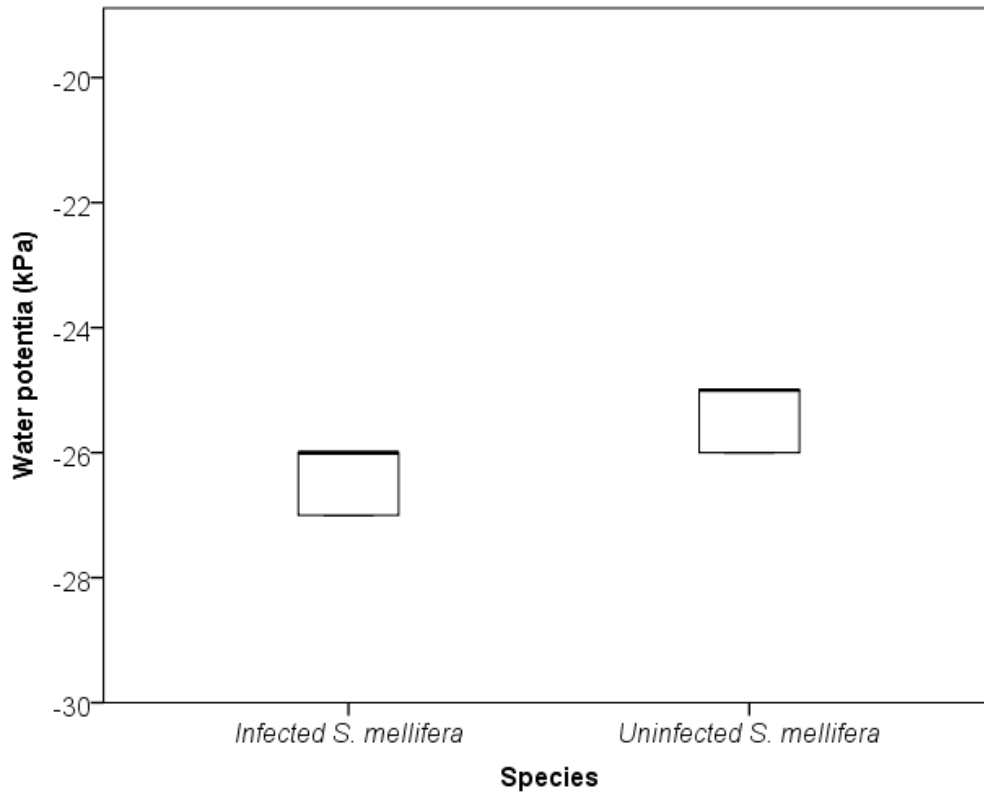
Figure 4.20 The median water potential (kPa) for the infected host (*S. mellifera*) and the control uninfected (*S. mellifera*) in the Botanic Garden.

4.11 Number of stomata on mistletoe - host pairs at the Garden

Tapinanthus oleifolius had a significantly high median number of stomata of 23 mm^{-2} compared to *S. mellifera* with 15 mm^{-2} (Wilcoxon signed - rank test, Z = -4.627, p<0.001; Figure 4.21). *S. mellifera* and *T. oleifolius* both displayed symmetry in the number of stomata which implies that the values are equally spread from the median to the lower and upper scale.
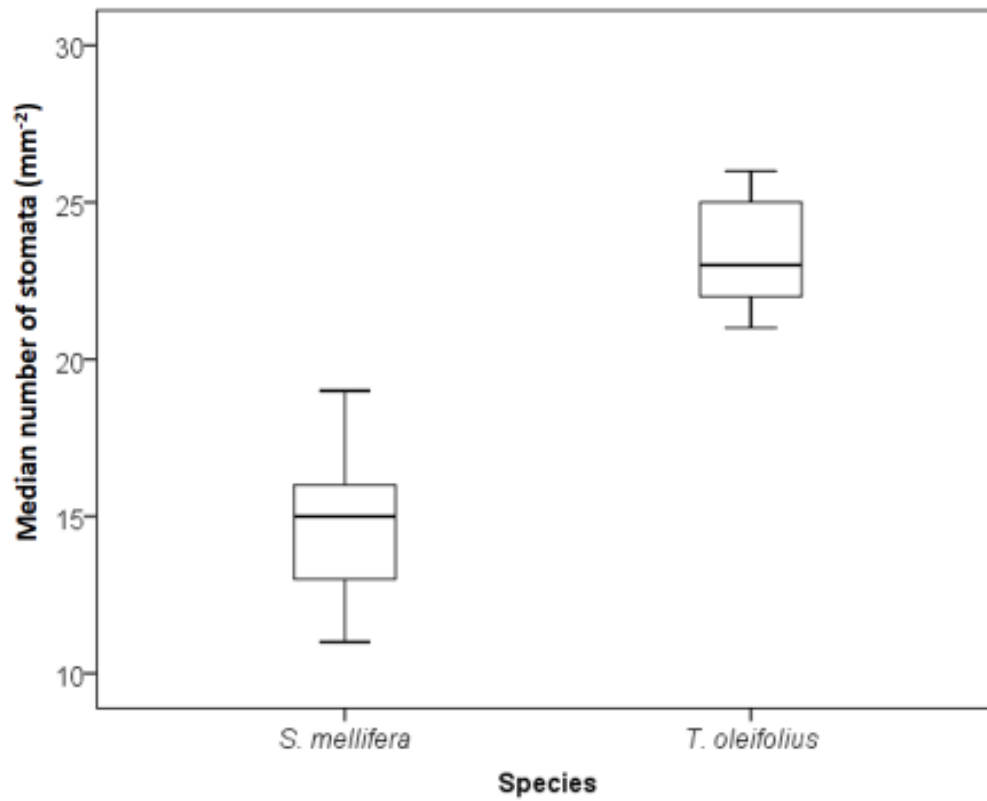
Figure 4.21 The median number of stomata (mm^{-2}) on the host *S. mellifera* and the

