Mistletoe effects on acacia species in western Saudi Arabia

Dhafer Albakre

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School of Biological Sciences
The University of Adelaide
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Table of Contents

Declaration ......................................................................................................................................................... 3

Acknowledgments .............................................................................................................................................. 4

Abstract .............................................................................................................................................................. 7

Chapter 1: General introduction ..................................................................................................................... 11
  Identification of mistletoes ............................................................................................................. 13
  Life history .................................................................................................................................... 13
  The effects of mistletoes on their hosts ........................................................................................... 14
  Physiological effects ...................................................................................................................... 17
  Effects on individuals ..................................................................................................................... 20
  Effects on populations and communities ......................................................................................... 21
  Research motivation and context .................................................................................................... 23
  Research questions ......................................................................................................................... 26
  Thesis structure .............................................................................................................................. 28
  References ..................................................................................................................................... 29

Statement of Authorship ................................................................................................................................... 34

Chapter 2: Incidence and abundance of Mistletoes as a function of altitude and host characteristics in desert landscapes in the western Arabian Peninsula.................................................................................................. 35
  Introduction ................................................................................................................................... 37
  Results ........................................................................................................................................... 44
  Discussion ...................................................................................................................................... 47
  Conclusion ..................................................................................................................................... 52
  References ..................................................................................................................................... 54
  Tables ............................................................................................................................................ 56
  Figures ........................................................................................................................................... 60

Statement of Authorship ................................................................................................................................... 72

Chapter 3: The effect of mistletoes on the nutrient status of their hosts: investigating several patterns of infestation in the western Arabian Peninsula .................................................................................................. 73
  Abstract ......................................................................................................................................... 73
  Introduction ................................................................................................................................... 75
  Statistical analysis .......................................................................................................................... 80
  Results ........................................................................................................................................... 82
  Discussion ...................................................................................................................................... 86
  Conclusion ..................................................................................................................................... 90
  References ..................................................................................................................................... 91
  Table .............................................................................................................................................. 93
  Figures ........................................................................................................................................... 94
### Statement of Authorship

- **Chapter 4: Host–mistletoe interactions on a branch scale: Investigating the nutritional relationship under coexistence of three mistletoe species on a single host tree, *Vachellia gerrardii*, in the western Arabian Peninsula**
  - Abstract ................................................................. 104
  - Introduction .................................................................. 105
  - Results .......................................................................... 111
  - Conclusion ...................................................................... 116
  - References .................................................................... 117
  - Table ............................................................................. 119
  - Figures ........................................................................... 120

- **Chapter 5: Effects of the mistletoe *Plicosepalus curviflorus* on the water status and photosynthetic performance of four host species in the western Arabian Peninsula**
  - Summary ................................................................. 125
  - Introduction .................................................................. 126
  - Materials and Methods .................................................. 128
  - Results .......................................................................... 131
  - Discussion ...................................................................... 136
  - References .................................................................... 140
  - Tables ........................................................................... 143
  - Figures ........................................................................... 148
  - Appendix ...................................................................... 154

- **Chapter 6: Heavy mistletoe infection increases water and light stress in *Vachellia gerrardii* in dryland regions of the western Arabian Peninsula**
  - Summary ................................................................. 156
  - Introduction .................................................................. 158
  - Results .......................................................................... 163
  - Discussion ...................................................................... 166
  - References .................................................................... 171
  - Tables ........................................................................... 173
  - Figures: ........................................................................... 175
  - Appendix ...................................................................... 179

- **Chapter 7: Conclusions**
  - Integration of findings .................................................... 186
  - Conclusion and future directions ......................................... 190
  - References .................................................................... 194
Declaration

I, Dhafer Albakre, certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Abstract

Mistletoes are rootless stem-parasitic plants that have various effects on their hosts. Although overabundance of mistletoes has become an ecological concern in the western Arabian Peninsula, the reasons for this as well as the consequences are unknown. In addition, the mechanisms underlying different effects of mistletoes on nutrient and water status of the host remain underexamined. Therefore, it is essential to determine whether abiotic factors such as drought, topographic variation, and host characteristics are increasing mistletoe prevalence, and what factors control the effects they have on their hosts. This study undertook a series of ecological and physiological assessments to contribute to answering those questions.

I assumed that the incidence of *Plicosepalus curviflorus* in Taif National Park, Saudi Arabia, is influenced by the characteristics of the four host species growing in various topographic positions. However, I found no differences in the host characteristics between infected and uninfected plants in three host species (*Vachellia flava*, *Vachellia gerrardii* and *Vachellia tortilis*). Only in the case of *Senegalia asak*, did the infected hosts have wider canopy areas than the uninfected plants. I suggest that *P. curviflorus* persists only on larger, more vigorous *S. asak* hosts. In Wadi Alshafa, I assessed the influence of altitude and host canopy volume on the prevalence (incidence/abundance) of three species of mistletoe coexisting on a single host species (*V. gerrardii*). The incidence and abundance of *Phragmanthera austroarabica* increased both with altitude and host canopy volume, while those of *Viscum schimperi* increased with altitude only, and those of *P. curviflorus* decreased with altitude and increased with host canopy volume. Moreover, the mistletoe species seemed to affect each other’s presence. *P. curviflorus* and *V. schimperi* appeared to compete, whereas *V. schimperi* appeared to facilitate the incidence and abundance of *P. austroarabica*. 
This suggests that biotic interactions among mistletoes could also affect their occurrence. Generally, the pattern of mistletoe species infection may be largely explained by different physiological responses to thermal stress in the summer season and water availability due to altitudinal effects.

I investigated whether the presence of *P. curviflorus* affected the mineral nutrition status of host species growing on different terrains by analysing leaf samples of infected and uninfected branches of parasitised and unparasitised hosts. There was a reduction in N and Mn concentrations in parasitised *S. asak*, and of P and Zn in parasitised *V. flava*, while no differences in nutrients were found in *V. tortilis*. Differences in nutrient status are more likely influenced by the host distribution in different terrains, and availability of nutrients in soil. In Wadi Alshafa, I examined the influence of different levels of infection on the nutrient status of the host. Compared with non-host trees and slightly infected hosts, the Ca and Mg concentrations were higher in hosts heavily infected with *V. schimperi*, while N, P, Na and Ca concentrations were higher in hosts heavily infected with *P. austroarabica*, and P and Na concentrations were higher in hosts heavily infected with *P. curviflorus*. Regardless of mistletoe species, my results suggest that high mistletoe load on the host reflects a response of the parasite to high nutrient availability in larger rather than smaller hosts, as the host characteristics are significantly different in the wadi system.

I examined whether three species of mistletoe coexisting on the same host and their respective infected branches differ in nutrient status. There was a significant difference between mistletoe species and their effects on distal branches of the host. Overall, K concentration was higher in mistletoe species than in the host branches. *P. austroarabica* showed higher levels of N and Na compared with *V. schimperi* and *P. curviflorus*; additionally, *P. austroarabica* accumulated
significantly more N and P than its respective host branches. This can be interpreted as a result of functional variation of morphological traits of the mistletoes, such as of the photosynthetic organ, reflecting their different requirements in nutrient acquisition.

In Taif National Park, I investigated the effect of *P. curviflorus* on water status and photosynthetic performance of four acacia species, *S. asak*, *V. flava*, *V. gerrardii*, and *V. tortilis*. The light-use efficiency of leaves and water status of twigs were assessed. Predawn and midday quantum yields, and water potential were assessed during the dry and wet periods. I compared mistletoe performance regarding their effect on distal branches of host species during dry and wet periods; in addition, I compared physiological performance of infected and uninfected branches of hosts and non-hosts. Mistletoes performed differently in the two periods on shrub-like *S. asak*, *V. flava*, and *V. tortilis*, which had lower predawn quantum yields and greater negative predawn water potentials in the dry season compared to the wet period; but mistletoe performance did not vary between periods on *V. gerrardii*. This is possibly because *V. gerrardii* grows in lower areas where more water may be available and is likely to have deeper roots to access reliable water in dry periods. Mistletoe infection increased the predawn water stress of infected branches of *S. asak*, *V. flava* and *V. tortilis* compared with uninfected branches or non-hosts during the dry period.

Interestingly, mistletoes had a greater $F_v/F_m$ ratio in the wet season and showed a negative impact on the infected branches of *S. asak*, which were water stressed, resulting in chronic photoinhibition even in the wet period. This could be due to the constant water demand of *P. curviflorus* to avoid host exclusion in the drier slope environment. Three different species of mistletoe infect a single host, *V. gerrardii*, in Wadi Alshafa. I examined water status and light utilisation at predawn and midday, and whether they were affected by different levels of infection (low, medium, heavy). Heavy mistletoe infection increased predawn water stress, and thus chronic photoinhibition,
compared with moderately and slightly infected hosts. The branches infected by *P. austroarabica* had a higher level of water stress than did the respective uninfected branch, but those branches infected by the other two species did not differ with heavy infection. On the mistletoe species level, loranthaceous mistletoes *P. austroarabica* and *P. curviflorus* had more negative predawn water potentials and a higher photosynthetic performance at daylight than *V. schimperi*. The disparity in mistletoe effects and their performance were most likely due to different levels of physiological stress in response to differences in temperature and moisture conditions in the Wadi.

This research reveals the crucial role of abiotic factors which drive the effects of mistletoes on hosts in two different ecosystems. My ecological and physiological data contribute new knowledge about potential biotic interactions between *V. schimperi*, *P. austroarabica*, and *P. curviflorus* in Wadi Alshafa. Results from this study can improve the scientific basis for management and control of mistletoe infection in natural drylands. Individual trees infected by high abundances of mistletoes, in particular, those trees infected by *P. austroarabica*, were suffering the most from physiological stress; therefore, the appropriate practice may be to remove mistletoe by pruning the infected branches. This practice can slow the spread of infection and maximise the growth rates of the trees as a short-term solution. For large-scale management, further investigations are essential to address the underlying biotic and abiotic causes in order to establish a longer-term solution.
Chapter 1: General introduction

There are approximately 4000 species of parasitic plants throughout the world and they are abundant in several habitats, particularly in systems where soil resources are limited. When abundant, they significantly influence the structure and function of plant communities. Even though the parasites have negative effects on their hosts, some can contribute to an increase in biodiversity of their communities. In particular, epiphytic parasites, such as mistletoes, often attract insects, mammals and about 66 families of birds as they provide food as fruits, flowers and foliage, and thus contribute to the biodiversity of their communities (Press & Phoenix, 2005). Parasitic plants vary greatly in form and function, and they can infect trees, shrubs, vines, or herbaceous plants. Some parasites can attach to the roots of their hosts, while others attach to the hosts’ branches. Some are incapable of photosynthesis (holoparasites) and derive all their nutrition from their hosts, while those that can photosynthesize (called hemiparasites), obtain mainly water and mineral nutrients from their hosts. It must be noted that this classification is somewhat arbitrary as there are intermediate strategies. Parasites affect many aspects of their hosts’ physiology such as water status, gas exchange, and mineral nutrient levels (Bell & Adams, 2011).

The abundance of parasitic plants may depend on a number of factors that contribute to seed dispersal, such as behaviour of birds (Del Rio, Silva, Medel, & Hourdequin., 1996; Aukema, 2003), topographic factors (Aukema, 2004), and host branch size (Sargent, 1995). Parasitic plants may differentially establish on several species of host, or they may have a preferred host because of co-evolutionary histories (Okubamichael, Griffiths, & Ward, 2016).
Parasitic plants attach to their hosts with a special organ called the haustorium (Yoshida, Cui, Ichihashi, & Shirasu, 2016). This structure extracts water and nutrients from the host tissues (Fig.1.1), and causes harm (Gill & Hawksworth, 2002). Mistletoe can cause negative effects on the host, which range from mild to severe reduction in growth (Ward, 2005; Shen, Prider, Facelli, & Watling, 2010) to, in extreme cases, death of the host (Watling, Facelli, & Prider, 2008). Overall, the symptoms of mistletoe infection are diminishing vitality, poor yields, diseases and premature death; and these effects depend on the hosts’ longevity and the intensity of parasitism (Gill & Hawksworth, 2002). These negative effects of parasitic plants are fundamentally due to their effects on the physiology of the host. For example, they reduce the water availability in the host, causing stomatal closure. As a result, diffusion of carbon dioxide is restricted and causes a reduction of photosynthesis in the host and eventually a reduction in growth (Watling & Press, 2001). At the community level, parasitic plants can reduce the abundance of their hosts, which allows the expansion of non-host species, thus changing vegetation structure (Press & Phoenix, 2005).

Besides their negative effects, mistletoes play an important positive role in supporting wildlife (Watson, 2001). They supply food and nesting sites for birds, support pollinators and many beneficial insects, and enhance nutrient circulation through the pattern of patchiness in their surroundings (March & Watson, 2010). For example, the beneficial effect of mistletoe was extensively reported for Eucalyptus trees in moderate and semi-arid environments in Australia, and African acacia trees in a savannah environment, where it was concluded that mistletoe litter increases N concentration in the soil underneath the host canopy, boosting productivity in the plant community as well as ecosystem functioning (Watson, McGregor, & Spooner, 2011; Muvengwi, Ndagurwa, & Nyenda, 2015).
Identification of mistletoes

Mistletoes are mostly flowering perennial plants in the Viscaceae and Loranthaceae families. They commonly grow on their host branches, constituting a particular structure that appears to be a leafy bushy shoot, which is popularly known as the "witches’ broom" (Kuijt, 1969). Many mistletoes are obligate hemi-parasites (Nickrent & Musselman, 2004; Mathiasen, Nickrent, Shaw, & Watson, 2008) since they have chlorophyll and thus are capable of photosynthesis. Holo-parasitic mistletoes, on the other hand, have no chlorophyll and completely depend on their hosts for carbohydrates, water and nutrients (Cullings & Hanely, 2010; Peh, Corlett, & Bergeron, 2015).

Life history

The effects of mistletoe infestation on the host depend on the abundance of mistletoes, which is influenced by many factors. The most important factor is often bird activity, which determines the seed dispersal of mistletoes, and the type, distribution and the abundance of potential hosts. In addition, abiotic factors that determine the resources available for the host significantly influence the performance and survival of mistletoe seeds once deposited on a branch (Aukema, 2003). The germination of mistletoe seeds is also influenced by the characteristics of the host (e.g. tree size, and height of tree, bark thickness) (Aukema & Del Rio, 2002).
For example, Roxburgh and Nicolson (2008) observed that tall trees of *Acacia sieberiana* are more susceptible to infection by mistletoe, because tall trees can attract more birds acting as dispersers, thus increasing the intensity of infection. The host distribution in the landscape is defined by the spatial patterns that attract birds, which contributes to seed dispersal. Thus, trees neighbouring infected ones are more likely to host mistletoes (Lavorel, Stafford Smith, & Reid, 1999). In African savannah, topographic factors affect the availability of water and nutrients for the hosts, thereby influencing the abundance of mistletoes (Griffiths, Ruiz, & Ward, 2017). Further, clusters of acacia plants in the water-abundant basin of Hadramaut in Yemen support higher densities of mistletoes (Donohue, 1995). There is another interesting aspect to the distribution and abundance of mistletoes, when two or more mistletoe species coexist on the same host and appear as patches on the tree (Fig. 1.2). This phenomenon is called multi-parasitism (Thriven, Shivamurthy, Amruthesh, Vijay, & Kavitha, 2010).

(Fig. 1.2) Multi-parasitism (*P. austroarabica* & *P. curviflorus*) on *V. gerrardii* in the western region of Saudi Arabia.

The effects of mistletoes on their hosts

Mistletoes can cause detrimental effects, ranging from negligible (Ward, 2005) to severe (Reid, Smith, & Venables, 1992). An example of a negligible effect was reported by Ward (2005), who
showed that while a high load of mistletoe resulted in statistically less foliage in their hosts, the difference between healthy and infected hosts was slight. Importantly, this study did not reveal any relationship between mistletoe load and dieback. On the other hand, severe symptoms can lead to the death of the host. The best examples of this are comprehensive studies of *Amyema preissii* in the arid zone of Australia, where the occurrence of mistletoe was reported to be extremely harmful for *Acacia victoriae*. Reid et al. (1992) monitored *A. preissii* infection for four years from 1985 to 1989 and showed that there was indeed a correlation between mistletoe size and the mortality of *A. victoriae*. Eventually, this infection was conducive to increased mortality in the host.

Generally, parasitic infection tends to reduce host growth and seldom kills the hosts, except under certain conditions (Bell & Adams, 2011). Mortality often happens when the host plants suffer from other sources of stress such as from abiotic factors (salinity, water stress) or biotic factors (human activities, insects) that lead to a reduction of healthy trees, and increased dieback (Landsberg & Wylie, 1988).

Saline soil affects the host’s water-status, leading to water stress. Miller, Watling, Overton, and Sinclair (2003) showed that the decline of predawn water potential in the infected hosts and signs of chronic stress, as measured by leaf $\delta^{13}$C, resulted in stressed hosts that were unsuitable for mistletoes. Furthermore, in the Mojave Desert, heavier mistletoe load triggered high mortality of the desert tree *Cercidium floridum* during episodic extreme drought (Spurrier & Smith, 2007).

Host plants can be threatened by human activities that in turn increase the incidence of mistletoe infection. For example, Donohue (1995) demonstrated that logging and overgrazing led to changes in the spatial distribution of *V. tortilis* in Wadi Hadramaut in Yemen. These stresses in turn increased infection on the host branches, thus accumulating mistletoe in the higher branches.
of large acacia trees, leading to deterioration of the vegetation. Moreover, the host plants can be injured by insects that have an association with mistletoes, increasing stress on the hosts. This happens with dwarf mistletoes, which attract bark beetles that bore into branches of the host, leading to mortality (Geils & Hawksworth, 2002).

Some parasitic plants infect a wide range of host species and can be classed as “generalists”, while others tend to infect exclusively certain hosts and act as “specialists” (Press & Phoenix, 2005). Even though the root hemiparasite *Rhinanthus minor* infects approximately 34 different host species, it still favours a small number of them, preferring plants in the Poaceae and Fabaceae families. Further, *R. minor* preferentially infects dominant hosts in the plant community. Another important factor is the ability of the host to supply nutrients, which determines that this parasite also preferentially infects leguminous plants even though they are less abundant than grasses. Because leguminous plants are capable of nitrogen fixation in soil, especially in N-deficient soils (Munzbergova & Ward, 2002), the nutrient value in this host attracts parasitic infestation (Dean, Midgley, & Stock, 1994).

Further variation in incidence of parasites between species results from the resistance of the host against the parasitic infection (Cameron, Coats, & Seel, 2006). Some studies have been conducted to explain host resistance. For example, forb species conserve their nutrients against the invasion of *R. minor*, because the forbs can suppress the penetration of the parasite into their tissues, which reduces the supply of nutrients to the parasite. As a result, the parasite may show weak growth compared with its growth on grass and legume species (Cameron & Seel, 2007).

Occasionally, parasite selectivity depends on its ability to penetrate the tissues of the host. *Cuscuta* species (dodder) are stem holoparasites that have a wide range of hosts, but still show a preference for some hosts (Kelly, Venable, & Zimmerer, 1988).
The rich nutritional status of the host enhances the ability of *Cuscuta* to proliferate on a preferred host (Kelly, 1992). Moreover, Runyon, Mescher, and De Moraes (2006) showed that *Cuscuta* seedlings grow towards tomato plants, which provide better nutrition, due to the volatile chemical cues produced by tomatoes, and thus the parasite is capable of finding a preferred host among potential hosts in a plant community.

**Physiological effects**

Primarily, all plant parasites penetrate the host’s tissues by their haustoria in order to obtain water and resources, causing a disruption of branch growth rather than killing their hosts (Press & Phoenix, 2005).