mistletoe *T. oleifolius* in the Botanic Garden.

CHAPTER 5. DISCUSSION

5.1 Prevalence and infectivity of mistletoes in the Botanic Garden and the Aloe Trail

The study showed a low prevalence and infectivity of mistletoes on trees at the Botanic Garden and a high prevalence at the Aloe Trail. The Botanic Garden is actively managed through conservation efforts and controlled by the NBRI staff. The conservation activities that take place in the Botanic Garden include removing the mistletoes form the host trees. The Trail is an open municipal area that is semi managed. It is not fenced off and it is open to the public at all times. It is highly disturbed due to human interferences such as littering. The plants grow freely in the wild and the area is used by the public as a recreation area for walks and picnics.

The Aloe Trail was more disturbed due to the people that visit it regularly for picnics and hiking; it was also observed to be very slopey, have a large quantity of dead wood, high number of thick bushes compared to the Botanic Garden. These conditions make it favorable for mistletoes for thrive well. This is supported by a study that was done by Mónica *et al.* (2013) to determine if disturbance determined prevalence of mistletoes. His results support the results of the current study. He pointed out that disturbed areas with slopes had high mistletoe prevalence and that there is an increament in the mistletoes abundance with increased disturbances and this is also the evident with the Aloe Trail as it is very slopey and highly disturbed.

5.2 Species association with mistletoes

Mistletoe infectivity is different between host tree species. The results from the study indicated that mistletoes were mainly associated with *Senegalia mellifera* and *S. erubescens*. Differences in infectivity between these host plants and other hosts could have resulted due to high mistletoe-host compatibility between the mistletoe and

these two species particular host (Roxburgh & Nicolson 2005). This compatibility is met because these species contain the specific chemicals that allow recognition between the mistletoe and the host and hence initiate haustoria penetration into the host's xylem (Fadini 2011).

The attractiveness of a tree to its disperser may be considered as an indicator of mistletoe infection. The behavior of seed dispersing seeds play a critical role (Overton 1994). Birds may prefer nesting and defecating more frequently on these species more than other species. The more they visit these species the more seeds they deposit leading to more infections (Messias *et al*. 2014; Roxburgh & Nicolson 2005).

Fadini (2011) pointed out that branch thickness affects contributes to haustorium development. These two species are characterized by their multi stems and bushy nature; they have many small branches that are not thin and this enhances haustorium penetration leading to infection. Watson (2001) pointed out that mistletoe survival depends on abilities of host plants to withstand stressful conditions. *Senegalia mellifera*, *S. erubescens* are drought-resistant species that are adapted to survive in dry conditions; this might be the reason why there is a strong association of *T. oleifolius*.

Senegalia erubescens and *S. mellifera* legumes; leguminous plants are known to accumulate little or no silica in their tissues. According to Yoshida *et al.* (1962) and Currie & Perry (2007) deposition of silica in plants reduce the plant susceptibility to enzymatic degradation and make them more prone to infection by parasites. Their low silica content increased their susceptibility to mistletoe infection hence explaining why they were mostly preferred by mistletoes (Fadini 2011).

Mistletoes show different degrees of host specificity, with generalists such as *T. oleifolius* which was found parasitizing a wide range of host trees including *Senegalia spp.* (Richter *et al.* 1995). According to Richter *et al.* (1995), *T. oleifolius* can grow on other trees and shrubs as an epiphyte, it is a host to birds since it is one of the few plants that flower in winter. The species is adapted to drier habitats, and because of these properties, it grows on many different hosts such as *D. cinerea* and *Senegalia spp.* Okubamichael *et al.* (2011) found that *V. rotundifolium* is least prevalent and very host specific of all *Viscum spp.* in southern Africa and it appears to be host specific in some locations. This is also evident from the results of this study. *V. rotundifolium* was found parasitizing only *B. albitrunca* and may be limited to one or a few hosts as reprted elsewhere (e.g. Roxburgh & Nicolson 2005).

5.3 The effect of host plant size on the abundance of mistletoes

Tree height and stem diameter can be used as a rough estimate of the relative size of trees (Aukema 2002). Host plant height has a considerable impact on the number of mistletoes that infect it. The results from this study showed that there is no linear relationship between tree host height and the number of mistletoe; as the host height increased, the number of mistletoes on the host tree decreased. Most studies indicated that; mistletoes normally grow on tall trees and the probability of encountering a parasitized tree increases with tree height (Roxburgh & Nicolson 2008). According to Aukema and del Rio (2002) and Okubamichael *et al.* (2011) taller trees are assumed to be nutrient and water-rich and because of these factors they are considered to have a higher number mistletoe infections; this is however not the same with this study.

As tree height increases the number of mistletoes decreases. The no linear relationship was established between tree height, diameter and number of mistletoes.

However, some studies also found no linear relationship between tree size and abundance of mistletoes (Overton 1994; Reid & Stafford-Smith 2000). Hence, variation in abundance of infections may be related to other factors such as availability of nutrients and water, and not host height.

Most of the trees that were encountered during data collection were thicket in form, shrubby and thorny with multiple stems. The shrubby and dense vegetation contributed to the large canopy diameter of the trees. The large canopy diameter gave the mistletoes enough space to grow and multiply and hence increasing their abundance. Canopy diameter might be a major contributing factor to mistletoe abundance than height and diameter (Lamont 1982).

5.4 Photosynthesis, chlorophyll and carotenoid content of mistletoe host plants

The study showed that hosts have a much higher photosynthetic rate than mistletoes. This results correlate with literature from a study by Strong *et al*. (2000) that also showed that photosynthesis in hosts are much higher than in mistletoes. Hosts saturate at higher electron transport rates to accelerate photosynthesis so that they will have enough photosynthetic products available for themselves after the mistletoes absorbs what they require.

Hosts saturate at higher light levels when host and mistletoe photosynthesis are compared at similar light levels; hosts are mostly exposed to sunlight and mistletoes are mostly shaded by the hosts canopy (Strong *et al*. 2000; Johnson & Choinski 1993). Because of its role in the light reactions, light is an essential factor that limits the rate of photosynthesis (Hopkins & Hüner 2008; Lambers *et al*. 2008). If a leaf absorbs insufficient light, there will not be enough ATP and NADPH to fuel the dark reactions. Mistletoes may be deprived of sufficient light to facilitate

photosynthesis and hence contribute to photosynthesize at low levels as opposed to hosts that are mostly exposed to full sunlight. The high light intensity that hosts are exposed to causes them to saturate at these high light intensities (Strong *et al.* 2000). Higher electron transport rates in hosts indicate a higher capacity for photosynthesis and may be the reason why hosts have higher photosynthetic rates than mistletoes (Strong *et al.* 2000; Johnson & Choinski 1993).

Even though low rates of CO_2 assimilation are normally associated with low rates of transpiration and stomatal conductance in the host, it is not clear if stomatal conductance is a source or a consequence of the observed effects on photosynthesis. Low rates of photosynthesis of mistletoes may be caused by the undifferentiated leaf mesophyll and the low number of plastids per mesophyll cell (Tuohy, Smith & Stewart 1986). According to Smith, Keys and Evans (1995), improved photorespiratory metabolism resulting from physical disruption of the bundle sheath cells may contribute to the lower rates of photosynthesis in mistletoes.

The results of this study showed that hosts had a higher chlorophyll and carotenoid content than mistletoes; this is supported by literature from Johnson and Choinski 1993; Seel *et al.* 1993. A study done on *T. vittatus* parasitizing *D. condylocarpon* also support the findings of this study by demonstrating that *T. vittatus* had a lower total chlorophyll content than its host based on a fresh weight basis (Strong *et al.* 2000). Blankenship (2010) indicated that carotenoids are essential for photo-protection and in many cases serve as key regulatory molecules. Overall, the current study showed that chlorophyll content is was not correlated to photosynthetic capacity and hence it could be that photosynthesis might be affected by other factors other than chlorophyll. These factors can be water or CO_2.