**The effect of mistletoes on the water status of hosts**

Mistletoes maintain their water potentials below that of the host, absorbing water from it, and often causing water stress in the host. This is evident in several symptoms such as lower water potential, increased concentration of abscisic acid (ABA), and eventually increment root: shoot ratios (Press, Scholes, & Barker, 1999). The reduced water potential in the host results first in stomatal closure that leads to reduced carbon assimilation, and therefore reduction of water use efficiency (Ehleringer, Cook, & Tieszen, 1986; Goldstein et al., 1989; Glatzel & Geils, 2008; Okubamichael et al., 2011). Ultimately, the whole host plant suffers from water deficiency, and in order to offset its water loss, it allocates greater growth to the roots in order to obtain water, rather than to its trunk, branches and leaves to intercept light (Chaves et al., 2002).
The effect of mistletoe on photosynthesis of the host

Few studies have addressed mistletoe effects on photosynthesis with respect to infected and uninfected plants, and most studies have compared the photosynthetic systems of mistletoes and hosts. Lüttge et al. (1998) and Burritt, Strong, and Bannister (2000) concluded that mistletoes often have a lower or equal photosynthetic capacity compared with their hosts. The ability of the plant to use carbon dioxide and light for photosynthesis depends on stomatal opening. Since parasitic plants affect the water potential of the host, which in turn triggers stomatal closure, this limits the diffusion of carbon dioxide into the parenchyma of the leaves, and therefore reduces the photosynthesis rate and water use efficiency of host leaves. Eventually, this diminishes host growth (Lambers, Chapin, & Pons, 2008; Shen et al., 2010). Further, stomatal closure restricts carbon dioxide from entering the leaves of the infected host (Goldstein et al., 1989), hence the Calvin cycle cannot turn, and the light energy transfer becomes interrupted. In this case, the excess light and the interruption of the Calvin cycle would in turn lead to photodamage in the leaves of infected hosts (Watling et al., 2008).

The response of plants to full sunlight depends on the concentration of pigments such as xanthophyll that can dissipate the excess light energy, therefore preventing photoinhibition (Demmig-Adams & Adams III, 1992). In infected hosts, light stress creates disturbance in the functioning of pigments that results from the high excitation, leading to tissue bleaching and cell death. For example, the root hemi-parasite Striga asiatica commonly infects many crops, particularly maize. Striga infection causes photodamage of infected hosts, and a consequent reduction of photosynthetic capacity (Watling & Press, 2001). In the stem hemi-parasite Cassytha pubescens, Shen et al. (2010) found that infection minimised the photosynthetic rate of the host (Cytisus scoparius). As a result of limited assimilation of carbon, which led to lower quantum yields, the infected host was more susceptible to photoinhibition, and suffered higher mortality.
Cirocco, Facelli, and Watling (2015) also studied *C. pubescens* and found that parasite infection negatively influenced midday quantum yield of the infected plant, but not pre-dawn maximum quantum yields.

Although photoinhibition is considered as a main component of the damage to photosystem (Björkman & Powles, 1984), it can be more pronounced when the plant experiences water stress in the field.

The effect of mistletoe on nitrogen content of the host

Mistletoes, not having true roots to obtain water and nutrients from the soil, directly depend on host plants for their mineral nutrition, including nitrogen (Ehleringer et al., 1986; Daryaei & Moghadam, 2012). Consequently, they act as a nitrogen drain on the host plants, which often suffer from nitrogen deficiency. Because nitrogen in the plants correlates to the nitrogen availability in soil, the negative impact of mistletoes may be more significant in nitrogen-poor areas (Hedberg, Borowicz, & Armstrong, 2005). The high transpiration rates of mistletoes facilitate importation of nitrogen from the host (Goldstein et al., 1989). As a result, the host suffers from nitrogen stress, often reflected in lower photosynthetic rates, which in turn diminishes the growth of the host.

Some researchers (Ehleringer et al., 1986; Daryaei & Moghadam, 2012) reported that mistletoe infestation causes a reduction in nitrogen concentration of the infected branches of the host. Ehleringer et al. (1986) demonstrated that the infected branches of *Juniperus osteosperma* that are affected by mistletoe *Phoradendron juniperinum* have low concentrations of nitrogen compared with that in uninfected branches. Eventually, the nitrogen decrease in the infected branches leads to disruption of the Calvin cycle, and thus suppression of photosynthesis in the leaves of distal branches, resulting in reduction of the growth, reproduction and survival of infected branches.
Effects on individuals

Survival of plants

The survival of plants is affected by numerous factors such as drought, insects, pathogens and parasites. These factors lead to a reduction in the supply of water and nutrients for plants, reducing their survival rates (Dowell, Gibbins, Rhoads, & Pallardy, 2009). In the case of mistletoe infection, the size of the host and degree of infection affect the survival rate of plants (Hawksworth & Geils, 1990). Hawksworth and Geils (1990) demonstrated that survivorship of Pinus ponderosa is affected by Arceuthobium vagintum. Over 90% of lightly infected hosts with stems 22.5 cm diameter at breast height survived for roughly 32 years, but in the case of heavy infection (over 22.5 cm dbh), only 5% of hosts were still alive at the end of this period. In temperate Australia, the mistletoe Amyema miquelii attacks two species of Eucalyptus, E. blakelyi and E. melliodora. Reid, Yan, and Fittler (1994) demonstrated that survival rate of the host was increased by mistletoe removal: at about 33 months, all disinfected hosts remained alive, while several uninfected hosts died.

Growth

By removing water and nutrition, mistletoes can inhibit the growth of the host. The negative effects of dwarf mistletoes on host morphology may include changing the host’s architecture via their swelling structures commonly known as the “witches’ broom” (Tinnin & Knutson, 1980). Also, severe infection by dwarf mistletoes leads to the demise of host branches (Filip, Colbert, Shaw III, Hessburg, & Hosman, 1993; Mathiasen, Edminster, & Hawksworth, 1990), resulting in
canopy loss and a lower chance of host survival (Tennakoon & Pate, 1996). In the south west of Australia, Tennakoon and Pate (1996) found that the mistletoe *A. preissii* negatively affected the growth of *Acacia acuminata*. This mistletoe sucked water and nutrition from the hosts’ vascular bundles in the haustorium area, resulting in a decrease in leaf area and diminished inner tissues of the distal branch compared with the tissues of the proximal branch. This situation caused the death of the distal branch. Invasion of mistletoe also leads to a reduction in foliage area, causing diminished photosynthesis. For example, *Viscum album* reduced the growth in two host species in Iran, where the mean foliage area in infected branches was much lower than uninfected branches of the host and other branches in healthy trees (Daryaei & Moghadam, 2012).

**Reproduction**

The negative impact of mistletoes may extend beyond reduced host growth to reduced reproduction (Silva & del Rio, 1996). The holoparasitic mistletoe *Tristerix aphyllus* leads to a decrease in yields of *Echinopsis chilensis*. Because of intense mistletoe infection, hosts produce less buds, flowers, and fruits (Silva & del Rio, 1996). In the desert of Israel, Ward, Shrestha, and Musli (2006) reported that *Ziziphus spina-christi* trees heavily infected by *Plicosepalus acacia* produced fewer fruits than healthy trees.

**Effects on populations and communities**

Relationships between mistletoes and their hosts have been extensively documented for pine trees in North America, *Eucalyptus* trees in Australia and African acacias species in semi-arid savannah in Zimbabwe. Numerous studies reported that mistletoes increased nutrient concentration in the soil underneath the host canopy, thus enhancing ecosystem function and accelerating the productivity of the plant community (Watson et al., 2011; Mvengwi et al., 2015).
A positive relationship is an indirect mistletoe effect, where mistletoes quickly diminish host growth, and hence the strong growth of mistletoes enhances nutrient return. In most cases the positive effects outweigh the negative effects, so the nutrient cycle can somewhat offset the negative impacts on host growth. However, a reciprocal relationship may be unstable and subject to variation in environmental factors, so these negative/positive effects are dependent on where mistletoes are found (Spasojevic & Suding, 2011).

As a consequence of the direct effect of parasitic plants on an individual host, parasitic plants also affect the plant populations. By removing water and nutrients from preferred hosts, parasitic plants modulate competition between their hosts and non-hosts for resources (Press, Scholes, & Barker, 1999). Some studies have asserted that *Salicorina virginica* is a preferred host for *Cuscuta salina* in a salt marsh community east of Santa Barbara, Calif., USA (Callaway & Pennings, 1998). As a result, the individuals of *S. virginca* are diminished by parasite infection, thus reducing the competitive effects on *Arthrocnemon subterminale*, allowing it to become more dominant in salt marshes (Callaway & Pennings, 1998). Similarly, it has been documented that *Rhinanthus* invasion decreased the abundance of a grass host, allowing non-host forb species to become more abundant (Press & Phoenix, 2005).

Parasitic plants can have negative effects on biotic relationships with mycorrhizal fungi and nitrogen fixing bacteria (Press et al., 1999). Parasitic plants play an important role in changing mutualistic relationships between their host and other organisms. Mycorrhizal fungi enhance nutrient availability to hosts, while the host provides photosynthetic output to mycorrhizae (Smith & Read, 2008). In the case of infected hosts, mycorrhizal fungi are also indirectly influenced. These organisms compete with parasitic plants for available carbon from the host plant.
For example, Gehring and Whitham (1992) suggested that a decrease in mycorrhizal fungi in infected junipers might result from competition between mistletoe (*P. juniperinum*) and mycorrhizal fungi for carbon.

Parasitic plants may also affect nitrogen fixation of bacteria in the nodules of the host. Because of suppression of photosynthesis in the host, nitrogen-fixing bacteria are not able to obtain an adequate supply of carbon from their host, and nitrogen fixation is reduced. For example, *Cuscuta reflexa*, a stem holoparasite, has a negative impact on its host, *Lupinus albus*, and therefore diminishes the supply of carbon, which causes a disruption in nitrogen-fixation by bacteria in nodules (Jeshke, 1994).

**Research motivation and context**

While there are already many studies about the effects of mistletoe infection, they often report inconsistent results.

As an indirect effect, mistletoe litterfall is advantageous as it enhances the nutrient cycle underneath host trees in many ecosystems. When mistletoe grows more aggressively on the host, it has a faster uptake of water and nutrients and more frequent leaf turnover, creating more litterfall (March & Watson, 2007; 2010). However, this process can be affected by mistletoe lifespan and leaf morphology. In nutrient-poor environments, desert mistletoes have small leaves or are leafless. Therefore, the opportunity for nutrient return is likely to be negligible in the drylands. In addition, strong winds, prolonged drought, and grazing could reduce the deposition of litterfall from the tree floor. With increased concern about environmental stresses on the relationship between host and mistletoe, there is a need to evaluate the direct effect of mistletoes on their hosts.
The scientific basis of this research depends on three underlying factors: taxonomic traits, spatial distribution and precipitation availability. To the best of my knowledge, no study has documented the coexistence of Loranthaceae and Viscaceae in arid lands, and exclusively co-infecting the same tree species. The present study adds new ecological information about mistletoe infection patterns. It presents interspecific interactions among three different species of mistletoe belonging to Loranthaceae and Viscaceae, which share the same individual tree. In other arid zones, all studies investigated one single mistletoe species versus a single host species to assess the direct effect of mistletoe. For example, studies include *Phoradendron californicum* (desert mistletoe) and its host, *Acacia greggii* in the Las Vegas Valley, Nevada (Lei, 1999), *P. acacia* on the host *Acacia raddiana* in the Negev Desert (Bowie & Ward, 2004) and desert mistletoe (*P. californicum*) vs. the host (*C. floridum*) in the Mojave Desert (Spurrier & Smith, 2007).

A combination of rainfall rate and geographic gradients control water availability in the soil, thus the spatial distribution of the host plant plays a core role in determining mistletoe effects. A number of studies have demonstrated clear links between long drought periods, mistletoe infection and host death, but it is not clear whether complex abiotic factors and infection patterns can influence host physiology. Here, I present the potential influence of mistletoe incidence in different topographic locations that are susceptible to dry and wet periods, which could explain water and nutrient dynamics in the host and mistletoe.

The influence of desert mistletoes on water, photosynthetic performance, and nutrients in the host is subject to the infection pattern. The coexistence of three different species of mistletoe and a single mistletoe species growing in different terrains present a unique pattern of mistletoe infection, which no research has addressed previously. The interaction of ecological factors and how they modulate the host - mistletoe relationship is not clear and needs further investigation.
As in many places, in the Kingdom of Saudi Arabia the vegetation suffers from deterioration because of long-term drought, soil erosion, logging and clearance, overgrazing, road building (El-Juhany, 2009), so the expansion of mistletoes may exacerbate the effects of environmental stress on host trees. There is also pressure from local people to protect the trees from parasitism because acacias spp. are important economic resources for firewood and livestock foraging (Magdy, personal communication, 10 Nov 2012). In recent years, mistletoe infection has also invaded fruit trees such as *Ficus carica* in farmlands. However, to date, little detail is known regarding the ecological functions of these mistletoes, or their effects on the host plants and plant community under different environmental circumstances in the western region of Saudi Arabia. This study will allow us to understand the fundamental relationships between the mistletoes and their hosts.

Taif National Park and Wadi Alshafa are tourist areas in the western Arabian Peninsula. Characterized by mountainous terrain with inter-fluctuating temperatures and an erratic rainfall pattern, these areas contain pristine landscapes, which are dominated by various vascular plants such as *Juniperus procera* and wild olive, (*Ziziphus* spp.). Acacias spp. are also widespread with trees and scrambling shrubs with thorns and leaflets. Plant communities are determined by their topographical position relative to water flow. Currently, changes in environmental factors in these areas may be affecting host-mistletoe interaction. I seek to protect remnant trees by understanding the interaction between mistletoes and their hosts. This will help with recommendations for appropriate mistletoe management (e.g., removal), to improve the host growth rate and productivity in arid and semi-arid lands of the peninsula. The research also provides basic information derived from the field observations, as a required objective to achieve sustainable natural resources management for Saudi Arabia according to the international Convention on Biological Diversity (CBD).
In Taif National Park, the mistletoe *P. curviflorus* infects a wide range of combinations in *Vachellia* and *Senegalia*, which grow on different topographic locations. It is unknown whether the water balance of the soil in the various sites drives the host-mistletoe interaction or whether mistletoes have negative impacts regardless of the environment. In Wadi Alshafa, coexistence of three mistletoes on a single host at different altitudes shows a complex pattern. Whether these species possibly compete with each other, or how they adapt to the water and nutrient balance of the host at different altitudinal locations remains unknown. It is necessary then to elucidate ecological and physiological aspects for the relationship between host and mistletoe.

**Research questions**

This project aims to answer the general research question: *What are the effects of mistletoes on the host plants under different environmental conditions in the arid and semi-arid region of Saudi Arabia?*

In order to answer this overall research question, the study has the following specific objectives that address aspects of the ecology, nutrient status and ecophysiology of *P. curviflorus*, *P. austroarabica*, and *V. schimperi* mistletoes and *S. asak*, *V. flava*, *V. tortilis*, and *V. gerrardii* host species at two study locations in Taif National Park, and Wadi Alshafa environment of western Saudi Arabia.
1. Ecological aspects

The objective was to determine the incidence and abundance of mistletoes on their hosts at different spatial and temporal scales and how this abundance changes with environmental factors and physical characteristics of the host plants.

2. Host and mistletoe nutrient status

The objectives were to determine the nutritional relationship between *P. curviflorus* and several hosts distributed on different landscape positions; to determine the effects of different levels of infection on nutrient concentration at the level of individual plants; and to determine whether nutrient status varies among three species of mistletoe that coexist on a single host.

3. Host and mistletoe ecophysiology

Focussing on plant water status and photosynthetic performance as indicators of physiological stress, the objectives were to determine the effect of *P. curviflorus* on different hosts during dry and wet seasons; to determine the effect of different levels of infection on physiological performance (Quantum yield, water potential) and whether three species of mistletoe have different influences on the host.

This knowledge is essential to determine if indeed mistletoes can be considered a pest, and if so to manage them to build sustainability and conserve the natural heritage of Saudi Arabia for future generations.
**Thesis structure**

Following the general introduction in Chapter 1, Chapters 2 to 6 have been prepared as manuscripts for publication, although the articles have not yet been submitted to journals for review. The second chapter has been written with the intention of submission to *Ecography*. The fifth chapter has been written following the style of *Annals of Botany* and the sixth chapter has been written following the style of *New Phytologist*. Because the studies for Chapters 2 to 6 draw on different data collected at the same two study sites in western Saudi Arabia, there is some unavoidable repetition in the introductions to the thesis chapters. Chapter 7 draws the findings of the study components together, with recommendations for application of the results and for further research.
References


29


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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

a. the candidate’s stated contribution to the publication is accurate (as detailed above);

b. permission is granted for the candidate in include the publication in the thesis; and

c. The sum of all co-author contributions is equal to 100% less the candidate’s stated contribution.

| Name of Co-Author | José M. Facelli |
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| Date |  |
Chapter 2: Incidence and abundance of Mistletoes as a function of altitude and host characteristics in desert landscapes in the western Arabian Peninsula

Dhafer A. Albakr* and José M. Facelli
School of Biological Sciences, the University of Adelaide, SA, 5005, Australia

*Author for correspondence: Dhafer A. Albakre, Email: Dhafer.albakre@adelaide.edu.au

This chapter has been written following the style of journal Ecography.

Abstract

Mistletoes are ecologically important species, but their overabundance may cause mortality of the host. This study aimed to understand the drivers of mistletoe distribution in two different sites with xeric conditions, Taif National Park and Wadi Alshafa in the Taif region of Saudi Arabia where overabundance of mistletoes has been reported recently. In Taif National Park, we studied the mistletoe and tree species, and measured the host morphological characteristics, In Wadi Alshafa, we modelled the dependence of the incidence and abundance of the three mistletoe species on the host canopy volume and altitude ranging from 1651 m to 2012 m (asl.). In Taif National Park, three shrublike species V. tortilis, V. flava, S. asak and one tree species V. gerrardii, support the mistletoe. Overall, the host characteristics (trunk diameter, canopy area and height) did not affect the incidence of Plicosepalus curviflorus on three of the Vachellia and Senegalia species. The only exception was S. asak: infected plants had wider canopies than uninfected ones. The difference between the species may be explained by the growing conditions of the host trees. S. asak grows on rocky slopes where large trees may have better access to water.
and nutrients than smaller trees. Three mistletoe species (P. curviflorus, Phragmanthera austroarabica and Viscum schimperi) coexist on a single Vachellia species, V. gerrardii, in Wadi Alshafa. The distribution of the three species showed different patterns. The incidence and abundance of V. schimperi and P. austroarabica increased with altitude whereas P. curviflorus decreased. The incidence of all three mistletoe species increased with host canopy volume. Furthermore, high abundance of P. austroarabica and P. curviflorus increased with host canopy volume, but high abundance of V. schimperi did not. Although competitive and facilitative relationships of the mistletoe species seemed to occur, the most important reason for the differences in mistletoe species distribution seemed to be the different physiological responses of the host trees, and the mistletoes, to temperature and water balance, which change along the altitudinal gradient.

**Key-words:**
Desert vegetation; Acacia; Mistletoe incidence; Mistletoe abundance; Altitude; Wadi Alshafa
Introduction

Mistletoes are ecologically important species found in a range of environments, from tropical forests to dry and temperate regions (Press and Graves 1995). They provide food and shelter for birds and insects, and they affect the dynamics of nutrients in the ecosystem (March and Watson 2010). For an individual host tree, the effect of mistletoes varies from negligible to severely harmful. For instance, for the pink gum *Eucalyptus fasciculosa* infected by box mistletoe *Amyema miquelii* in South Australia, a high mistletoe load resulted in less foliage in hosts but the difference in canopy condition between infected and uninfected trees was only slight (Ward 2005). However, the overabundance of mistletoes can lead to host death, as in the arid zone of Australia where the growth of *Amyema preissii* increased the mortality of *Acacia victoriae* (Reid, Smith and Venables 1992). Elsewhere, namely in North America and India, mistletoe spread has caused serious economic damage reducing the reproduction of the hosts or killing them (Geils and Hawksworth 2002, Thriveni et al. 2010).

In the Arabian Peninsula, vegetation is currently deteriorating due to logging, overgrazing, road building, soil erosion and severe drought (El-Juhany 2009). Particularly in the western region, the expansion of mistletoe is a serious problem because it is harmful to economically important species of acacias that provide firewood and forage for livestock. More information is needed to decide whether some management is required, and if so, the possible management options. To date, there is little knowledge regarding the environmental factors that control the prevalence of mistletoes in the Arabian Peninsula.