Shukla, Sharma and Shukla (2014) pointed out that N is essential for the formation of amino acids which are the building blocks of proteins and is essential for plant cell division. (Shukla *et al.* 2014). Evans (1989) pointed out that N is an essential component of chlorophyll and rubisco. And that N content is correlated to chlorophyll content. Studies done by Bannister (1989), Ehleringer and Schulze (1985) indicated that hosts had high N compared to mistletoes. They results can also be linked to the results of current study such that the high N content in hosts enable for more chlorophyll formation and concentration in the hosts.

With high carotenoid contents in hosts than in mistletoes; hosts are better protected from the effects of harmful photo-oxidative processes and from harmful toxic oxygen species formed within the chloroplast (Blankenship 2010). They are better protected by quenching triplet state chlorophyll molecules, their structural components of the photosynthetic antenna and reaction centre complexes are enhanced contributing to their high photosynthetic rates (Bartley & Scolnik 1995; Blankenship 2010). Bartley and Scolnik (1995) pointed out that in the absence of carotenoids, plants suffer severe photo-oxidative damage. This shows that carotenoids are important and play a crucial role in the development and survival of the plant. The implication of low carotenoid content for mistletoes is that they are face the risk of being exposed to harmful photo-oxidative processes that will negatively hinder they development and growth onto the host, ultimately leading to their death.

5.5 Transpiration of mistletoe and host plants

Transpiration is the evaporation of water from the surface of leaf cells in actively growing plants. Through transpiration water is lost from the plant in the form of water vapor. Water is absorbed by roots from the soil and transported as a

liquid to the leaves via xylem. In the leaves, small pores allow water to escape as vapor (Hopkins & Hüner 2008).

The study showed that mistletoes had a much higher transpiration rate than hosts. Most studies have concluded that the transpiration rate of mistletoes is higher than hosts, hosts have better water use efficiency than mistletoes (Johnson & Choinski 1993; Okubamichael *et al.* 2011; von Willert & Popp 1995). These water use efficiency mechanisms include; closing their stomata to limit the amount of water lost to the atmosphere. But von Willert and Popp (1995) further went on to say that transpiration is influenced by the availability of water, optimal soil moisture and other stress conditions. Johnson and Choinski (1993) pointed out that high transpiration rates prevent stomatal closure, the mistletoes might have the inability to control stomatal closure and as a result more water is lost through the stomata to the atmosphere. As the host tree is acting as a buffer between the parasite and its environment, there is less evolutionary pressure for mistletoes CO_2 assimilation to respond as strongly to environmental factors (Strong *et al.* 2000). This has caused hosts to be more adapted to the harsh environmental factors as opposed to mistletoes which would be vulnerable to these factors.

A study conducted by Schulze and Ehleringer (1984) indicated that, mistletoes have higher transpiration rates than their host. Stem parasites depend on the xylem sap as their sole source of N, which suggests that they have no connection to the host phloem. The high transpiration rates serve as a mechanism to gather N for growth of mistletoes (Schulze & Ehleringer 1984) - this was evident in this study.

5.6 Water potential and stomata of mistletoes and host plants

Mistletoes are deprived of the uptake of water and minerals of a normal plant root system and rely upon the haustorium for connection to the host for water and nutrients (Glatzel & Geils 2008). The study showed that mistletoes had a much lower Ψ than their hosts and this is supported by several studies (e.g. Glatzel & Geils 2008; von Willert & Popp 1995). The low Ψ in mistletoes are caused by low levels of abscisic acid which is the stress hormone that regulates water relations of the plants on the stomatal level and hence they might be facing the challenge of regulating water relations of the plants on the stomatal level (Popp 1987). The high abscisic acid levels in hosts are stimulated more because of strong water potential gradient that is maintained by the constant absorption of water from the host to the parasite

In mistletoes, the flow of nutrients through the transpiration stream is mostly one way, from the mistletoe to the host and never in the opposite direction (Türe *et al*. 2010). This one way movement of nutrients contributes to the high concentration of mobile nutrients which causes a low Ψ in mistletoes (Popp 1987; Türe *et al*. 2010). The main driving force for water movement is greater negative water potential. It is beneficial for mistletoes to maintain a low Ψ and a high transpiration rate than their host so that they can maintain the gradient in leaf Ψ forcing water to move toward areas with less water. This will enable the constant flow of water from the host to the mistletoe, which they do by keeping their stomata open (von Willert & Popp 1995).

The physiology of stomata has evolved as a compromise between the two contradicting functions of allowing CO_2 uptake during photosynthesis and limiting water loss during transpiration. The study showed that mistletoes have more stomata than their hosts. These results are supported by von Willert and Popp (1995) who

also found higher stomatal quantity on mistletoes than on their host plants. As a result of the high stomatal quantity in mistletoes, mistletoes lose a lot of water through transpiration. However, mistletoes have evolved mechanisms to control water loss (Perry 1995). These mechanisms include the closely packed stomata that counteract water loss by maximizing the uptake of gases because of the increased surface area (Perry 1995; von Willert & Popp 1995).

Mistletoes are mostly shaded by host plants. Young *et al*. (2004) pointed out that shaded plants are deprived of sunlight because all parts of the leaf are not lit with sunlight, when a tree does not get much light there is more stomata, so that the intake of CO_2 is enough to complete photosynthesis. Host plants are exposed to more light because most of the leaves are lit by sunlight and are able to bring in more CO_2 with less stomata. This results in a low stomatal density because they are more exposed to sunlight during the course of the day and are able to complete photosynthesis more often (Young *et al*. 2004).

CHAPTER 6. CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

The Aloe Trail had a high prevalence and infectivity of mistletoes than the Botanic Garden. This is attributed to the better conservation management measures such as the removal mistletoes in the Botanic Garden as opposed to the Aloe Trail. Mistletoes strongly preferred to infect *Senegalia mellifera* and *Senegalia erubescens* because of their strong compatibility with the mistletoes. There was no linear relationship between host plant size (height and diameter) and the abundance of mistletoes; variation in abundance of infections may be related to other factors such as canopy diameter availability of nutrients and water, and not host height. The photosynthetic measurements made on mistletoe host pairs suggest that hosts have greater capacity to assimilate CO_2 and hence greater photosynthetic capabilities than mistletoes. The high chlorophyll content in hosts than mistletoes could imply that hosts have greater amounts of N allowing for more chlorophyll formation. The high carotenoid content in hosts could imply that hosts are enhanced in terms of photo protection from harmful photo oxidative processes and from harmful toxic oxygen species formed within the chloroplast. The greatest resource problem for a tree is the loss of water to mistletoes; the mistletoes had a high Ψ than hosts and this is because hosts make use of water efficiency mechanisms to conserve water.

6.2 RECOMMENDATIONS

Further studies are needed to compare mistletoe host interactions between different mistletoe host pair species apart from *S. mellifera*, *T. oleifolius* and *B. albitrunca - V. rotundifolium* that were investigated during this study.

Since the current study was carried out in the highland savanna in the Khomas Region, the findings cannot be generalised as being applicable to other savannas in Namibia hence, it is recommended that a similar study be done to collect data from the different types of savannas in the country.

I recommend that a study must be carried out over a period of years targeting the most dominant plant species in an area to determine whether mistletoes contribute to the mortality of those specific plant species and also to monitor other effects that mistletoes might have on the plant species and on that specific environment.

I recommend that a study be done to find out the specific traits that host plants possess that make them more vulnerable to mistletoe infection. So as to understand the reasons and factors that expose hosts to mistletoe infection.

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Appendix 1: List of woody species encountered at the Botanic Garden and at the

Aloe Trail. A √ represents presence and x represents absence.

Host species	Botanic Garden	Aloe Trail
Albizia anthelmintica	√	√
Boscia albitrunca	√	√
Carchonanthus camphoratus	√	x
Catophractus alexandrii	√	√
Combretum apiculatum	√	x
Commiphora glandulosa	√	x
Dichrostachys cinerea	√	√
Dombeya rotundifolia	√	x
Ehretia alba	√	√
Elephantorrhiza sufruticosa	√	√
Euclea undulanta	√	x
Grewia flava	√	√
Grewia flavescens	√	√
Lycium boscifolium	√	x
Lycium iini	√	√
Malnuleopsis dinteri	√	x
Montinia caryophyllacea	√	√
Searsia lancea	√	x
Senegalia erubescens	√	√

Senegalia hereroensis	√	√
Senegalia mellifera	√	√
Vachellia reficiens	√	√
Ziziphus mucronata	√	√

Appendix 2. List of Symbols

Ψ = Water potential

Ψ_{Π} = Osmotic potential

Ψm = Matrix potential

$\Psi\rho$ = Hydrostatic pressure

Ψg = Gravitational potential