The prevalence of mistletoes is regulated by a set of interacting ecological factors (Aukema 2003). To be able to understand these factors, both the incidence and abundance of mistletoes must be studied, at both the landscape scale as well as on the individual host trees.
One important controlling factor is the activity of birds. Birds disperse mistletoe seeds effectively, and thus, the behaviour of birds greatly affects the incidence and abundance of mistletoe (Pratt and Stiles 1983). Birds prefer large trees for shelter, food and perch sites and, therefore, the establishment of mistletoe can be greater on large trees (Reid and Smith 2000). Therefore, the characteristics of the host plant, such as canopy volume, trunk diameter and height potentially affect mistletoe prevalence (Ndagurwa et al. 2012).

Moreover, the co-occurrence of mistletoe species is known to affect the mistletoe prevalence on individual hosts. One species can facilitate or out compete the growth of other mistletoe species. Both facilitative and competitive mechanisms greatly affect the co-infecting of mistletoes on individual trees (Queijeiro-Bolaños et al. 2017). The pattern of infection seems to be complex and it depends not only on the mistletoe species present but also on other underlying factors, such as the density of the hosts and their competition for soil resources (Matula et al. 2015).

At the landscape scale, the prevalence of mistletoe can be influenced in complex ways by topographical factors, such as altitude, slope and aspect. For example, a relationship between altitude and the infection of the mistletoe Phoradendron californicum was observed both at low (< 1500 m) and high (> 4000 m) altitudes in semi-desert habitat in Arizona (Aukema 2004). Topography affects temperature, moisture and growing conditions in general. Therefore, altitudinal gradients can control the distribution of mistletoe through effects on the physiological response of mistletoe to temperature and moisture (Dobbertin et al. 2005).

This study investigated factors that potentially affect the incidence and abundance of mistletoe in desert landscapes and on individual host trees. In particular, we aimed to study (1) whether morphological characteristics of infected and uninfected hosts differ, (2) how the prevalence of mistletoe species changes along an altitude gradient, and (3) how the prevalence of three mistletoe
species is related to the canopy volume of the host, *V. gerrardii*. The first questions were studied in Taif National Park and the latter two in Wadi Alshafa, Arabian Peninsula.

**Methods**

**Study area and plant species**

The two study sites were located in an arid mountainous sector of the Arabian Peninsula western region, Taif National Park (21° 18’ 07.93”N 40° 29’ 38.49”E) and Wadi Alshafa (21° 12’ 11.66 N 40° 20’ 44.72”E) (Fig.1). The environment of this region experiences extreme fluctuations in climate, with both seasonal and inter-annual fluctuations in temperature and erratic patterns of rainfall. The coolest month is January, when the average minimum and maximum temperatures are 6°C and 29°C, respectively. For the hottest month (July) the respective values are 20°C and 39°C (Al-Shaibani 2008). Annual precipitation varies considerably from year to year. From 2003 to 2013, the average annual rainfall was close to zero in Taif National Park and 129 mm in Wadi Alshafa.

Human activities are restricted in Taif National Park. The 50 km² area of the site is fenced from grazing animals and has a diverse landscape that includes mountains, dry floodplains and flat plains, which support rich plant diversity. Vegetation composition in the area is xerophytic plants and the most dominant plant family is Fabaceae, including two genes *Vachellia* and *Senegalia* that host mistletoes. highlands (Fig. 2) (Collenette 1999). A preliminary survey indicated that these four species were infected by the mistletoe *P. curviflorus*. 
Wadi Alshafa is located in a valley in a rural area. The upstream valley starts in the southwest at an altitude of 2012 m and continues downstream towards the northeast to an altitude of 1651 m. It comprises a distinct 11 km of dry stream bed. The dominant tree species, *V. gerrardii*, grows mainly in the dry stream beds. A preliminary survey indicated that *A. gerrardii* trees were often infected by three mistletoe species, *P. curviflorus, P. austroarabica* and *V. schimperi* (Fig. 3). The three species have distinctive characteristics. *P. austroarabica* attaches through a single haustorium and has considerably large leaves reaching some 40 cm$^2$. *P. curviflorus* also attaches through a single haustorium but has much smaller leaves (up to 3.5 cm$^2$). *V. schimperi* on the other hand is aphyllous.

**Study design**

**Sampling at Taif National Park**

Four belt transects (20 m wide x 100 m long) representing different topographic areas were marked at Taif National Park. In each of the four transects, 12 infected trees of each of the four host tree species were randomly chosen. In addition, for each tree, the nearest uninfected host tree of the same species was chosen for comparison. A total of 96 trees were chosen. For each individual tree, trunk diameter at breast-height, height and canopy area were measured. The average measurement of the stems was used for multi-stemmed trees *V. gerrardii, S. asak, V. flava* and *V. tortilis*. Host height was measured using a clinometer, with the angle and distance to the tree used to calculate the height.

Host canopy area was determined by measuring the widest diameter (a) and the diameter perpendicular to the widest (b). Area (A) was then calculated using the formula of an ellipse ($A = \pi \times a \times b$) (Ward 2005).
**Sampling at Wadi Alshafa**

Wadi Alshafa was sampled from the altitude of 2012 m to 1651 m, this being the range from the highest to the lowest point where the host tree *V. gerrardii*, grows. Roadsides and agricultural edges were avoided to ensure that the sampling was as homogeneous as possible. Host location was recorded by GPS. A total of 240 host trees were randomly chosen. *V. gerrardii* is a large tree, with a main trunk and a sizable canopy, which is a prominent morphological trait. The canopy volume of each host tree was determined by measuring maximum canopy diameter, canopy diameter perpendicular to the maximum diameter, and canopy height using a clinometer (Bowie and Ward 2004). Volume was calculated with the equation for the volume of a spheroid.

For each tree, the presence or absence (incidence) of each mistletoe species was recorded. The abundance of each mistletoe species present in each canopy was determined using a modified mistletoe abundance index that comprises descriptive standards (introduced by Sangüesa- Barreda et al., 2013): Host (L) indicates ‘slightly infected host’, with roughly one-third of the host canopy occupied by mistletoe; Host (M) indicates ‘moderately infected host’, with roughly two-thirds of the host canopy occupied by mistletoes; and Host (H) indicates ‘heavily infected host’, with most of the host canopy occupied by mistletoes, and (N) indicates no mistletoe infection.

**Statistical analysis**

The differences in mean diameter, height and canopy area between infected and uninfected trees in Taif National Park were analysed with an independent-t-test.
All treatments were first assessed for normality and variance homogeneity, where Brown-Forsythe test was run to determine if standard deviation was significantly different. Data for *S. asak* canopy area was log transformed, whereas *V. flava* trunk diameter and *V. tortilis* height were square root transformed to equalize variances.

In Wadi Alshafa, binary logistic regression models were constructed for the incidence, and multinominal logistic regression for the abundance. Regression models were constructed to estimate the influence of altitude, host canopy volume, and mistletoe species as explanatory variables on the incidence or abundance of mistletoe as a response variable. Pearson’s correlation coefficient was calculated to study any multicollinearity. Since Pearson’s tests showed data as reciprocal correlations, we diagnosed variance of the inflation factor, indicating no major collinearity in data (VIF<1.5). Preliminary data analysis suggested that the incidence and abundance models should be constructed separately. Regressions were run for all combinations of predictors (altitude, host canopy volume, the incidence or abundance of co-existing mistletoe species). A backwards stepwise process was used to eliminate predictors one by one. Improvement of the model was assessed by log-likelihood (Field 2013). An omnibus test (incidence) and a likelihood ratio test (abundance) were used to choose the best model. The higher value of -2 log likelihood (-2LL) indicated the best-fit model (Field 2013). The odds (B) values were converted to the probability according to Field (2001). Probability = Odds/(1+Odds). Data analyses were performed with SPSS package V.23.
Results

Effect of the host characteristics on the incidence of *P. curviflorus* in Taif National Park

Host morphological variables were general not significantly different between infected and uninfected trees. The only significant difference was observed for *S. asak*, and only for canopy area (p = 0.007; t-test). The mean canopy area of infected trees (19.2±8.2 cm², n = 12) was significantly higher than that of uninfected trees (11.4±3.6, n = 12) (Fig. 4a). None of the host morphological variables were significantly different between infected and uninfected trees for *V. gerrardii*, *V. flava* or *V. tortilis* (p > 0.05, t-test, Fig. 4a, b, c).

Mistletoe incidence and abundance as a function of abiotic and biotic factors

The best-fit binary logistic model for *P. austroarabica* incidence contained altitude, host canopy volume and an interaction term (host canopy volume × *V. schimperi* incidence; $X^2 = 28.24, p < 0.001$; Omnibus test) (Tables 1, 2, 3). An increase of 1 m in altitude corresponded to 0.6% ($B=0.006$, Exp($B)=1.006$; $p = 0.02$) increase in the probability of *P. austroarabica* incidence (Table 3; Fig 5a). The interaction between the host canopy volume and *V. schimperi* incidence ($B=0.004$, Exp($B)=1.004$; $p = 0.032$) indicates that the probability of *P. austroarabica* incidence increased with increasing host canopy volume more when *V. schimperi* was present (Fig. 5b, right subplot) than when it was absent (Fig. 5b, left subplot; Table 3).

The best-fit multinomial logistic regression model for *P. austroarabica* abundance contained altitude and an interaction term (host canopy volume × *V. schimperi* abundance) ($X^2 = 49.42, p < 0.001$; Likelihood ratio test; Tables 1, 2, 4). An increase of 1 m in altitude corresponded to 1.1% increase in the probability of high *P. austroarabica* abundance (index H) (Fig. 6a, top subplot) ($B=0.011$, Exp($B)=1.011$, $p<0.001$) (Table 4). Fig. 6b shows the interactions and the complexity of the dependence of *P. austroarabica* abundance on the predictor variables used in the model.
In general, the probability of high *P. austroarabica* abundance (index H) increased with altitude (Fig. 6a, top subplot), host canopy volume and *V. schimperi* abundance (Fig. 6b, top subplots). In Fig. 6b lower subplots, where *P. austroarabica* abundance was low (abundance indices N, L, M), *V. schimperi* abundance did not significantly affect the relationship between *P. austroarabica* abundance and host canopy volume; *V. schimperi* and host canopy volume interaction was not significant (Table 4). For high *P. austroarabica* abundance, the interaction between *V. schimperi* and host canopy volume was significant and positive (B = 0.011, Exp(B) = 1.011, p < 0.001, Table 4). This positive interaction indicates that *P. austroarabica* increased with host canopy volume, and at the same time, *P. austroarabica* increased more when *V. schimperi* abundance was high than when it was low or medium, as can be seen in Fig. 6b top subplots.

The best-fit binary logistic model of *P. curviflorus* incidence contained altitude, *V. schimperi* incidence and a marginal interaction (host canopy volume $\times$ *P. austroarabica* incidence) ($X^2 = 38.139$, p < 0.001; Omnibus test) (Tables 1, 2, 3). An increase of 1 m in altitude corresponded to 0.7% (B = -0.007, Exp(B) = 0.993, p = 0.011) decrease in the probability of *P. curviflorus* incidence (Table 3; Fig. 7a). The probability of *P. curviflorus* incidence was significantly lower (50%) when *V. schimperi* was present than when it was absent (B = -1.022, Exp(B) = 0.360, p = 0.020) (Table 3; Fig. 7b). The interaction between host canopy volume and *P. austroarabica* incidence, shown in Fig. 4c, indicates that the probability of *P. curviflorus* incidence increased with host canopy volume more when *P. austroarabica* was absent (Fig. 4c, left subplot) than when it was present (Fig 7c, right subplot) (B = -0.005, Exp(B) = 0.995, p = 0.085 (Table 3).
The best-fit multinomial logistic regression model for *P. curviflorus* abundance contained host canopy volume and an interaction term (altitude × *V. schimperi* abundance; \(X^2 = 78.89, p < 0.001;\) Likelihood ratio test) (Tables 1, 2, 4). An increase of 1 m³ in host canopy volume corresponded to 0.5% \((B= 0.005, \text{Exp}(B) = 1.005; p = 0.004)\) increase in the probability of high *P. curviflorus* abundance (index H) (Table 4; Fig. 8a, lowest subplots). Fig. 8b indicates the complex interactions and shows that the probability of high *P. curviflorus* abundance (index H; lowest subplots) decreased in general with altitude. The decrease was, however, different depending on *V. schimperi* abundance.

The best-fit binary logistic regression for *V. schimperi* incidence contained altitude, host canopy volume, and an interaction term (host canopy volume × *P. curviflorus* incidence; \(X^2 = 85.55, p < 0.001;\) Omnibus test) (Tables 1, 2, 3). An increase of 1 m in altitude corresponded to 3.2% \((B= 0.032, \text{Exp}(B)=1.033, p<0.001)\) increase in the probability of *V. schimperi* incidence (Table 3; Fig. 9a). The interaction indicated that the probability of *V. schimperi* incidence increased with increased host canopy volume more when *P. curviflorus* was absent (Fig. 9b, lower subplot) than when it was present \((B= -0.013, \text{Exp}(B) = 0.987; p = 0.039)\) (Table 3; Fig. 9b, upper subplot).

The best-fit multinomial logistic regression model for *V. schimperi* abundance contained only altitude \((X^2=77.31, p< 0.001;\) Likelihood ratio test) (Tables 1, 2, 4). For all levels of infection (abundance indices H, M, L), the probability of *V. schimperi* abundance increased significantly with altitude (3.1% for the low infection, \(B= 0.031, \text{Exp}(B)=1.032\); 3.2% for the medium infection, \(B= 0.032, \text{Exp}(B)=1.032\); 3.5% for the high infection, \(B=0.035, \text{Exp}(B)=1.036\); \(p<0.001\)) (Table 4; Fig. 9).
Discussion

Effects of host morphological characteristics on *P. curviflorus* incidence in Taif National Park

Our results indicated that the host morphological characteristics (trunk diameter, height, and canopy circumference) did not affect the incidence of the mistletoe *P. curviflorus* on three (*V. flava*, *V. gerrardii*, and *V. tortilis*) out of the four host tree species growing in Taif National Park. Thus, it seemed that the host characteristics did not, in general, control the incidence of the mistletoe *P. curviflorus*. This finding contrasts with most other studies showing that larger and taller hosts are more likely to support higher mistletoe incidence (Donohue 1995; Smith and Reid 2000; Roxburgh and Nicolson 2008). Our results are arguably more robust than previously reported results as we sampled pairs of infected and uninfected hosts that were always close to each other and were thus more likely to be growing in very similar conditions of soil nutrients and water. These three host species inhabited low-lying areas that receive run-on water. Therefore, their growing conditions may differ considerably from the fourth species, *S. asak*. Increased canopy area of *S. asak* was indeed related to high incidence of mistletoe. This may be due to the topographic position where the species grows. *S. asak* grows on rocky slopes, where free water run-off creates very dry and harsh growing conditions. The mistletoe *P. curviflorus* may only successfully establish and persist on the more vigorous individuals that have large canopies.

Relationship between the prevalence of mistletoe species and altitude in Wadi Alshafa

We found that the prevalence (incidence and abundance) of all three mistletoe species (*P. austroarabica*, *P. curviflorus*, *V. schimperi*) growing on *V. gerrardii* in Wadi Alshafa changed along the altitudinal gradient in different ways.
The incidence and abundance of both *V. schimperi* and *P. austroarabica* increased with altitude. In contrast, the incidence and abundance of *P. curviflorus* decreased with altitude. These changes in mistletoe prevalence most likely resulted from changes in temperature and moisture conditions along the altitudinal gradient. Altitude is considered a key factor influencing vegetation patterns in arid and semi-arid climates, such as in the Arabian Peninsula (Hegazy et al. 1998; Brinkmann et al. 2009). Altitude controls temperatures and water availability, and thus creates different levels of physiological stress for plants. Mistletoes and their hosts growing in the lowlands of Wadi are likely to experience higher day temperatures compared with those growing in higher regions. In Wadi Alshafa, the climatic effects of altitude are marked, especially in the summer season.
Relationship between the prevalence of different mistletoe species and canopies of *V. gerrardii* in Wadi Alshafa

Our logistic regression models suggest that the incidence and abundances of the three mistletoe species growing on *V. gerrardii* in Wadi Alshafa have a complex interdependence with each other and the canopy volume of the host tree. The host canopy volume in general positively affected incidence of the three mistletoe species. This is not surprising, since large canopy size, which is expected in older, more vigorously growing trees, provides more branches for perching birds (Lavorel et al. 1999, Aukema and Del Rio 2002; Tsopelas et al. 2004). This can lead to greater seed deposition and should result in increased mistletoe infection in larger trees, which is a phenomenon documented in several studies (Donohue 1995; Lei 1999; Smith and Reid 2000; Roxburgh and Nicolson 2008).

While the incidence of mistletoe increased with host canopy volume for all three species consistently, the abundance of mistletoe showed a different pattern. The abundance of *P. austroarabica* and *P. curviflorus* increased while that of *V. schimperi* was not affected by host canopy volume. It seems that *P. austroarabica* and *P. curviflorus* grow better on larger host trees, as previously documented with other mistletoes (Roxburgh and Nicolson 2008). These two species differ substantially in their morphology, especially in leaf area, from *V. schimperi*. Presumably, the leafy mistletoes (*P. austroarabica* and *P. curviflorus*) may have a higher transpiration rate, requiring more water than the leafless mistletoe (*V. schimperi*). Because the leafy mistletoes may need more water, the water status of the host is more important for them. Host trees with larger canopies are likely to have deeper root systems that can supply sustained water and nutrients to the host as well as to a greater number of mistletoes (Ward et al. 2006). Therefore, we conclude that the abundance of the leafy mistletoes seems to be driven by the availability of water in Wadi.
We found a negative relationship between the prevalence of *V. schimperi* and *P. curviflorus* suggesting that they might compete for space in their hosts' canopies. This competitive relationship is consistent with observations in Australia and central Mexico (Norton et al. 1997; Queijeiro-Bolaños et al. 2013).

A negative relationship can be due to the haustorial endophytic structures that allow for different levels of penetration and resource acquisition, but this explanation needs further anatomical studies.

Interestingly, a facilitative relationship also potentially occurred; the high abundance of *V. schimperi* may have facilitated the increase of the high abundance of *P. austroarabica*, although not *vice versa*. Our modelling indicated that when *P. austroarabica* abundance was low or medium, the interaction between *V. schimperi* abundance and host canopy volume was absent. When *P. austroarabica* abundance was high, the probability of its high abundance increased with the increased canopy volume of the host as well as with the abundance of *V. schimperi*. We interpret this positive interaction between *V. schimperi* abundance and host canopy volume to reflect the positive response of *P. austroarabica* abundance to host canopy volume, rather than the dependence of *V. schimperi* abundance on the host canopy volume. It seems that *V. schimperi* abundance was influenced by factors we could not determine. We speculate that because these mistletoes have different fruiting seasons, they do not compete with each other, since overlapping fruiting seasons would induce competition to attract birds (Herrera 1981). These interpretations, although somewhat speculative and needing further study, are supported by the model of *V. schimperi* abundance that included neither host canopy volume nor *P. austroarabica* abundance. Two possibilities may explain the seemingly facilitative relationship of the two mistletoe species, *P. austroarabica* and *V. schimperi*. 
First, *P. austroarabica* could have shifted its parasitism from a tree to *V. schimperi* mistletoe. This is a form of hyperparasitism, and its frequent occurrence is supported by observations by the first author during the fieldwork. An alternative explanation is that the coexistence of these mistletoes represents intra-guild mutualism (Queijeiro-Bolaños et al. 2017). This means that the resistance of the host is weakened by one mistletoe species, enabling the infection of another mistletoe species. We suggest that the infection of *V. schimperi* has facilitated the infection of *P. austroarabica* through hyperparatism and favoured its growth in Wadi.
Conclusion

This study determined that, overall, host morphological characteristics seemed to affect the prevalence of mistletoes both in Taif National Park and in Wadi Alshafa. However, this was not always the case. In Taif National Park, the morphological characteristics did not explain the incidence of the mistletoe *P. curviflorus* in the three species (*V. flava, V. gerrardii, V. tortilis*). These species grew in low-lying areas, such as valleys and bench lands. In these lower areas, water and finer sediments accumulate creating better growing conditions and possibly providing improved conditions not only for uninfected trees but also for infected trees to grow. Therefore, host characteristics and topographic traits are closely connected and could override the effects of host size. They seemed to also be connected when the host characteristics, i.e. large canopy area of *S. asak*, seemed to favour *P. curviflorus* incidence. We suggest that *P. curviflorus* persist better on larger, more vigorous *S. asak* hosts because this tree species grows on rocky slopes, where free draining water creates dry and harsh conditions. We conclude that *P. curviflorus* presence may be indirectly influenced by topographical traits that most likely control the distribution of resources, and thus, the growth of the host trees. We were able to model both the incidence and abundance of the mistletoe species and explain them with altitude, host canopy volume and the other mistletoe species growing in Wadi Alshafa. Their incidence and abundance depended on these factors, but the interactions were complex.

The distributions of the three mistletoe species studied were fitted by different logistic regression models, both for their incidence and abundance indicating that they respond to different environmental conditions. The incidence and abundance of *P. austroarabica* increased both with altitude and host canopy volume, while those of *V. schimperi* increased with altitude and those of *P. curviflorus* decreased with altitude and increased with host canopy volume. Moreover, the
mistletoe species seemed to affect each other’s prevalence. *P. curviflorus* and *V. schimperi* seemed to compete with each other, whereas *V. schimperi* seemed to facilitate the prevalence of *P. austroarabica*. The pattern of mistletoe species distribution may largely be explained by their morphological traits, most simply by their leaf areas; *P. austroarabica* has large leaves, *P. curviflorus* has small leaves, and *V. schimperi* is leafless. Leaf properties can affect the physiological response of mistletoes to varying temperatures and water availability through the host tree. Temperature and water availability, in turn, are largely controlled by topographic traits, especially by altitude. Therefore, we conclude that topographic traits largely explain the prevalence of mistletoes in desert landscapes, but biotic interactions amongst mistletoes also affect their distribution.
References


March, W. A., & Watson, D. M. (2010). The contribution of mistletoes to nutrient returns:


55
### Tables

Table 1. Spearman's rho correlations for, altitude, host canopy volume, and incidence and abundance of mistletoe species. *P < 0.05, **P < 0.01.

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<th>P. curvifolius abundance</th>
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Table 2. Stepwise process of logistic regression using log-likelihood to rank the models for incidence and abundance of mistletoe species. *P. austroarabica* incidence (PhragIncidence), *P. austroarabica* abundance (PhragNumber), *P. curviflorus* incidence (PlicoIncidence), *P. curviflorus* abundance (PlicoNumber), *V. schimperi* incidence (VisIncidence), *V. schimperi* abundance (ViscNumber), Host canopy volume (H.VOL).

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<tr>
<th>Mistletoe species</th>
<th>Backward Stepwise method</th>
<th>Model Fitting Criteria</th>
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<td><em>P. austroarabica</em> incidence</td>
<td>Altitude + H.VOL + H.VOL × VisIncidence + H.VOL × PlicoIncidence + Altitude + PlicoIncidence + Altitude × VisIncidence</td>
<td>298.816</td>
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<td>Altitude + H.VOL + H.VOL × VisIncidence + H.VOL × PlicoIncidence + Altitude × VisIncidence</td>
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<td>Altitude + H.VOL + H.VOL × PlicoIncidence + H.VOL × PlicoIncidence + Altitude × VisIncidence</td>
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<td>Altitude + H.VOL × VisIncidence</td>
<td>381.192</td>
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<tr>
<td><em>P. austroarabica</em> abundance</td>
<td>Altitude × (H.VOL) + H.VOL × ViscNumber</td>
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<td><em>P. curviflorus</em> incidence</td>
<td>Altitude + H.VOL + Altitude × PhragIncidence + Altitude × ViscIncidence + H.VOL × PhragIncidence + H.VOL × ViscIncidence</td>
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<td>Altitude + H.VOL + H.VOL × PlicoIncidence + H.VOL × PhragIncidence + Altitude × PhragIncidence</td>
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<td>206.548</td>
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<td>Altitude + H.VOL + H.VOL × PlicoIncidence</td>
<td>287.659</td>
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<tr>
<td><em>V. schimperi</em> abundance</td>
<td>Altitude × (H.VOL) + (H.VOL × PlicoNumber)</td>
<td>564.217</td>
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<td></td>
<td>Altitude</td>
<td>660.786</td>
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a. H.VOL was removed from the *P. austroarabica* abundance model because it was redundant.
b. (H.VOL × VisIncidence) was removed from the full model of *P. curviflorus* incidence because the Hosmer and Lemeshow test was very close to the statistical significance p=0.056.
c. Altitude & (H.VOL × PhragNumber) were removed from *P. curviflorus* abundance model because the Pearson goodness of fit test was significant p=0.040.
d. H.VOL & (H.VOL × PlicoNumber) were removed from *V. schimperi* abundance model because they were redundant.
Table 3. Summary of the binary logistic regression outputs for the best-fit models using altitude (1651-2012 m), host canopy volume (H.VOL - m3) and the different mistletoe species incidence as predictors in the Wadi Alshafa in Western Saudi Arabia. * The reference category of each model is: non-incidence.

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<tr>
<th>Mistletoe species</th>
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<th>Parameters in Equation (45-54)</th>
<th>95% C.I for EXP(B)</th>
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<td>Model summary (-2 Log Likelihood &amp; Nagelkerke R²)</td>
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<td>P. astrocarpa</td>
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<td>Altitude</td>
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<td>P=0.425</td>
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<td>H.VOL+ P. astrocarpa</td>
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Table 4. Summary of the multinomial logistic regression outputs for the best-fit models using altitude, host canopy volume and the abundance of different mistletoe species as predictors in the Wadi Alshafa. The mistletoe abundance is described as the abundance index (Low, Medium, High). * The reference category of each model is: none.

<table>
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<th>Pearson Goodness of fit test ($p&gt;0.05$)</th>
<th>Mistletoe abundance index</th>
<th>Parameters in Equation (df=1)</th>
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<td>Intercept</td>
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<td>.003</td>
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<td>Host canopy volume + E. schleperi</td>
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<td>.002</td>
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<td>P. schleperi</td>
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<td>Altitude + E. schleperi</td>
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59
(Fig. 1) Study sites in Taif National Park, and Wadi Alshafa, western region of Saudi Arabia.
(Fig. 2) Senegalia and Vachellia species growing on different terrains in Taif National Park, western Saudi Arabia.
(The resource of the background image is Google Earth 2013)
(Fig. 3) The mistletoe species (a) *V. schimperi*, (b) *P. austroarabica*, (c) *P. curviflorus* infection on (d) *V. gerrardii* (the host tree) in Wadi Alshafa in the western region of the Arabian Peninsula.
(Fig. 4) The host characteristics of (a) host canopy area (m²), (b) diameter of trunk at breast height (cm) and (c) host height (m) for S. asak, V. flava, V. tortilis and V. gerrardii for infected (shaded bars) and uninfected (open bars) host trees. Error bars represent SD (n = 12). Different letters above the bar charts denote a significant difference (P < 0.05; t-test) between infected and uninfected trees within a species.
(Fig. 5) The best-fit binary logistic regression model for *P. austroarabica* incidence with a) altitude and b) the interaction of host canopy volume x *V. schimperi* incidence as predictors. Shaded areas represent 95 CI% envelopes. 

*n* = 240 trees.
(Fig. 6) The best-fit multinomial logistic regression model for *P. austroarabica* abundance with a) altitude and b) the interaction of host canopy volume x *V. schimperi* abundance as predictors. The abundance is described as the level of infection (abundance index): (H) high, (M) medium, (L) low, and (N) no mistletoe. Shaded areas represent 95 CI% envelopes. n = 240 trees.
(Fig. 7) The best-fit binary logistic regression model for *P. curviflorus* incidence with a) altitude, b) *V. schimperi* incidence, and c) the interaction of host canopy volume x *P. austroarabica* incidence as predictors. Shaded areas represent 95 CI% envelopes. *n* = 240 trees.
(Fig. 8) The best-fit multinominal logistic regression model for *P. curviflorus* abundance with a) host canopy volume and b) the interaction of altitude x *V. schimperi* abundance as predictors. The abundance is described as the level of infection (abundance index): (H) high, (M) medium, (L) low, and (N) no mistletoe. Shaded areas represent 95 CI% envelopes. *n* = 240 trees.
(Fig. 9) The best-fit binary logistic regression model for *V. schimperi* incidence with a) altitude and b) the interaction of host canopy volume x *P. curviflorus* incidence as the predictors; c) the best-fit multinominal logistic regression model for *V. schimperi* abundance with altitude as the predictor. The abundance is described as the level of infection (abundance index): (H) high, (M) medium, (L) low, and (N) no mistletoe. Shaded areas represent 95 CI% envelopes. $n = 240$ trees.
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## Principal Author

<table>
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<tr>
<th>Name of Principal Author (Candidate)</th>
<th>Dhafer A. Albakre</th>
</tr>
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<tbody>
<tr>
<td>Contribution to the Paper</td>
<td>D.A.B conceived the ideas and methodology, analysed data, and wrote manuscripts and acted as corresponding author.</td>
</tr>
<tr>
<td>Overall percentage (%)</td>
<td>80%</td>
</tr>
<tr>
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<tr>
<th>Name of Co-Author</th>
<th>José M. Facelli</th>
</tr>
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<tr>
<td>Contribution to the Paper</td>
<td>J.M.F conceived the ideas, interpreted data, critically assessed to drafts and issued final approval for publication.</td>
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Chapter 3: The effect of mistletoes on the nutrient status of their hosts: investigating several patterns of infestation in the western Arabian Peninsula

Dhafer Albakre* and José M. Facelli
School of Biological Sciences, the University of Adelaide, Adelaide, SA 5005, Australia.
*Author for correspondence: Dhafer A. Albakre, Email: Dhafer.albakre@adelaide.edu.au

Abstract

Mistletoes are a core component of most terrestrial ecosystems because they affect nutrient dynamics, among other ecological and physiological effects. We investigated the direct effect of mistletoes on the nutrient (N, P, K, Na, Ca, Mg, Fe, Mn, Zn) status of their hosts at two different sites in arid and semi-arid lands in western Saudi Arabia. In the arid shrubland and varied terrain of Taif National Park, we compared the nutrient status of three xeric shrub species, *Senegalia asak*, *Vachellia flava*, and *Vachellia tortilis*, parasitised or not by *Plicosepalus curviflorus*. Mistletoe negatively affected N and Mn levels in *S. asak* and P and Zn levels in *V. flava*, while the nutrient content of *V. tortilis* was unaffected by parasite status. Mistletoes affected the nutrient contents of infected branches but did not affect the nutrient status of uninfected host branches. The disparity in nutrient levels between parasitised host species can be largely explained by the environmental conditions in which they grow, as these conditions may influence the nutrient uptake mechanisms of the mistletoes. In a dry valley, Wadi Alshafa, we compared the nutrient status in *Vachellia gerrardii* for non-host trees vs. host trees infected by different loads of mistletoe species (heavy, medium, low). Compared with non-host trees and slightly infected hosts, the Ca and Mg concentrations were higher in hosts heavily infected with *V. schimperi*, only Ca concentration was higher in moderately infected than uninfected hosts, but it was not significantly different compared to slightly and heavily infected hosts.
while N, P, Na, and Ca concentrations were higher in hosts heavily infected with *P. austroarabica*, and P and Na concentrations were higher in hosts heavily infected with *P. curviflorus*. Our results suggest that increasing the mistletoe load on the host represents a response of this parasite to high nutrient availability, which differs significantly among the hosts according to their characteristics in the Wadi system.

Key words: Arabian Peninsula, mistletoe infestation load, host characteristics, nutrient uptake
Introduction

Mistletoes are aerial parasites in the Loranthaceae and Viscaceae families that are widespread throughout most habitats. These plants are found in tropical to alpine and subarctic habitats and from mesic to arid habitats. Mistletoes are hemi-parasites that typically attach to host branches through a haustorium (Press & Graves, 1995). These plants are capable of photosynthesis, but they depend on the host for water and mineral nutrients (Glatzel, 1983).

Mistletoes maintain one-way transport of mineral elements from the host xylem to their own vessels through the haustorium. To acquire sufficient minerals, particularly nitrogen, mistletoes often have high transpiration rates, which they achieve by having a lower water potential than the host (Ehleringer, Cook, & Tieszen, 1986; Press et al., 1990; Tennakoon & Pate, 1996). Therefore, mistletoes can passively take up nutrients from the host xylem, and they usually have much higher nutrient concentrations than the host (Lamont & Southall, 1982).

Researchers such as Panvini and Eickmeier (1993) and Bowie and Ward (2004) have shown that mistletoes can also acquire nutrients actively from the host phloem, a conclusion drawn indirectly. For instance, Okubamichael et al. (2011) used unique criteria to compare nutrient accumulation in mistletoe with that of the host to investigate whether mistletoe derives its nutrients from the host xylem or phloem by examining the N:Ca ratio in mistletoe tissues. Since nitrogen is highly mobile in the plant, whereas calcium is immobile, the authors reasoned that a ratio of $\leq 1$ indicates that the nutrients were obtained from the host xylem (passive nutrient uptake), whereas an N:Ca ratio of $> 1$ indicates nutrient uptake from the host phloem (active nutrient uptake) (Panvini & Eickmeier, 1993; Bowie & Ward, 2004; Okubamichael et. al., 2011). Different nutrient acquisition mechanisms are thought to underlie differences in host–mistletoe compatibility.
Favourable ambient environments with available water and soil nutrients can determine host-mistletoe compatibility, and thus the preferred host for mistletoes. Under optimal conditions for the host tree, when water and nutrients are available, the mistletoe may be able to obtain nutrients directly from the host xylem, which is a passive nutrient uptake mechanism. However, if there is a reduction in water and nutrient supply the host experiences physiological stress; in this case, mistletoe can avoid the stress through taking water and nutrients from the host phloem. This latter mechanism requires energy and is called active nutrient uptake (Lambers et. al., 2008).

Mistletoes are known to be a nitrogen drain on their host plants. Consequently, they often reduce the nitrogen concentration in infected branches, which leads to a reduced growth rate in these branches (Ehleringer et al., 1986; Tennakoon & Pate, 1996; Daryaei & Moghadam, 2012). However, cases where infected branches or plants had higher nutrient levels than their uninfected counterparts have also been reported (Türe, Böcük, & Aşan ., 2010), probably because there is an increased likelihood that mistletoes will become established on the branches of a host with high nutrient availability. Some studies (Bowie & Ward, 2004; Okubamichael et al., 2011) have shown that the nitrogen concentration in mistletoe is lower than that of the host. For example, in the Negev Desert, Bowie and Ward (2004) found that the host (Acacia raddiana) had a negative impact on the nitrogen concentration in the mistletoe Plicosepalus acacia, which had 65% less N than the host plant, Acacia. Mistletoes are profligate users of water and nutrients from the host (Schulze & Ehleringer, 1984; Goldstein et al., 1989). Therefore, the presence of mistletoes can also serve as a biological indicator of the host’s health, as these plants rely on the host for survival and growth, and a healthy host with good access to nutrients and water can support many healthy mistletoes.
(Watson, 2008). Nevertheless, the high abundance of mistletoes on an individual host can ultimately harm it, reducing its viability (Ward et al., 2006).

In the current study, we investigated how the presence of mistletoe affects nutrient contents in several host species in an extremely arid environment and the nutritional relationship between proximal and distal branches of the host and mistletoe. We also determined whether the nutrient acquisition by mistletoe was by passive or active uptake under various ecological circumstances. We examined the variation in mistletoe abundance on the host, and whether there was a difference in the nutrient status of a single host when it is parasitised at different levels by multiple mistletoe species.

Understanding the variation in nutrient concentrations during these complex interactions between acacias and mistletoe species (which are all valuable natural resources in western Saudi Arabia) will add insights into the dynamics of nutrient distribution, especially in poor-nutrient environments. These findings are essential for government organisations that need information to create better management practices in arid and semi-arid ecosystems.
Methodology

Study sites and plant species

Two study sites in western Saudi Arabia were selected. One was located in Taif National Park, northeast of Taif city (21° 18' 07.93" N, 40° 29' 38.49" E). This site is at an altitude of 1570 m, and the average annual rainfall is approximately 120 mm, although the rainfall from 2003 to February 2013 at this site was close to zero. The three shrubby host species studied grow in a limited range of topographic positions: S. asak often grows on upper rocky slopes, V. flava often grows on lower slopes, and V. tortilis often grows in bench lands (flat lands next to valleys). These species are often infected by the stem-parasitising mistletoe, P. curviflorus (Collenette, 1999). This site was used to address the question of how the presence of mistletoe affects the nutrient content of host species.

The second study site was located in the long dry valley, Wadi Alshafa, southwest of Taif city (21° 12' 11.66" N, 40° 20' 44.72" E). The average annual rainfall is approximately 275 mm, although from 2003 to 2013, it averaged ~129 mm. Three different mistletoe species, P. austroarabica, P. curviflorus, and V. schimperi, occur in this area and exclusively infect V. gerrardii, which is distributed along the Wadi. This site was studied to address the question of whether nutrient status of a single host species differs when it is parasitised by different levels of three mistletoe species.

Sample collection

The field research was conducted in February 2013 and involved two sample designs and sets of measurements.
In Taif National Park, *S. asak* samples were collected in mountainous terrain, *V. flava* samples were collected in the vicinity of floodplains, and *V. tortilis* samples were collected on plains. Plots of 50 m × 50 m were set up at three different locations as follows (rocky hill, lower slope, and bench land – the flat land next to valley). In each plot, for each host species (*S. asak*, *V. flava*, and *V. tortilis*) six individuals with mistletoes (*P. curviflorus* at this site) and the nearest-neighbour six without were selected. Leaf samples (to obtain at least 100 g of dry material) were collected from the selected hosts with mistletoes and from specimens without the parasite as a control (HB). Leaves were collected from infected branches (IB) of the parasitised hosts at the distal area from the haustorium, from uninfected branches (UB), and from the parasitising mistletoe, the latter sample was used for determining the nutrient acquisition. In the Wadi Alshafa, 100 m long line transects were randomly placed at six different sites of the Wadi, and the mistletoe load on *V. gerrardii* was assessed along these lines. In each transect, four hosts with the parasite and the nearest-neighbour four without it were selected. For the marked parasitised hosts, the species of mistletoe was recorded: *P. austroarabica*, *P. curviflorus*, or *V. schimperi*. Mistletoe load on a single host was quantified using the following classes (Sangüesa-Barreda, Linares, & Camarero, 2013): Host (L) indicates ‘slightly infected host’, with roughly one-third of the host canopy occupied by mistletoe; Host (M) indicates ‘moderately infected host’, with roughly two-thirds of the host canopy occupied by mistletoes; and Host (H) indicates ‘heavily infected host’, with most of the host canopy occupied by mistletoes.

Leaf samples were collected from infected branches from all infection classes, as well as from unparasitised hosts (Non-Host) and the mistletoe, using the same collection protocol as for the previous field site. All samples were packed in paper bags and uniquely labelled. The samples were transported to the laboratory of the Ministry of Environment, Water and Agriculture under the supervision of King Saud University (Riyadh) for analysis. For each sample, 0.5 g of dry,
ground tissue was prepared and digested as described in Chapman (1966). The total nitrogen (N) content was measured by Kjeldahl distillation. Phosphorus (P) levels were measured using a colorimetric method. The concentrations of potassium (K) and sodium (Na) were measured with a photoelectric flame photometer, while calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), and zinc (Zn) were measured with an atomic absorption spectrometer.

**Statistical analysis**

**Data validation**

*Homogeneity of variance*

Graph Pad Prism 6.0 was used to analyse the data. For all treatments in both sites, the Brown-Forsythe and Bartlett tests were used to determine if the standard deviations were significantly different between parasitised and unparasitised hosts. If the data failed the Brown-Forsythe and Bartlett tests for equal standard deviations and they could not be adjusted using transformation, the non-parametric Kruskal-Wallis test was used to detect any differences between treatments. Since the data for K and Fe concentrations in *V. flava* and Ca concentration in *V. tortilis* in Taif National Park failed the assumption of homogeneity of variance, the Kruskal-Wallis test was used to determine the exact $p$-value. The data for Zn concentration in *V. flava* were square-root transformed to equalise variances.

**Data analyses**

For the Taif National Park collection sites, the nutrient concentrations for parasitised and unparasitised host species were analysed by one-way analysis of variance (ANOVA) and Tukey’s multiple comparison tests. When the difference in nutrient concentrations was found to be statistically significant, a linear regression was run to establish the shape of the relationship between the nutrient content in the mistletoe and the host. A paired t-test was used to assess the potential nutrient accumulation according to the passive and active uptake theory (Bowie & Ward,
2004), in which N is considered a highly mobile element, and Ca an immobile element in plants. The N concentration in mistletoe relative to that in the host tree was determined in study 1. In addition, the N:Ca ratio in mistletoe was calculated to determine whether the mistletoe acquired nutrients by passive or active uptake. The samples size for *S. asak* = 6, but the samples size for *V. flava* and *V. tortilis* = 5 for each species; they lost one sample from each species during the analysis in the lab. For the second study (performed at the Alshafa Wadi site) a similar approach was taken, using one-way ANOVA and Tukey’s multiple comparison tests.
Results

Comparison of nutrient concentrations in parasitised vs. unparasitised hosts

For *S. asak*, no significant difference in P, K, Ca, Na, Mg, Fe, or Zn concentration was found between parasitised and unparasitised hosts (*p* > 0.05) (Fig. 3-1 b, c, d, e, f, g, i). However, the N concentrations were significantly different between parasitised and unparasitised hosts (*F* (2, 15) = 7.439, *p* = 0.0057). Control branches of unparasitised hosts had higher N concentrations than infected branches and uninfected branches of parasitised hosts (Fig. 3-1 a). The difference in Mn concentration between unparasitised hosts and infected branches of parasitised hosts were also statistically significant (*F* (2, 15) = 4.934, *p* = 0.0226). Post hoc comparisons using Tukey’s HSD test indicated that the Mn concentration was lower in infected branches of parasitised hosts than in control branches of unparasitised hosts, whereas no statistically significant difference was found in Mn concentration between control branches of unparasitised hosts and uninfected branches of parasitised hosts (Fig. 3-1 h).

For *V. flava*, there was no difference in nutrient concentration between unparasitised and parasitised hosts for N, K, Ca, Na, Mg, Fe, and Mn (*p* > 0.05) (Fig. 3-2 a, c, d, e, f, g, h). By contrast, the difference in P concentration between control branches of unparasitised hosts and infected branches of parasitised hosts was statistically significant (*F* (2, 13) = 4.653, *p* = 0.0299). Control branches of unparasitised hosts had higher P concentrations than infected branches of parasitised hosts, but this difference was not statistically significant between control and uninfected branches (Fig. 3-2 b). The difference in Zn concentration between unparasitised and parasitised hosts was not statistically significant, but it was statistically significant between infected and uninfected branches of parasitised hosts (*F* (2, 12) = 4.853, *p* = 0.0285); the Zn concentration was higher in uninfected host branches than infected host branches (Fig. 3-2 i). For
For S. asak, we found a positive relationship between the N concentration in mistletoe and the N concentration in infected host branches \((p = 0.0023, R^2 = 0.922)\) (Fig. 3-4 a1), but not between mistletoe and uninfected host branches \((p = 0.0891, R^2 = 0.555, \text{slope equation (Fig. 3-4 a2). There was a significant relationship between the Mn concentration in mistletoe and that of infected host branches \((p < 0.0001, R^2 = 0.987)\), but not between mistletoe and uninfected host branches \((p = 0.711, R^2 = 0.0379)\).\)

For V. flava, there was a positive relationship between the P concentration in mistletoe and that of infected host branches \((p = 0.0409, R^2 = 0.7988)\) (Fig. 3-5 a1), but not between mistletoe and uninfected host branches \((p = 0.1389, R^2 = 0.5721)\) (Fig. 3-5 a2). The foliar Zn concentration in mistletoe was positively correlated to that in infected branches \((p = 0.0285, R^2 = 0.8405)\) (Fig. 3-5 b1), but not to that in uninfected host branches \((p = 0.3496, R^2 = 0.2895)\) (Fig. 3-5 b2).

**Nutrient acquisition mechanisms by mistletoe**

The N concentrations and N:Ca ratios of the host species significantly differed from those of mistletoes. The mistletoe had a significantly higher N concentration than S. asak; thus, the mistletoe: host ratio was greater than 1 (Table 1). The N concentration was lower in mistletoe than in V. flava and V. tortilis, but these differences were not significant (Table 1). The N:Ca ratio of mistletoe growing on S. asak was significantly greater than 1, while mistletoe growing on V. flava and V. tortilis had significantly lower ratios, with N: Ca < 1 (Table 1).
Mistletoe abundance on Vachellia gerrardii in the Wadi Alshafa

Comparison of nutrient concentrations in hosts under different levels of Viscum schimperi infection

There were no statistically significant differences in N, P, K, Na, Fe, Mn, or Zn concentrations between parasitised and unparasitised hosts in the Alshafa Wadi (P > 0.05) (Fig. 3-6 a, b, c, e, g, h, i). However, the Ca concentration significantly differed between heavily infected hosts and unparasitised hosts or slightly infected hosts (F (3, 12) = 14.97, p = 0.0002); heavily infected hosts had higher Ca concentrations than unparasitised or slightly infected hosts (Fig. 3-6 d). In addition, there was a significant difference in Ca concentration between moderately infected hosts and unparasitised hosts, with Ca levels being higher in moderately infected hosts than in unparasitised hosts (Fig. 3-7 d). We detected a statistically significant difference in Mg concentration in heavily infected hosts compared to unparasitised and slightly infected hosts (F (3, 12) = 5.823, p = 0.0108); the heavily infected hosts had a higher Mg concentration than unparasitised and slightly infected hosts (Fig. 3-6 f).

Comparison of nutrient concentrations in hosts under different levels of P. austroarabica infection

There was a statistically significant difference in N concentration between treatments (F (3, 12) = 20.57, p < 0.0001); post hoc comparisons using Tukey’s HSD test indicated that the N concentration was higher in moderately and heavily infected hosts than in slightly infected and unparasitised hosts (Fig. 3-7 a). We detected statistically significant differences in P and Na concentrations between heavily infected hosts and unparasitised hosts; heavily infected hosts had higher P and Na concentrations than unparasitised hosts (Fig. 3-7 b, e). Additionally, a significant difference in Ca concentration was found between moderately and heavily infected hosts vs. unparasitised hosts (F (3, 12) = 11.43, p = 0.0008); Ca concentrations were higher in moderately and heavily infected hosts than in unparasitised hosts (Fig. 3-7 d). Finally, the K, Mg, Fe, Mn, and Zn
concentrations did not significantly differ between treatments ($p > 0.05$) (Fig. 3-7 c, f, g, h, i).

Comparison of nutrient concentrations in hosts under different levels of $P$. curviflorus infection

There was a statistically significant difference in P concentration between heavily infected hosts vs. slightly infected and unparasitised hosts ($F_{(3, 12)} = 5.055, p = 0.0172$); post hoc comparisons using Tukey’s HSD test indicated that heavily infected hosts had higher P concentrations than slightly infected hosts and unparasitised hosts (Fig. 3-8 b). Additionally, we detected a significant difference in Na concentration among groups ($F_{(3, 12)} = 4.203, p = 0.0300$); heavily infected hosts had a higher Na concentration than unparasitised hosts (Fig. 3-8 e), whereas N, K, Ca, Mg, Fe, Mn, and Zn concentrations did not significantly differ among treatments ($p > 0.05$) (Fig. 3-8 a, c, d, f, g, h, i).

Comparison of nutrient concentrations in hosts under different levels of $P$. curviflorus infection

There was a statistically significant difference in P concentration between heavily infected hosts vs. slightly infected and unparasitised hosts ($F_{(3, 12)} = 5.055, p = 0.0172$); post hoc comparisons using Tukey’s HSD test indicated that heavily infected hosts had higher P concentrations than slightly infected hosts and unparasitised hosts (Fig. 3-8 b). Additionally, we detected a significant difference in Na concentration among groups ($F_{(3, 12)} = 4.203, p = 0.0300$); heavily infected hosts had a higher Na concentration than unparasitised hosts (Fig. 3-8 e), whereas N, K, Ca, Mg, Fe, Mn, and Zn concentrations did not significantly differ among treatments ($p > 0.05$) (Fig. 3-8 a, c, d, f, g, h, i).
Discussion

Comparison of nutrient concentrations in parasitised vs. unparasitised hosts

Among infected branches, we detected a reduction in N and Mn contents in *S. asak* and a reduction in P and Zn contents in *V. flava*. *P. curviflorus* usually has a more direct negative influence on infected distal branches (Tennakoon & Pate, 1996). In the current study, we detected a positive linear relationship in the levels of these nutrients between mistletoe and infected host branches, providing evidence that mistletoe has a direct effect on infected branches rather than influencing uninfected branches in both *S. asak* and *V. flava*. Mistletoes often maintain a higher transpiration rate than that of the host, so they may be able to gather significant amounts of nitrogen (Schulze & Ehleringer 1984; Marshall, Dawson, & Ehleringer, 1994; Prider, Watling et al. 2009). This should lead to signs of nitrogen stress in hosts, particularly those growing where nitrogen supply is limiting. Additionally, the foliar N concentration in uninfected branches was considerably lower in parasitised *S. asak* than in unparasitised hosts, although *P. curviflorus* did not affect N concentration in uninfected host branches. These results suggest that mistletoe infection is not the main determinant in the reduction in N levels in the entire host plant. However, this finding suggests that mistletoes may have indirect effects on *S. asak*. We found that mistletoes import nutrients through both passive and active uptake mechanisms. This strategy, combined with the harsh, drier conditions on the slope, can have a negative impact on total N content in the host. This hypothesis is in accordance with Galiano et al. (2011), who found that severe drought, combined with the depletion of N in the leaves of the host plant by mistletoe, had negative effects on Scots pine trees. Specifically, these factors limit the carbon reserve by reducing the amount of photosynthetic tissue in the trees (i.e., defoliation of the canopy), eventually leading to host mortality. This finding suggests that *P. curviflorus* may decrease the amount of photosynthesis occurring in infected host branches, thereby diminishing carbon assimilation through limiting
stomatal conductance in the leaves. Furthermore, mistletoes are dependent on the host phloem to some degree, as it requires substantial amounts of N which, when accompanied by drought stress, can minimize the carbon budget throughout the parasitised host plant. In the present study, we did not measure the photosynthetic capacity of *S. asak*. However, as a probable consequence of mistletoe infection, the interaction between the plant host *S. asak* and its nitrogen fixing rhizobia might be affected by mistletoe infection. This could reduce the host’s production of carbohydrates for the rhizobia, making them unable to fix atmospheric N in the host roots. This reciprocal effect may result from the indirect effect of mistletoe and the simultaneous occurrence of a long drought, which might explain the reduced N levels throughout parasitised *S. asak* plants. This explanation is consistent with a study by Cirocco et al. (2015) showing that hemiparasitic *Cassytha pubescens* has a negative effect on nodule biomass in the root system of the leguminous shrub host *Ulex europaeus*. The disparity in the effects of mistletoe on nutrient status in the host species, including its effect on N and Mn in *S. asak* and on P and Zn in *V. flava*, but not in *V. tortilis*, may result from the position of the host in the landscape; *S. asak* grows on upper slopes, whereas *V. flava* mostly grows on lower slopes, and *V. tortilis* grows in the bench lands of tributaries. Therefore, the nutrient balance may vary in soils among upper and lower slopes and bench lands, which would affect the nutritional relationship between mistletoe and its host in Taif National Park. Furthermore, the variation in nutrient status of the host and mistletoe may be attributed to the different nutrient uptake mechanisms used by mistletoe, which may be affected by the different environmental conditions where the host species grows.

We detected an N:Ca ratio of > 1 in mistletoe infected *S. asak*. Mistletoe may use both passive and active uptake to access high levels of N from the xylem and phloem of *S. asak*, suggesting that mistletoe survives on this plant with the help of active and passive uptake, because *S. asak* grows in harsh, dry conditions on slopes. By contrast, mistletoes that infected other host species growing
at lower elevations had an N:Ca ratio of < 1. These mistletoes mainly use passive uptake to obtain nutrients from the host xylem. This behaviour suggests that these parasites easily acquire nutrients from the host plants, which likely receive an adequate supply of water and nutrients at lower elevations. Moreover, haustorial endophytic structure of mistletoe that allow for different levels of penetration and nutrient acquisition, may be influenced by the variation in genes of Senegalia and Vachellia, coupling with ecological circumstances of the location where hosts grow. This later explanation needs to be examined.

**Nutrient status and the degree of infection for three different species of mistletoe on V. gerrardii**

A major finding of this study is that host trees that were infected by a large number of mistletoes had higher foliar concentrations of some nutrients compared to those infected by a few mistletoes and unparasitised host trees. However, this was not the case for other elements, most notably Ca and Na, in trees infected by *V. schimperi*; N, P, Ca, and Na in trees infected by *P. austroarabica*; and P and Na in trees infected by *P. curviflorus*. The increased nutrient concentrations in heavily infected hosts might occur because such hosts are experiencing rapid hydraulic conductivity in terms of water movement, transpiration rate, and stomatal conductance, allowing the parasites to extract more nutrients from the soil compared to host plants infected by few or no mistletoes. The response of heavily infected hosts may be driven by the mistletoe, which relies on having a higher transpiration rate than the host tree to maintain sufficient nutrient flow from its host, as reported in several studies (Schulze et al., 1984; Ehleringer, Cook, & Tieszen, 1986; Goldstein et al., 1989; Press & Whittaker, 1993; Bannister, Strong, & Andrew, 2002). Mistletoes have a transpiration rate that is 2-3 times higher than their hosts. As a consequence, large groups of mistletoes can accumulate higher concentrations of mineral elements than their hosts (Panvini & Eickmeier, 1993). Therefore, heavily infected hosts may withdraw nutrients from the soil and supply them to
these mistletoes. This mechanism would account for the finding that high levels of nutrients accumulate in heavily infected hosts, which grow more rapidly than trees infected by a few mistletoes and uninfected hosts, as reported by Marias et al. (2014). These findings suggest that the variation in nutrient levels might be due to the reciprocal influence between mistletoes and their hosts. Mistletoes directly affect their hosts by obtaining nutrients from these plants, as mentioned above. Another possible indirect effect is the increase in nutrient levels in host plants heavily infected with *P. austroarabica* and *P. curviflorus* due to the presence of mistletoe litterfall. Since these mistletoes have leaves and can therefore increase nutrient recycling, heavily infected hosts produce high rates of litterfall compared to slightly infected hosts and uninfected hosts. Therefore, nutrient concentrations increase in the soil below the host canopy, enhancing nutrient return for heavily infected hosts. By contrast, host plants heavily infected with the leafless mistletoe *V. schimperi* might produce less litterfall underneath the host canopy, thereby reducing nutrient availability in the soil, particularly for major nutrient elements, and hence, reducing nutrient return for the host. This interpretation is consistent with the finding of Ndagurwa, Dube, and Mlambo (2014) that the density of the mistletoes *Erianthemum ngamicum* and *Plicosepalus kalachariensis* (leafy mistletoes) have a stronger positive influence on litterfall, nutrient return, and nutrient concentrations in soil beneath the host (*Acacia karroo*) than *Viscum verrucosum* (leafless mistletoe). As we noted that different morphological of leaves/stems of mistletoe species may lead to variable nutrient acquisition.

Irrespective of the direct and indirect effects of mistletoe species on the nutrient status of the host, our data for host size indicate that trunk diameter and height were greater in heavily infected hosts than in plants infected by a few mistletoes and unparasitised hosts in the Wadi Alshafa. This result suggests that host size can serve as a good indicator of host quality or nutrient availability in the soil (i.e., valleys) (Watson, 2008). In this case, heavily infected hosts (which are larger and taller
than other host plants) in the Wadi are more likely to access greater amounts of nutrients than other plants, since nutrient elements are primarily available in soils such as wadis in arid lands (Alatar, El-Sheikh, Thomas, Hegazy, & El-Adawy, 2015); these conditions could increase host size, and hence mistletoe number, as reported by (Norton, Ladley, & Owen, 1997; Ndagurwa, Mundy, Dube, & Mlambo, 2012).

**Conclusion**

*P. curviflorus* had various effects on *Senegalia* and *Vachellia*; this mistletoe acquired N and Mn from *S. asak* and P and Zn from *V. flava*, but it did not affect nutrient levels in *V. tortilis*. Also, mistletoe acquired nutrients actively from *V. asak* (i.e. N: Ca > 1), but passively from *V. flava* and *V. tortilis* (i.e. N: Ca < 1). The diversity in mistletoe behaviour to exploit nutrients may reflect the needs of this parasitic plant for specific nutrient elements, and the availability of nutrients in the soil is most likely influenced by the host distribution in various types of topography.

N, P, Na, and Ca levels were higher in host plants heavily infected by *P. austroarabica* and *P. curviflorus*, whereas Mg and Ca levels were higher in hosts heavily infected by *V. schimperi*. The varying physiological responses of mistletoe might be due to different morphological traits such as the morphology of photosynthetic organs, which may lead to variable requirements for nutrients. Regardless of the mistletoe species, the degree of infection is indeed driven by host characteristics, where most of the heavily infected hosts are larger and older than moderately and slightly infected or uninfected hosts, as these host plants can produce a reliable supply of nutrients for a large number of mistletoes.
References


(Table 1) The ratio of nitrogen (N) in mistletoe (*P. curviflorus*)/nitrogen (N) in the host species (*S. asak, V. flava, V. tortilis*), and N:Ca ratio in mistletoe. Mistletoe accumulated higher nutrient concentrations than *S. asak* (N of mistletoe:N of host > 1), while mistletoe accumulated lower nutrient concentrations than *V. flava* and *V. tortilis* (N of mistletoes:N of hosts < 1). Mistletoe on *S. asak* had a N:Ca ratio that was significantly > 1 (active nutrient uptake), while mistletoe on other host species had N:Ca ratios that were significantly < 1 (passive nutrient uptake). Paired t-test, data are mean ± SD, n = 5–6.

<table>
<thead>
<tr>
<th>Habitat</th>
<th>Host species</th>
<th>N of mistletoe: N of host</th>
<th>N: Ca Mistletoes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper slope</td>
<td><em>S. asak</em></td>
<td>1.095±0.015**</td>
<td>1.139±0.0423*</td>
</tr>
<tr>
<td>Lower slope</td>
<td><em>V. flava</em></td>
<td>0.916±0.059 ns</td>
<td>0.461±0.154**</td>
</tr>
<tr>
<td>Bench land</td>
<td><em>V. tortilis</em></td>
<td>0.916±0.135 ns</td>
<td>0.452±0.210*</td>
</tr>
</tbody>
</table>

* P ≤ 0.05, ** P ≤ 0.01, ns = not significant.
Mineral element concentrations in non-parasitised and parasitised *S. asak*, including a) nitrogen (N), b) phosphorus (P), c) potassium (K), d) calcium (Ca), e) sodium (Na), f) magnesium (Mg), g) iron (Fe), h) manganese (Mn), and i) zinc (Zn) in infected host branches (IB, green fill), uninfected branches (UB, gray fill) from parasitised hosts, and control branches (HB, no fill) from unparasitised hosts. Different letters indicate statistically significant differences ($p < 0.05$), while similar letters indicate no statistically significant difference ($p > 0.05$) for nitrogen and magnesium. “ns” indicates no statistically significant difference, “sig” indicates a statistically significant difference, (%) indicates the percentage of mg/g dry weight, (ppm) represents part per million, (—) the line in the middle of the box-whisker represents the median, (+) represents the mean, the boxes denote the interquartile range, and the whiskers denote the minimum and maximum values of the dataset, n = 6.
(Fig. 3-2) Mineral element concentrations in non-parasitised and parasitised *V. flava*, including a) nitrogen (N), b) phosphorus (P), c) potassium (K), d) calcium (Ca), e) sodium (Na), f) magnesium (Mg), g) iron (Fe), h) manganese (Mn), and i) zinc (Zn) in infected host branches (IB, green fill), uninfected host branches (UB, gray fill) of parasitised hosts, and control branches (HB, no fill) of unparasitised hosts. Different letters indicate statistically significant differences (\(p < 0.05\)), while similar letters indicate no statistically significant difference (\(p > 0.05\)) for phosphorus and zinc. “ns”, indicates no statistically significant difference, “sig” indicates a statistically significant difference, (%) indicates the percentage of mg/g dry weight, (ppm) represents part per million, (—) line in the middle box-whisker represents the median, (+) represents the mean, the boxes denote the interquartile range, and the whiskers denote the minimum and maximum values of the dataset, \(n = 4–6\).
(Fig. 3-3) Mineral element concentrations in non-parasitised and parasitised *V. tortilis*, including a) nitrogen (N), b) phosphorus (P), c) potassium (K), d) calcium (Ca), e) sodium (Na), f) magnesium (Mg), g) iron (Fe), h) manganese (Mn), and i) zinc (Zn) in infected host branches (IB, green fill), uninfected branches (UB, gray fill) of parasitised hosts, and control branches (HB, no fill) of unparasitised hosts. “ns”, indicates no statistically significant difference (p > 0.05) in nutrient concentration, (%) indicates the percentage of mg/g dry weight, (ppm) represents parts per million, (—) line in the middle box-whisker represents the median, (+) represents the mean, the boxes denote interquartile range, and the whiskers denote the minimum and maximum values of the dataset, n = 4–6.
Linear regression analysis of nitrogen (N) and manganese (Mg) concentrations in mistletoe (*P. curviflorus*) and infected and uninfected branches of the host (*S. asak*). The slope shows the nutritional relationship between mistletoe and its host. 

- **a1)** Balance of N concentration between mistletoe and infected branches ($p = 0.0023$, $R^2 = 0.922$, slope equation $Y = 1.614 \times X - 0.6391$), **a2)** balance of N concentration between mistletoe and uninfected branches ($p = 0.0891$, $R^2 = 0.555$, slope equation $Y = 1.815 \times X - 0.7807$).
- **b1)** balance of Mn concentration between mistletoe and infected branches ($p < 0.0001$, $R^2 = 0.987$, slope equation $Y = 0.7690 \times X + 6.632$), **b2)** balance of Mn concentration between mistletoe and uninfected branches ($p = 0.711$, $R^2 = 0.0379$, slope equation $Y = -0.06983 \times X + 21.95$).

“sig” indicates a statistically significant relationship, “ns” indicates not statistically significant, $n = 6$. 

(Fig. 3-4)
(Fig. 3.5) Linear regression analysis of phosphorus (P) and zinc (Zn) concentrations in mistletoe (*P. curviflorus*) and infected and uninfected branches of the host (*V. flava*). The slope shows nutritional relationships between mistletoe and its host. 

a1) Balance of P concentration between mistletoe and infected branches ($p = 0.0409$, $R^2 = 0.7988$, slope equation $Y = 2.645X - 0.3350$), a2) balance of P concentration between mistletoe and uninfected branches ($p = 0.1389$, $R^2 = 0.5721$, slope equation $Y = -0.9543X + 0.6508$), b1) balance of Zn concentration between mistletoe and infected branches ($p = 0.0285$, $R^2 = 0.8405$, slope equation $Y = 0.9605X + 7.089$), b2) balance of Zn concentration between mistletoe and uninfected branches ($p = 0.3496$, $R^2 = 0.2895$, slope equation $Y = -0.5008X + 51.63$).
Tukey’s multiple comparisons test of mineral element concentrations in the host *V. gerrardii* and the mistletoe *V. schimperi* for a) nitrogen (N), b) phosphorus (P), c) potassium (K), d) calcium (Ca), e) sodium (Na), f) magnesium (Mg), g) iron (Fe), h) manganese (Mn), and i) zinc (Zn) using infection classes that quantify different levels of infection by the mistletoe. Non-host (no fill) indicates non-parasitised hosts, host (L, gray fill) indicates ‘slight infection’ (a single mistletoe per tree, n = 1), host (M, green fill) indicates ‘moderate infection’ (2–3 mistletoes per tree), and host (H, red fill) indicates ‘heavy infection’ (≥4 mistletoes per tree). Different letters indicate statistically significant differences (*p* < 0.05), while the same letters indicate no statistically significant difference. “ns”, indicates no statistically significant difference (*p* > 0.05), box plots represent interquartile range, whiskers represent minimum and maximum values, (—) line in the middle box and whiskers denotes the median, (+) indicates the mean, (ppm) represents parts per million, and (%) indicates the percentage of foliar concentration of elements (mg/g dry weight), n = 4.
Tukey’s multiple comparisons test of mineral element concentrations in the host *V. gerrardii* infected by the mistletoe *P. austroarabica* for a) nitrogen (N), b) phosphorus (P), c) potassium (K), d) calcium (Ca), e) sodium (Na), f) magnesium (Mg), g) iron (Fe), h) manganese (Mn), and i) zinc (Zn) using infection classes that quantify different levels of infection by the mistletoe. Non-host (no fill) indicates non-parasitised hosts, host (L, gray fill) indicates ‘slight infection’ (a single mistletoe per tree, n = 1), host (M, green fill) indicates ‘moderate infection’ (2–3 mistletoes per tree), and host (H, red fill) indicates ‘heavy infection’ (≥4 mistletoes per tree). Different letters indicate statistically significant differences (p < 0.05), while the same letters indicate there no statistically significant difference. “ns”, indicates no statistically significant difference (p > 0.05), box plots represent interquartile range, whiskers represent minimum and maximum values, (—) line in the middle box and whisker denotes the median, (+) indicates the mean, (ppm) represents parts per million, and (%) indicates the percentage of foliar concentration of elements (mg/g dry weight), n = 4.
(Fig. 3-8) Tukey’s multiple comparisons test of mineral element concentrations in the host *V. gerrardii* infected by the mistletoe *P. curviflorus* for a) nitrogen (N), b) phosphorus (P), c) potassium (K), d) calcium (Ca), e) sodium (Na), f) magnesium (Mg), g) iron (Fe), h) manganese (Mn), and i) zinc (Zn) using infection classes that quantify different levels of infection by the mistletoe. Non-host (no fill) indicates non-parasitised hosts, host (L, gray fill) indicates ‘slight infection’ (a single mistletoe per tree, n = 1), host (M, red fill) indicates ‘moderate infection’ (2–3 mistletoes per tree), host (H) indicates ‘heavy infection’ (≥4 mistletoes per tree). Different letters indicate statistically significant differences (*p* < 0.05), while similar letters indicate no statistically significant difference. “ns”, indicates no statistically significant difference (*p* > 0.05), box plots represent interquartile range, whiskers represent minimum and maximum values, (—) line in the middle box and whisker denotes the median, (+) indicates the mean, (ppm) represents parts per million, and (%) indicates the percentage of foliar concentration of elements (mg/g dry weight), n= 4.
Trunk diameter, height, and canopy area in trees under different levels of mistletoe infection and in uninfected trees. For host height, the *P. curviflorus* infection class is significant at \(^*p < 0.05, F = 4.665, R^2 = 0.538\); the *P. austroarabica* infection class is significant at \(^**p = 0.0007, F = 11.56, R^2 = 0.743\); the *V. schimperi* infection class is significant at \(^***p = 0.0018, F = 9.388, R^2 = 0.7\). For trunk diameter, the *P. curviflorus* infection class is significant at \(^***p = 0.05, F = 14.46, R^2 = 0.783\); the square root transformed data for *P. austroarabica* infection class are significant at \(^****p < 0.0001, F = 18.52, R^2 = 0.822\); square root transformed data for *V. schimperi* infection class are significant at \(^***p = 0.0005, F = 12.60, R^2 = 0.75\). For the host canopy area, the *P. curviflorus* infection class is significant at \(^***p < 0.0001, F = 32.41, R^2 = 0.8901\); square root transformed data for *P. austroarabica* infection class are significant at \(^***p < 0.0001, F = 42.81, R^2 = 0.9145\); square root transformed data for *V. schimperi* infection class are significant at \(^***p = 0.0002, F = 8.8001, R^2 = 16.01\). Different letters indicate significant differences between treatments, while similar letters indicate no significant difference between treatments. Mistletoe infection classes are as follows: (None, no fill) unparasitised hosts, (L, speckled fill) slightly infected hosts, (M, gray fill) moderately infected hosts, and (H, black fill) heavily infected hosts, \(n = 4\), ANOVA).
Statement of Authorship

Title of Paper
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Dhafer A. Albakre

Contribution to the Paper
D.A.B conceived the ideas and methodology, analysed data, and wrote manuscripts and acted as corresponding author.

Overall percentage (%)
80%

Certification:
This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.

Signature

Date
27 March 2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate’s stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate to include the publication in the thesis; and
iii. The sum of all co-author contributions is equal to 100% less the candidate’s stated contribution.

Name of Co-Author
José M. Facelli

Contribution to the Paper
J.M.F conceived the ideas, interpreted data, critically assessed to drafts and issued final approval for publication.

Signature

Date

Please cut and paste additional co-author panels here as required.
Chapter 4: Host–mistletoe interactions on a branch scale:
Investigating the nutritional relationship under coexistence of three
mistletoe species on a single host tree, *Vachellia gerrardii*, in the
western Arabian Peninsula

Dhafer Albakre* and José M. Facelli
School of Biological Sciences, the University of Adelaide, Adelaide, SA 5005, Australia.
*Author for correspondence: Dhafer A. Albakre, Email: Dhafer.albakre@adelaide.edu.au

Abstract

Differences in mineral nutrition in various species of mistletoes are limited by the availability of
suitable infection patterns that rule out confounding factors. In Wadi Alshafa in the Arabian
Peninsula, three different species of mistletoe (*Viscum schimperi*, *Plicosepalus curviflorus*, and
*Phragmanthera austroarabica*) coexist on a single host tree species (*V. gerrardii*). We investigated
whether the N, P, K, Na, Ca, and Mg concentrations differed in these mistletoes, and assessed the
relationship between the nutrient contents in the mistletoes and the infected branches. We found
that there were important differences in the concentrations of various nutrients in the three
mistletoe species. N and Na levels were highest in *P. austroarabica*, and P level was higher in *V.
schimperi* than in *P. curviflorus*. In addition, N and P contents were higher in *P. austroarabica*
than in the host branch. While the K content was higher in all mistletoe species than in the host
branch, the K content was lower in branches infected by *V. schimperi* than in branches infected by
other mistletoe species. By contrast, Ca levels were higher in host branches infected by *V.
schimperi* compared to other species, while Mg levels were higher in *V. schimperi* than in the host
branch. These results suggest that the disparity in nutrient contents among mistletoe species and/or
in the host branch versus mistletoe pairs might
represent a response to the variation in the morphological traits of mistletoes that help them adapt and survive to harsh conditions in the Wadi.

**Key words:** mistletoe coexistence, mineral nutrition, branch scale, Wadi, Arabian Peninsula,

**Introduction**

Mistletoes play an important role in nutrient dynamics in most ecosystems (March & Watson, 2007). Leaf morphology is a key component that shapes nutrient cycling (Ndagurwa et al., 2014) and therefore determines the availability of essential nutrients, which influence plant productivity, diversity, and growth through modifying the homogeneity of nutrients in the soil beneath the host plant (Facelli & Pickett, 1991). The effect of mistletoe infection on the nutrient status of the host is variable and mostly depends on the health of the host, which in turn is affected by the environment. Therefore, trees with greater access to water and nutrients from the soil are more likely to support mistletoes than are other trees (Watson, 2008).

Mistletoes maintain the one-way transport of mineral elements from the host xylem to their own vessels through the haustorium. They consequently reduce the nutrient concentration in the infected branches, which leads to a reduced growth rate in the infected branches of the hosts (Ehleringer et al., 1986; Tennakoon & Pate, 1996; Daryaei & Moghadam, 2012).

However, the reverse has also been shown, whereby the host’s surrounding environment (Türe et al., 2010) and the host’s nutrient quality can have an even more significant effect on the likelihood a host will become infected with mistletoe (Watson, 2008). Therefore, some studies (Bowie & Ward, 2004; Okubamichael et al., 2011) found that the nitrogen concentration in mistletoe was lower than that of the host.
For example, in the Negev Desert, Bowie and Ward (2004) found that the host *Acacia raddiana* had a negative impact on mistletoe *Plicosepalus acacia*, and that the nitrogen concentration of mistletoe was 65 per cent less than that of the host acacia.

In the past, research has focused on the seasonal intake of mineral elements of mistletoe and its host (Glatzel, 1983; Bannister et al., 2002), while other studies focused on the issue of nutrient uptake mechanisms by mistletoe, i.e. whether mistletoe takes up nutrient from the host xylem (Ehleringer et al., 1986; Tennakoon & Pate, 1996), or from the host phloem (Bowie and Ward, 2004, Okubamichael et al., 2011). The effect of mistletoe on nutrient concentration in the host also depends on the taxonomic characteristics of the mistletoe species. Some studies (Shaw, Watson, & Mathiasen, 2004; Marina & Wright, 2015) have suggested that visaceous mistletoes have a greater impact on their hosts than loranthaceous mistletoes; Marina and Wright (2015) found that across various environments worldwide, Viscaceae mistletoes, but not Loranthaceae mistletoes, have a higher nitrogen concentration than their hosts. However, the accuracy of comparing the contents of nutrients between mistletoe species on a global scale is limited, since nutrient levels are affected by variations in the surrounding environments, such as the availability of water and nutrients in soil for the host, which often differs from one place to another. Studies comparing different species of mistletoe growing on the same host species under the same environmental conditions are needed to obtain detailed information about this question.

In the present study, we compared nutrient concentrations in three species of mistletoe with different morphological traits. These mistletoes belong to two families: Loranthaceae, with two species, the woodrose *P. austroarabica*, which has very large, dark-green leaves (leaf area up to 40 cm²) and *P. curviflorus*, which has much smaller, sparse, narrow grey-green leaves (leaf area up to 3.5 cm²); and Viscaceae, with one species, *V. schimperi*, which is leafless and has succulent green stems (Fig. 4.1a, b, c) (Collenette, 1999).
These species coexist under similar environmental conditions and on the same host, an unusual pattern of mistletoe infection that exclusively occurs on *V. gerrardii*, a preferred host. This pattern of infection allows us to address the following questions: a) based on the different families and leaf morphological traits of the three mistletoe species, do the concentrations of nutrients in their tissues differ? and b) do the nutrient levels of infected branches vary according to the mistletoe species infecting them?
Methods

Study area

Wadi Alshafa is a dry valley located in the western region of the Arabian Peninsula at approximate latitude of 21° 12’ 11.66” N and longitude of 40° 20’ 44.72” E. The average annual rainfall for 2003–2013 was approximately 128.76 mm. The average temperature varies between 20 and 39°C in summer and between 6 and 29°C in winter (Al-Shaibani, 2008). The Wadi Alshafa is approximately 12 km long and gradually slopes down from the southwest to the northeast, from an elevation of 2000 masl to 1690 masl. *V. gerrardii* is a leguminous tree found along the stream bed in the Wadi. This tree is widely distributed clay to gravelly sand soils throughout the Wadi. *V. gerrardii* is preferred host mistletoes and is infected by three different species of mistletoe.

Study design

Sampling protocol

Six 50 m transects were distributed every 2 km along 12 km of the Wadi. Along the transects, we marked all trees that hosted three different species of mistletoe and excluded trees infected by one or two species; thirty trees were selected for analysis. We collected the leaf samples from young mistletoes which have the same sizes, and growing on the peripheral of the host canopy, those samples were selected at approximately 3 meter in height among all host trees. Leaf samples or photosynthetic stems in the case of *V. schimperi* were taken from each mistletoe and from branches infected by these mistletoe species (Fig. 4.1). Chemical analysis of the samples was conducted by the laboratory of the Ministry of Environment, Water and Agriculture under the supervision of King Saud University. For each sample, 0.5 g of dry ground tissue was prepared and digested using the method described in Chapman (1966). The total nitrogen (N) content was measured by Kjeldahl distillation. Phosphorus (P) was measured via a colorimetric method. The concentrations
of potassium (K) and sodium (Na) were measured with a photoelectric flame photometer, while calcium (Ca) and magnesium (Mg) were measured with an atomic absorption spectrometer (Chapman, 1966).

Samples were labelled using “Tree ID”, which indicated the identity of the host tree. Each host tree produced the second main effect, “Branch character”, which was composed of two categories indicating if the samples had been taken from a mistletoe (M) or from the host branch (H). Each mistletoe (M) and host branch infected by a given species (H) formed the third main effect, the “Mistletoe species” variable, which was composed of *V. schimperi*, *P. curviflorus*, or *P. austroarabica*.

Analytical approach

We assessed whether nutrient acquisition was associated with mistletoe species, infected branch, and the entire host tree. The concentrations of six nutrients, including nitrogen (% N), phosphorus (% P), potassium (% K), calcium (Ca ppm), sodium (Na ppm), and magnesium (Mg ppm), represented response variables, which were taken from each sample. We organised samples as a hierarchical model in order to detect the influence of variables on the nutrient contents of the host. Three explanatory variables formed the model, “Tree ID”, “Branch character”, and “Mistletoe species”, which also involved the interactive effect resulting from the variables “Branch character” and “Mistletoe species”.

Data diagnosis

The R package (3.2.2) was run to evaluate univariate normality using a Q-Q plot. The data for N and P did not require transformation for analysis, but the other variables did, and the data were consequently transformed to fit the normal distribution as follows: sqrt(K), Ca^ (0.6), log (Mg), Na^ (0.25). In addition, the transformed data were examined for multivariate outliers to ensure that
the analyses would not be biased by overly influential points. Adjusted quantile plots and bivariate
correlation plots from the “mvoutlier” package in R were used. Therefore, the outliers were not
serious enough to produce misleading results.

**Data analysis**

Once we demonstrated the validity of the data, ANOVA was implemented with a linear model to
examine the fit of the model on independent variables using residual plots, showing residual vs.
fitted values, normal Q-Q plot, scale-location, and constant leverage residual vs. factor levels. For
all nutrients, the outputs of fit model included all explanatory variables as follows:

\[
\text{Nutrients} \sim \text{TreeID} + \text{Branch character} \times \text{Mistletoe species}
\]

The outputs of the fit model were presented through the effect of a plot with a 95% confidence
interval that was examined using the default effect function in R, a robust method for showing the
main and interactive effects between independent variables, by plotting the ANOVA results in a
graph (Fox, 2003). The effect plots also showed how the data appeared after ANOVA had
accounted for the effects of the entire host tree “Tree ID” as an independent variable.
Results

The concentrations of N and Na were higher in *P. austroarabica* than in *P. curviflorus*, but these concentrations were not high in *V. schimperi* compared to *P. austroarabica* (Fig. 4.2a; Fig. 4.3a). The concentration of P was higher in *V. schimperi* than in *P. curviflorus*, but it was not high in *P. austroarabica* compared to *V. schimperi* (Fig. 4.2b).

Foliar concentrations of N and P were higher in mistletoe *P. austroarabica* than in the host branch (Fig 4.2 a, b). No interactive effect was detected for N and P concentrations (Table 4.1). Notably, the K concentration was considerably higher in all mistletoe species than in the host branches (Fig 4.2 c). Furthermore, an interactive effect was detected for K (Table 4.1), with lower concentrations of K in host branches infected by *V. schimperi* (mean = 0.16, 95% CI [0.14, 0.18]) than in host branches infected by *P. austroarabica* (mean = 0.21, 95% CI [0.19, 0.23] and *P. curviflorus* (mean = 0.23, 95% CI [0.21, 0.25]) (Fig 4.2 c); however, no difference in K content was detected among the mistletoe species. The Ca concentration was lower in mistletoe *V. schimperi* than in the host branches (Fig 4. 3b), and an interactive effect was also found for Ca, as host branches infected by *V. schimperi* had a higher Ca concentration (mean = 251.1, 95% CI [228.1, 274.2]) than host branches infected by *P. curviflorus* (mean = 201.2, 95% CI [176.0, 226.4]). No difference in Ca was found between host branches infected by *V. schimperi* and those infected by *P. austroarabica*, nor among mistletoe species (Fig. 4.3b). We found higher concentrations of Mg in mistletoe *V. schimperi* (mean = 6.8, 95% CI [6.6, 6.9] than in the host branches (mean = 6.3, 95% CI [6.5, 6.1]) (Fig 4.3c), with an interactive effect between branch character and mistletoe species showing a higher concentration of Mg in mistletoe *V. schimperi* (mean = 6.8, 95% CI [6.6, 6.9] than in mistletoe *P. austroarabica* (mean = 6.232, 95%CI [6.090, 6.374]) and mistletoe *P. curviflorus*.
(mean = 6.3, 95%CI [6.2, 6.5]) (Fig. 4.3c). No differences were detected among host branches for all mistletoe species examined (Fig. 4.3c).

**Discussion**

**Mistletoe species**

The three mistletoe species examined have rather different mineral nutrition patterns, as they accumulate nutrients at different levels when growing on identical hosts. Our data show that *P. austroarabica* accumulates N and Na. The selective uptake of these nutrients might be a function of the leaf traits of these mistletoes. The woodrose *P. austroarabica* produces very large leaves, fruits, and haustoria. Therefore, *P. austroarabica* might require high levels of N to increase metabolic processes such as photosynthesis in order to produce higher biomass (Schulze & Ehleringer, 1984; Ehleringer et al., 1986). Additionally, the higher levels of Na in *P. austroarabica* suggest that this species encounters higher water potential gradients than the other species. Na acts as a partial substitute for K (Taiz & Zeiger, 2002), regulating the opening and closing of stomata and thus internal water balance. Therefore, the large leaves of *P. austroarabica* might undergo higher rates of transpiration than smaller leaves, which would require Na to support the osmotic potential of this mistletoe. Indeed, the Australian stem-hemiparasite *Cassytha pubescens* has higher concentrations of Na when the host experiences limiting water conditions (Cirocco et al., 2015). We found that *V. schimperi* reaches a higher concentration of P than the other species. This mistletoe has a distinctive growth form; the absence of leaves and the high moisture content of its succulent shoots prevent desiccation, especially when the host plant suffers from water stress (Atwell, Kriedemann, & Turnbull, 1999). It is possible that the reduced transpiration rate and the higher concentrations of stored P are due to active uptake of P by this mistletoe under harsh
conditions in the Wadi. On the other hand, the low concentrations of N, Na, and P in *P. curviflorus* might be due to a mechanism in this mistletoe that helps it receive these nutrients from the host. This effect is especially pronounced in the context of mistletoe coexistence, where *P. austroarabica* and *V. schimperi* might use both active and passive uptake mechanisms to obtain nutrients from the xylem and phloem tissues of the host, whereas *P. curviflorus* might only use passive uptake from the host xylem to obtain essential nutrients. The present study suggests that the variation in the morphological characteristics of mistletoes, and the response of the mistletoes to the environmental condition such as harsh condition, can lead to active nutrient uptake, thus determining their demand for nutrients.

**Mistletoes and nutrient status of the host branches**

We found that the presence of *P. austroarabica* greatly reduced N and P levels in the host. This mistletoe, which produces large leaves and fruits, is thought to have a higher rate of transpiration than the host.

Indeed, several studies (Glatzel, 1983; Ullmann et al., 1985) have shown that the transpiration rate of mistletoe is often higher than that of the host, which allows the parasite to continuously import nutrients from the host.

On the other hand, P and N levels did not differ between both *V. schimperi* and *P. curviflorus*, and the infected branches of the host. This result is consistent with the findings of Marina and Wright (2014) involving a wide range of mistletoe species and their hosts, particularly the finding that N concentration is not a limiting factor for mistletoes, but instead mistletoes are driven by water availability. This explanation is reasonable in light of the response of the host trees to water stress in the Wadi. The distinct morphological traits of *V. schimperi* (the absence of leaves) and *P. curviflorus* (small leaves) might be compatible with the limited physiological performance of these mistletoes in response to the limited availability of water in a site or in the host. This can affect
nutrient exploitation and result in these mistletoes having the same nutrient concentrations as their host branches. Notably, the concentration of K, which regulates osmotic potential (Taiz & Zeiger, 2002), was higher in all mistletoe species than in the host branches; this can be connected to mistletoe species often experiencing lower water potential than the host branches (Glatzel & Geils, 2009). In addition, the enriched levels of K in mistletoes might be due to the passive accumulation of this element. Typically, only the xylem connects mistletoe to the host branch. When K moves through the host xylem from the roots to leaves, the mistletoe acts as a sink for K (Glatzel, 1983; Glatzel & Geils, 2009; Gebauer, Volařík, & Urban, 2012).

We found that branches infected by *V. schimperi* had lower K levels than did those infected by *P. austroarabica* and *P. curviflorus*. The strong acquisition of K by *V. schimperi* may result from active accumulation, and perhaps this mistletoe can deplete K from the host phloem as well as the host xylem. However, this interpretation requires further evidence. We detected an interactive effect for Ca accumulation, which was higher in host branches infected by *V. schimperi* than for host branches infected by *P. austroarabica* and *P. curviflorus*.

This difference can be attributed to the higher accumulation of Ca when the branch supports *V. schimperi*, providing some evidence that this mistletoe may facilitate a higher level of Ca concentration in the host branches, enhancing the vitality of host branches, as a strategy for this mistletoe to maintain nutrient circulation with host branches. Alternatively, this mistletoe might demand a specific quantity of Ca as an adaptive strategy, especially in nutrient-poor soils in arid lands. In arid environments of Africa, *Viscum rotundifolium* depletes nutrients from the phloem and xylem of the host (passive and active uptake together). This mistletoe mechanism provides an advantage in the face of a lack of nutrients in dry land (Okubamichael et al., 2011).
Furthermore, a significant interactive effect was detected for a higher level of Mg in *V. schimperi* vs. its host branch and the other mistletoe species. Mg is an important component of chlorophyll (Taiz & Zeiger, 2002). It is possible that due to an important functional aspect of *V. schimperi*, namely, that this mistletoe depends on its greenish succulent stems as a photosynthetic organ (Kuijt, 1969), this species utilizes more Mg to constitute chlorophyll than the other species, especially when it encounters limited light in the host canopy. Alternatively, the high concentration of Mg in this species may be attributed to the element status, with an insoluble form more highly concentrated in mistletoe than its host (Press & Graves, 1995). At the tree level, we found variations in the concentrations of P, Na, Ca, and Mg, whereas the concentrations of N and K did not vary among host trees infected with different species of mistletoe, pointing to the varying level of available nutrients in the soil supporting the targeted trees. N (only for *P. austroarabica*) and K (for all mistletoe species) may be the most important nutrients for the survival of mistletoes in the Wadi system. Thus, the morphological traits of various mistletoe species appear to influence the patterns of nutrient accumulation in the mistletoes and the host branches they infect, and both the morphology and the nutrient content of mistletoes represent adaptations to arid conditions.
Conclusion
The woodrose *P. austroarabica* had the most negative impact on nutrients especially N, P, K in the infected branches. The three species accumulated different levels of nutrients. *P. austroarabica* had higher concentrations of N and Na, and *V. schimperi* had a higher concentration of P than *P. curviflorus*. Furthermore, *V. schimperi* accumulated much more Mg than the other mistletoes. Also, Ca levels were higher in branches infected by *V. schimperi* than in those infected by *P. austroarabica* or *P. curviflorus*. However, the mistletoes only had a slight effect on the nutrient content of their host branches; these values did not differ, except that *P. austroarabica* had higher levels of N and P than the host, and all mistletoes had higher concentrations of K than their host branches. Regardless of whether the variability in nutrient accumulation resulted from different levels of passive or active uptake of nutrients among mistletoes, the functional variation in morphological traits of these mistletoes (such as photosynthetic organs) can shape nutrient acquisition.
References


Table 4.1 Summary of (ANOVA) outputs showing the fit to a linear model for the effect of three different species of mistletoe (as explanatory variables) on six nutrient elements (as response variables) in a single host in the Wadi Alshafa.

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<th>Branch character (DF = 1)</th>
<th>Mistletoe species (DF = 2)</th>
<th>Interaction Branch character × Mistletoe species (DF = 2)</th>
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<td>F</td>
<td>Mean sq.</td>
<td>Mean sq.</td>
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‘***’, **, and *: Significant at p<0.001, 0.01, and 0.05, respectively.
Figures

(Fig. 4.1) The three species of mistletoe, (a) *V. schimperi* (leafless), (b) *P. curviflorus* (small leaves), and (c) *P. austroarabica* (very large leaves), which coexist on the dominant host species *V. gerrardii* throughout the study area in Wadi Alshafa. Black circle denotes *V. schimperi* infection, blue circle denotes *P. austroarabica* infection, yellow circle denotes *P. curviflorus* infection. Abbreviations are (H) haustorium, (Mis) mistletoe, and (InB) infected branch.
(Fig. 4.2) The concentrations of (a) nitrogen, (b) phosphorus, and (c) potassium in three species of mistletoe (P. curviflorus, P. austroarabica, and V. schimperi) and in their host’s (V. gerrardii) branches. Nutrient concentrations were determined in two samples taken from the distal branches of the host (H) and from mistletoe (M). The vertical axis shows nutrient concentration in (%) mg/g dry weight. The red dashed lines are pointwise 95% confidence intervals drawn around the estimated effect.
(Fig. 4.3) The concentrations of (a) sodium, (b) calcium, and (c) magnesium in three species of mistletoe (P. curviflorus, P. austroarabica, and V. schimperi) and in their host’s (V. gerrardii) branches. Nutrient concentrations were determined in two samples taken from the distal branches of the host (H) and from mistletoe (M). The vertical axis shows nutrient concentrations in mg/mL (ppm) dry weight. The red dashed lines are pointwise 95% confidence intervals drawn around the estimated effect.
# Statement of Authorship

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<th>Effects of the mistletoe <em>Plicosepalus curviflorus</em> on the water status and photosynthetic performance of four <em>Acacia</em> species on the western Arabian Peninsula</th>
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## Principal Author

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<tr>
<th>Name of Principal Author (Candidate)</th>
<th>Dhafer A. Albakre</th>
</tr>
</thead>
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<tr>
<td>Contribution to the Paper</td>
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</tr>
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<td>Overall percentage (%)</td>
<td>80%</td>
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<td>Certification:</td>
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**Date** 27 March 2019

## Co-Authors Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate’s stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. The sum of all co-author contributions is equal to 100% less the candidate’s stated contribution.

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<th>José M. Facelli</th>
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<tr>
<td>Contribution to the Paper</td>
<td>J.M.F conceived the ideas, interpreted data, critically assessed to drafts and issued final approval for publication.</td>
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**Signature**

**Date**

Please cut and paste additional co-author panels here as required.

123
Chapter 5: Effects of the mistletoe *Plicosepalus curviflorus* on the water status and photosynthetic performance of four host species in the western Arabian Peninsula

Dhafer Albakre* and José M. Facelli
School of Biological Sciences, the University of Adelaide, Adelaide, SA 5005, Australia.
*Author for correspondence: Dhafer A. Albakre, Email: Dhafer.albakre@adelaide.edu.au

This chapter has been written following the style of the *Annals of Botany*. 

124
Summary

• Rationale: In arid regions where xeric plants endure severe drought conditions, parasitism by mistletoe adds another physiological stress to host plants. We investigated the effects of mistletoe infection on four host species in dry and wet periods.

• Methods: The photosynthetic performance and water potential status of mistletoe (*Plicosepalus curviflorus*) and four hosts (*Senegalia asak*, *Vachellia flava*, *Vachellia gerrardii*, and *Vachellia tortilis*) were measured. The light use efficiency of leaves and water status of twigs were assessed. Predawn and midday quantum yields, and water potential gradients were assessed during the dry and wet periods.

• Main results: *P. curviflorus* growing on *S. asak*, *V. flava*, and *V. tortilis* had lower predawn quantum yields and greater negative predawn water potentials in the dry period than in the wet period. Independent of the period, mistletoes had lower or equal $F_v/F_m$ ratios and midday quantum yields than those of the hosts’ infected branches, except in *S. asak* during the wet period, when mistletoe had a greater $F_v/F_m$ ratio. The predawn and midday water potentials in *P. curviflorus* were lower than those of infected branches during the dry and wet period, except in mistletoe on *S. asak* during the dry period. There were no differences in midday quantum yields among infected host branches, uninfected host branches, and uninfected hosts in either period.

• Conclusions: Mistletoe performed differently on the four hosts, possibly because of the hosts’ distribution or morphological characteristics that affect the water balance of these species. Mistletoe infection had a greater impact on the water stress of infected branches of *S. asak*, *V. flava*, and *V. tortilis* compared to *V. gerrardii* during the dry period. The infected branches of *S. asak* were water-stressed even in the wet period and were more prone to chronic photoinhibition, possibly because of the constant water demand of *P. curviflorus* to avoid host exclusion in the drier slope environment.

Key words: Chronic photoinhibition, light use efficiency, quantum yield, water potential, mistletoe, Arabian Peninsula.
**Introduction**

Mistletoes can have important effects on individuals, populations, communities, and ecosystems. Their different effects on various species can affect species abundance and the diversity and function of the ecosystem (Norton and Carpenter, 1998; Press and Phoenix, 2005; Mathiasen et al., 2008). Documenting and understanding effects of mistletoes on different species can increase our understanding of the ecological properties of a system.

The host and mistletoe performance would be greatly consistent in their wet condition; however, it is useful to look at the physiological performance in response to drought. Presumably, acacias, as xeric wood plants, can tolerate severe drought periods. They typically develop an extensive root system rather than the green parts above the soil, drawing water from deep soil, increasing the levels of abscisic acid (ABA) at the same time—a plant hormone which triggers the stomatal closure. This restricts water loss and limits CO2 from entering, thereby diminishing the photosynthesis, especially during noon (Chaves et al., 2002; Lambers and Chapin et al. 2008). By removing water from the mistletoe, the host tree may experience a double stress or an additive effect (drought plus infection), so this situation exacerbates the symptoms of chronic water stress. For the mistletoe, however, if the host experiences water stress, this may also induce water stress in the parasite (Mathiasen et al., 2008).

At predawn time or shade condition may mitigate the negative effects of mistletoe on the water status of the host by reducing the evaporative rate, the mistletoe can maintain the negative water potential lower than the host; and also transpire rapidly, thus removing water and probably inducing water stress in the host (Whittington and Sinclair, 1988; Strong and Bannister 2002).
As mentioned above, water stress in the host and a downregulation of stomatal conductance reduce CO2 assimilation into the host leaf. This can interrupt photochemical activities (light use efficiency) for chlorophyll florescence, causing a breakdown of the photosynthesis known as a photoinhibition (Demmig-Adams and Adams III, 1992).

Although mistletoes always have low water potentials to maintain water movement from the host (Ehleringer and Marshall, 1995), the water relationship between the host and mistletoe may undergo periodic and geographical changes. In the Nevada desert in the USA, *Acacia gregii* trees parasitized by the mistletoe *Phoradendron californicum* were under greater stress at terrace sites than at stream bed sites during summer (Lei, 1999). As mistletoe continually depletes water from its host, the impacts of mistletoe on the host vary from minor physiological effects such as reducing CO2 uptake in host leaves (Goldstein *et al*., 1989; Těšitel *et al*., 2010; Galiano *et al*., 2011) to severe effects, especially when the host is under environmental stress such as prolonged drought. In such cases, mistletoe has been shown to cause the death of the host (Spurrier and Smith, 2007; Sangüesa-Barreda *et al*., 2013). However, several studies did not detect any effect of mistletoe on the water status of the host, whether they concentrated on a single species (Miller *et al*., 2003; Bowie and Ward, 2004; Ward *et al*., 2006), or on a community in a single habitat (Bannister *et al*., 1999; Okubamichael *et al*., 2011). The preferred hosts of the mistletoe *P. curviflorus* are *Senegalia* and *Vachellia*, which occupy a broad geographical range on the Arabian Peninsula (Dean *et al*., 1994; Griffiths *et al*., 2017). The host species have differing water requirements and hence distributions. *S. asak* specifically inhabits rocky upper slopes, *V. flava* inhabits lower slopes, *V. gerrardii* grows in valleys, and *V. tortilis* grows on bench lands (terraces). These host species also show morphological variations; *V. gerrardii* is a single stemmed tree up to 7 m high, and *S. asak*, *V. flava*, and *V. tortilis* are shrub-like trees with multiple branches emerging from ground level.
Therefore, mistletoe may have differential effects among these hosts. This study focused on the water relations and photosynthetic performance of mistletoe and its hosts distributed in different landscapes during dry and wet periods. Water status and photosynthetic performance in the host trees are influenced by two direct factors, the effect of water availability (dry/wet periods) on the host, and the effect of mistletoe, which is known to be a more profligate user of water than its host. Consequently, the study sought to address the following questions: 1) does mistletoe have consistent performance on different host species during dry and wet periods? 2) does mistletoe infection affect the physiological performance of infected branches of hosts? and 3) are infected hosts more prone to physiological stress compared to non-hosts?

Materials and Methods

Study site

This study was conducted at Taif National Park, which is located northeast of Taif city (21° 18' 07.93" N, 40° 29' 38.49" E) in western Saudi Arabia at an altitude of 1,570 m. The average minimum and maximum temperatures in this region are 6°C and 29°C, respectively, in January, the coolest month, while temperatures reach 20°C–39°C in July, the hottest month (Al-Shaibani, 2008). The annual precipitation varies from year to year, averaging approximately 119 mm/year (Fadl et al., 2015). Before the study, there was an unusually long and severe drought period from January 2003 to February 2013, with a total average rainfall of almost zero (Fig. 1a). Each host species is distributed in a different part of the landscape: upper slopes, lower slopes,
valleys, and bench lands (terraces). These hosts are often infected by the stem-parasitizing mistletoe, *P. curviflorus*.

**Sample selection and plant species**

The fieldwork was conducted in two periods: the end of a severe dry period in February 2013 (no rainfall was recorded for 10 years from 2003 to the end of the winter season in Feb 2013, see Fig. 1a) and the wet period (spring season) from March to mid-May 2013 after 60 mm of rainfall (Fig. 1b). Mistletoe and *S. asak* samples were collected from the top slope, *V. flava* samples from the bottom slope, *V. tortilis* samples from the bench land next to the valley, and *V. gerrardii* samples from the floodplain (Appendix Fig. 7).

In each of the locations where the four host species were found (*S. asak*, *V. flava*, *V. tortilis*, and *V. gerrardii*), we randomly marked six individuals infected with mistletoe (*P. curviflorus*) and six without. From the parasitized hosts, we chose samples of infected branches in the distal area relative to the haustoria, from uninfected branches, and from the parasitizing mistletoe. We then measured the photosynthetic performance of leaf samples and the water potential of their respective shoots. We repeated the same procedures twice on the same set of plants in the dry and wet period.

**Chlorophyll fluorescence and water potential**

We used a Mini pulse amplitude modulated chlorophyll fluorometer (MINI-PAM Walz, Effeltrich, Germany) to assess predawn and midday photochemical quantum yield ratios as indications of the light use efficiency of the hosts and mistletoes. The detailed equations for quantum yield ratios have been published previously (Maxwell and Johnson, 2000; Klughammer and Schreiber, 2008). Hosts and mistletoes were measured after the overnight dark recovery.
period before sunrise at 5 am. We repeated the measurement on the same set of samples between 12 and 1 pm on days when the sky was clear, to calculate photosystem II (PSII) efficiency ($\Phi_{\text{PSII}}$) (Klughammer and Schreiber, 2008). The host and mistletoe samples were fitted with a leaf-clip holder (2030-B, Walz, Effeltrich, Germany) facing the sunlight to maximize exposure to sunlight. This achieved a maximum photosynthetic photon flux density of approximately 1,200 μmol quanta m$^{-2}$ s$^{-1}$. Data were collected in February (dry period) and May in 2013 (wet period).

The same samples were used to assess the water stress status of the hosts and the parasite using a Scholander pressure chamber (Scholander et al., 1965). Predawn and midday water potential measurements for hosts and mistletoes were performed twice during the dry and wet periods (February and May 2013) on the same set of plants.

**Data analysis**

Measurements were made twice on the same plant samples; therefore, there is no possibility of violating sphericity (O’Brien and Kaiser, 1985; Cole and Grizzle, 1966). Homogeneity of variances was checked with Bartlett’s test. We assessed mistletoe infection on water status and photosynthetic performance of hosts/non-hosts in the dry period, and again measured the same variables in the wet period, thus the model effect was determined by a repeated measurement analysis.

The period (dry and wet) was the default additive effect, while branch characteristic (mistletoe-infected branch pairs), infection (infected and uninfected branches of hosts, and uninfected hosts), and the host species (mistletoe on *S. asak*, *V. flava*, *V. gerrardii*, and *V. tortilis*), were considered as independent effects. A Tukey’s honestly significant difference post-hoc comparison test was run for pairwise comparisons where the interactive effects between branch characteristic × period, infection × period, and host species × period were significant.
If these interactions were not present, then we stated there was a significant additive or independent effect. All data were analyzed by Graph Pad Prism ver. 7.2 at $\alpha = 0.05$.

**Results**

**P. curviflorus performance on different host species**

There was a significant interaction between host species $\times$ period for the predawn $F_v/F_m$ of mistletoe growing on different host species ($F = 27.18, P < 0.0001$) (Table 1). The mistletoe-infected *S. asak, V. flava*, and *V. tortilis* had lower $F_v/F_m$ ratios in the dry period than in the wet period (Fig. 2a). However, the $F_v/F_m$ of mistletoe on *V. gerrardii* did not differ between dry and wet periods (Fig.2a).

An interactive effect (species $\times$ period) was also detected for the predawn water potential of mistletoe ($F = 43.82, P < 0.0001$) (Table 1). Significant differences in the water potentials of mistletoe were found on *S. asak, V. flava* and *V. tortilis* between dry and wet periods, indicating a greater negative water potential in the dry period than in the wet period (Fig. 2b). However, the predawn water potentials of the mistletoes on *V. gerrardii* were not different between dry and wet periods (Fig. 2b).

**Light use efficiency and water potential gradient: Mistletoe vs. infected host branches**

A significant period $\times$ branch characteristic interaction for the predawn $F_v/F_m$ was detected in *S. asak* ($F = 85.36, P < 0.0001$) (Table 2). The predawn $F_v/F_m$ of mistletoe was lower than that of the infected host branch during the dry period. Conversely, mistletoe had a higher $F_v/F_m$ than that of the infected host branch in the wet period (Fig. 3a). There was a marginal effect of the interaction on the $F_v/F_m$ in *V. flava* ($F = 3.598, P = 0.087$) (Table 2), showing a lower predawn water potential of mistletoe in the dry period compared with that of the infected host branch.
However, in the wet period the mistletoe’s $F_v/F_m$ was not different than that of the infected host branch (Fig. 3b). No significant interactions were found for *V. gerrardii* ($F = 0.578$, $P = 0.464$) and *V. tortilis* ($F = 1.078$, $P = 0.323$) (Table 2). The $F_v/F_m$ of mistletoe was not different compared with the respective infected branch during either period for *V. gerrardii* and *V. tortilis* (Fig. 3c, d).

For the $\Phi_{PSII}$, no significant interaction between period × branch characteristic was detected for *S. asak* ($F = 0.040$, $P = 0.844$) (Table 2). Although the period effect was significant, Tukey’s test showed that the $\Phi_{PSII}$ values of mistletoe and infected branches were not different between the periods, but the branch characteristic effect was significant. The $\Phi_{PSII}$ of mistletoe was lower than that of the infected branch during both the dry and wet periods ($P = 0.0003$; Fig. 3e). An interactive effect was detected for the midday $\Phi_{PSII}$ of *V. flava* ($F = 5.047$, $P = 0.0485$) (Table 2), with a lower mistletoe $\Phi_{PSII}$ than that of the infected branch ($0.351 \pm 0.041$) in the dry period. In the wet period, mistletoe also had a lower $\Phi_{PSII}$ than that of the infected branch. Additionally, the $\Phi_{PSII}$ values of the mistletoe and infected branch were significantly different between dry and wet periods (Fig. 3f). Despite the period and the branch characteristic effects being significant for *V. gerrardii*, no significant interaction was found ($F = 0.108$, $P = 0.748$) (Table 2) when the $\Phi_{PSII}$ of the mistletoe or infected branch was lower in the dry period compared with the wet period. The $\Phi_{PSII}$ of the mistletoe was lower than that of the infected branch of *V. gerrardii* during both the dry and wet periods (Fig. 3g). There was a marginally significant interaction for *V. tortilis* ($F = 4.519$, $P = 0.059$) when the $\Phi_{PSII}$ of the mistletoe was lower than that of the infected branch in the dry period. However, they were not significantly different in the wet period (Fig. 3h). None of the host species showed an interactive effect between period × branch characteristic for the midday water potential of mistletoe or infected branches ($P > 0.1$; Table 3). Although, the model detected a significant additive period effect, and branch characteristic was an independent effect ($P < 0.01$; Fig. 4a, b, c, d). Mistletoe infecting *V. flava*, *V. gerrardii*, and *V. tortilis* had more negative water potentials
compared with those of their respective infected branches for both the dry and wet periods (Fig. 4b, c, d). However, for S. asak, the midday water potential was not significantly different between the mistletoe and the infected branch during the dry period (Fig. 4a). A significant interaction for the predawn water potential was detected for the mistletoe and the infected branches of S. asak (F = 5.631, P = 0.039; Table 3), with the mistletoe having a greater negative water potential than the infected branches in the dry period. Consistently the mistletoe had a lower predawn water potential compared with the infected branches in the wet period (Fig. 4e). No significant period × branch characteristic interactions of the predawn water potential were observed for V. flava, V. gerrardii, and V. tortilis (P > 0.05) (Table 3). Irrespective of a significant additive effect for period, the branch characteristic’s independent effect was significant. Tukey’s test showed that the predawn water potentials of mistletoe were consistently more negative than those of the infected branches for these species (P < 0.05) (Fig. 4f, g, h).
**Light use efficiency and water potential gradient: infected branch vs. uninfected branches**

A significant period × infection interaction was found for *A. asak* (F = 18.53, P < 0.0001) (Table 4). The infected branch of the host had a lower $F_v/F_m$ compared with the uninfected branch of the host or uninfected host in the wet period, but they were not significantly different in the dry period (Fig. 5a). No significant interactions were found for *V. flava, V. gerrardii,* and *V. tortilis* (P > 0.1) (Table 4). For *V. flava,* the independent infection effect and the additive period effect were significant. Tukey’s test did find a difference during the wet period, when the $F_v/F_m$ of the infected branch of the host was lower than that of the uninfected host. Additionally, the uninfected host had a higher $F_v/F_m$ ratio in the wet period than the dry period (Fig. 5b). The model detected a significant additive period effect for *V. tortilis,* in which only the uninfected host had a higher $F_v/F_m$ ratio in the wet period than in the dry period (Fig. 5d). For *V. gerrardii,* no differences were found between any treatment combinations (Fig. 5c).

No interactive effects of $\Phi_{PSII}$ were observed between infection and period for any of the host species (P > 0.1) (Table 4). Although the model detected a significant additive period effect, Tukey’s test showed that the $\Phi_{PSII}$ was not significantly different between infected and uninfected branches of the host or uninfected host for *S. asak,* and *V. tortilis* (Figs. 5e, h), respectively. Infected branches had a higher $\Phi_{PSII}$ level in the wet period compared with the dry period for only *V. flava* and *V. gerrardii* (Fig. 5f, g). No interactive effect of period × infection was detected for the midday water potential of the infected and the uninfected host branches, and the uninfected host, for all of the host species (P > 0.1) (Table 5). The model did not find a difference in the midday water potential for the independent infection effect (P > 0.05), but the additive period effect was significant (P < 0.0001).
The infected branches showed no difference in the mean midday water potential compared with their respective uninfected host branches or uninfected hosts during both the dry and wet periods (Fig. 6a, b, c, d).

There was a significant interaction between period × infection on the predawn water potential of *S. asak* (*F* = 3.718, *P* = 0.048) (Table 5). The trends of the infected host branches and the uninfected hosts were significantly different in the dry and wet periods. Infected branches had greater negative predawn water potentials compared with those of uninfected hosts for both the dry and wet periods. Also, infected branches had lower predawn water potentials compared with those of uninfected branches in the wet period, but they had similar predawn water potentials in the dry period (Fig. 6e). An interactive effect between period and infection was detected for *V. flava* (*F* = 6.064, *P* = 0.011) (Table 5), in which the infected branches had lower predawn water potentials than those of the uninfected host branches and uninfected hosts during the dry period, but they were not different during the wet period (Fig. 6f). An interactive effect between period and infection was detected for *V. gerrardii* (*F* = 4.004, *P* = 0.040) (Table 5). Although an additive period effect was significant (*P* < 0.0001), the independent infection effect was not significant (*P* = 0.561), with the infected branches not showing any differences in the predawn water potentials compared with those of their respective uninfected host branches or uninfected hosts for both the dry and wet periods (Fig. 6g). For *V. tortilis*, there was an interaction between period and infection effects (*F* = 7.769, *P* = 0.004) (Table 5). Infected branches had more negative water potentials compared with those of their respective uninfected host branches and uninfected hosts during the dry period; however, they were not different during the wet period (Fig. 6h).
Discussion

Mistletoe performance on acacia species

Our data demonstrate that mistletoe growing on *S. asak*, *V. flava*, and *V. tortilis* had lower water potentials and $F_v/F_m$ values in the dry period than the wet period, while those parameters did not vary between periods for mistletoe growing on *A. gerrardii*. This disparity in mistletoe performance may reflect not only the topographic position of the host, but also its morphology, and thus the effects of dry and wet periods on the water balance of the host. We speculated that although the shrub-like *S. asak*, *V. flava*, and *V. tortilis* occupy different topographic positions, their shallow root systems may be affected by prolonged drought stress, leading to limited water supply to the host and reduced mistletoe performance on these species. However, mistletoe may not encounter the same conditions on *V. gerrardii*, which is a large tree, because it has a longer and wider root system that draws water efficiently from deeper soil levels of the floodplain (Ward, 2016). Thus, mistletoes were able to maintain consistent performance on *V. gerrardii* by reliably obtaining water and nutrients, even under severe drought stress. Period effects (soil water availability to host species), and the ability of root architecture to import soil water may explain the variations in mistletoe performance in the study area. Here, mistletoe performance could be an indicator of the drought tolerances of different host species in arid lands. This explains the initial observation in the field that *V. gerrardii* could host several individuals of *P. curviflorus*, while the shrub-like species hosted only one or two. *V. gerrardii* has a large canopy, thus the microclimatic conditions beneath canopy may reduce moisture loss of mistletoes. Therefore, *V. gerrardii* possesses various features to adapt with drought, representing the host quality which determines mistletoe survival and vitality as reported by (Watson, 2008).
Mistletoes vs. infected host branches

*P. curviflorus* had a similar or lower $F_v/F_m$, and $\Phi_{PSII}$ compared with those of the respective host’s infected branches during both the dry and wet periods. *P. curviflorus* may reduce Rubisco activity and chlorophyll content, which are general features of functional adaptations of parasitic plants (Hibberd et al., 1998; Close et al., 2006). In particular, during the wet period, the $F_v/F_m$ ratio of *P. curviflorus* and those of the infected branches of *V. flava*, *V. gerrardii*, and *V. tortilis* were similar to those of loranthaceous mistletoes and their hosts in tropical Brazil, where light utilization was equal in both the host and mistletoe (Lüttge et al., 1998). Similarly, mistletoe had a lower $F_v/F_m$ ratio than those of infected branches of *S. asak* during the dry period, but not in the wet period when mistletoe had a significantly higher predawn $F_v/F_m$ value. *S. asak* appeared to grow on drier slopes in the study area, where water availability is severely limited. The low values of $F_v/F_m$ ($\leq 0.6$) indicated that the host and mistletoe may experience chronic photoinhibition during the dry period, but the effect was stronger in the host than in the mistletoe. This was not the case during the wet period, suggesting that mistletoe may rapidly exploit more nutrients, especially nitrogen, from its host to increase its growth rate during the short wet period. This would explain the lower $F_v/F_m$ ratios in leaves of infected branches in the wet period compared to the dry period. The lower $F_v/F_m$ ratios could also result from the mistletoe infection as an additive effect; that is, mistletoe may increase the stomatal closure of leaves on infected branches, reducing the internal CO$_2$ assimilation, which in turn suppresses the efficiency of photosynthesis (Press et al., 1999). *P. curviflorus* had lower midday and predawn water potentials than those of infected branches of all species.
In most mistletoes, more negative water potential gradients are attributed to high transpiration rates and stomatal conductance of mistletoe, which maintains the water and nutrient flow from the host (Ullmann et al., 1985; Ehleringer et al., 1986; Goldstein et al., 1989; Ehleringer and Marshall, 1995). However, there was no difference in the midday water potential gradient during the dry period between mistletoe and infected branches of S. asak. This may result from the increased drought stress in the slope environment during this period, leading to stomatal closure in the host. Hence, mistletoe may track its host’s response to drought conditions, and close its stomata to minimize the inevitable stress caused by high transpiration rates at midday.

During the dry period, P. curviflorus had similar water potentials at midday and predawn, while the host recovered its water status by predawn. During prolonged drought, mistletoe may experience water stress on S. asak; therefore, it may have a lower water potential at midday, which it maintains during its host’s recovery time, in order to be able to survive on the drier slope environment. Further investigations of the transpiration rate and stomatal conductance will be undertaken.

**Host infected branches vs. host uninfected branches or uninfected hosts**

The leaves of infected branches of S. asak and V. flava had lower Fv/Fm ratios than those of uninfected host branches or uninfected hosts only during the wet period. This suggested that P. curviflorus may have a significant negative impact because it appropriates more resources for growth during the wet period, reducing the light use efficiency of the infected branch. This is supported by the fact that the infected branches, especially those of S. asak, had lower water potentials than uninfected branches or uninfected hosts during both the dry and wet periods. Because mistletoe performance was better during the wet period, we hypothesized that infection with P. curviflorus may worsen the water stress levels of distal branches during the wet period.
This would extend the period of stomatal closure and decrease CO2 diffusion, thus causing the lower photosynthetic performances of the infected branches. This is consistent with the results of a glasshouse experiment on the Australian stem hemiparasite *Cassytha pubescens* growing on *Ulex europaeus* under high and low irrigation levels (Cirocco et al., 2016): the parasitic plant more strongly inhibited the photosynthetic performance of the host under high irrigation levels than under low irrigation levels.

In this study, we observed that the $F_v/F_m$ ratios of infected and uninfected hosts were similar during the dry period, possibly because of the direct effects of extreme drought on the physiological performance (such as PSII and water status stress) of the mistletoe–host association, impeding mistletoe’s effect on the host.

A comparison between branches of the host species *V. gerrardii* and *V. tortilis* did not reveal any difference in $F_v/F_m$, implying that mistletoes did not affect the distal branches of these species. An $F_v/F_m$ ratio of 0.8 for *V. gerrardii* and of 0.7–0.8 for *V. tortilis* suggested that the morphological characteristics of these hosts may supply more reliable resources to mistletoes and distal branches. These species have larger and deeper roots for obtaining ground water, enabling the survival of mistletoes (Roxburgh and Nicolson, 2008).

The period (dry and wet) had a clear influence on infected and uninfected host physiology, while the infection effect had no influence on $\Phi_{\text{PSII}}$ or midday water potential gradients for all species during both the dry and wet periods. These findings suggested that infected and uninfected hosts may close their stomata, avoiding high evaporation rates during long exposure to sunlight. In turn, this would reduce midday $\Phi_{\text{PSII}}$ values and increase water potentials simultaneously in all the branches, regardless of the infection effect.
References


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method. PAM application notes, 1(2), 201-247.


Table 1 Results of a repeated measurement two-way analysis of variance (ANOVA) on the additive effects of period (dry/wet), *P. curviflorus* performance on *S. asak*, *V. flava*, *V. gerrardii*, and *V. tortilis* (host species), and their interaction (period × host species) on predawn quantum yields ($F_v/F_m$) and predawn water potentials ($\Psi$). Analyses were run separately; for period, degrees of freedom (df) = 1; host species, df = 3; and period × host species interaction, df = 3; n = 6 for all parameters.

<table>
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<th>$P$</th>
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Table 2 Results of a repeated measurement two-way ANOVA on the additive effects of period (dry/wet), *P. curviflorus* mistletoe and infected host branches (branch characteristic), and their interaction (period × branch characteristic) on predawn (*Fv/Fm*) and midday (ΦPSII) quantum yields for each host species, *S. asak*, *V. flava*, *V. gerrardii*, and *V. tortilis*. Analyses were run separately; for period, degree of freedom (df) = 1; branch characteristic, df = 1; and period × infection interaction, df = 1; n = 6 for all parameters.

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<th>Parameters</th>
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Table 3 Results of a repeated measurement two-way ANOVA on the additive effects of period (dry/wet), *P. curviflorus* mistletoe and infected host branches (branch characteristic), and their interaction (period × branch characteristic) on predawn and midday water potentials (Ψ) for each host species, *S. asak*, *V. flava*, *V. gerrardii*, and *V. tortilis*. Analyses were run separately; for period, degree of freedom (df) =1; branch characteristic, df =1; and period × infection interaction, df = 1; n = 6 for all parameters.

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Table 4 Results of a repeated measurement two-way ANOVA on the additive effects of period (dry/wet), infected and uninfected host branches, and non-host (infection), and their interaction (period × infection) on predawn ($Fv/Fm$) and midday ($\Phi_{PSII}$) quantum yields for each host species, *S. asak*, *V. flava*, *V. gerrardii*, and *V. tortilis*. Analyses were run separately; for period, degree of freedom (df) = 1; infection, df = 2; and period × infection interaction, df = 2; n = 6 for all parameters.

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Table 5 Results of a repeated measurement two-way ANOVA on the additive effects of period (dry/wet), infected and uninfected host branches, and non-host (infection), and their interaction (period × infection) on predawn and midday water potentials (Ψ) for each host species, *S. asak*, *V. flava*, *V. gerrardii*, and *V. tortilis*. Analyses were run separately; for period, degree of freedom (df) = 1; infection, df = 2; and period × infection interaction, df = 2; n = 6 for all parameters.

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(Fig. 1) Precipitation data for Taif National Park study site, (Source: Power & Water Ministry in the Kingdom of Saudi Arabia); (a) Average annual rainfall (b) Average monthly rainfall. Physiological measurements of mistletoe *P. curviflorus* and host species were performed in February, and after 60 mm of rainfall in May of 2013, as indicated by the red arrows.
(Fig. 2) The (a) predawn \((F_v/F_m)\) quantum yields and (b) predawn water potentials \((-\psi = \text{KPa})\) of mistletoe \(P.\ curviflorus\) growing on the different host species \(S.\ asak, V.\ flava, V.\ gerrardii,\) and \(V.\ tortilis\) (H). Shaded bars denote samples from the wet period, and white bars denote samples from the dry period (S). Different lowercase letters denote significant differences; data are means (+ SD), \(n = 6\).
(Fig. 3) The predawn ($F_v/F_m$) and midday ($\Phi_{PSII}$) quantum yields of *P. curviflorus* and four hosts, using infected host branches (B). The hosts were *S. asak*, *V. flava*, *V. gerrardii*, and *V. tortilis*. Shaded bars denote samples from the wet period, and white bars denote samples from the dry period (S). Different lowercase letters denote significant differences; data are means (± SD); n = 6.
(Fig. 4) Midday and predawn water potentials (−Ψ = KPa) of *P. curviflorus* and four hosts, using infected host branches (B). The hosts were *S. asak*, *V. flava*, *V. gerrardii*, and *V. tortilis*. Shaded bars denote samples from the wet period, and white bars denote samples from the dry period (S). Different lowercase letters denote significant differences; data are means (± SD); n = 6.
(Fig. 5) The predawn ($F_{v}/F_{m}$) and midday ($\Phi_{PSII}$) quantum yields of four hosts, using infected and uninfected host branches, and uninfected hosts (I). The hosts were $S.\ asak$, $V.\ flava$, $V.\ gerrardii$, and $V.\ tortilis$. Shaded bars denote samples from the wet period, and white bars denote samples from the dry period (S). Different lowercase letters denote significant differences; data are means (± SD); $n=6$. 
(Fig. 6) Midday and predawn water potentials (−Ψ = KPa) of four hosts, using infected and uninfected host branches, and uninfected hosts (I). The hosts were S. asak, V. flava, V. gerrardii, and V. tortilis. Shaded bars denote samples from the wet period, and white bars denote samples from the dry period (S). Different lowercase letters denote significant differences; data are means (± SD); n = 6.
Appendix

V. gerrardii tree

S. asak shrub

Mistletoe *P. curviflorus*

V. tortilis shrub

*V. flava* shrub

(Fig. 7): Mistletoe *P. curviflorus* infecting tree and shrub *Senegalia* and *Vachellia* growing on different topography.
Statement of Authorship

| Title of Paper | Heavy mistletoe infection increases water and light stress in *Acacia gerrardii* in dryland regions of the western Arabian Peninsula |
| Publication Status | | |
| | Published | Accepted for Publication |
| | Submitted for Publication | Unpublished and Unsubmitted work written in manuscript style |
| Publication Details | Not published |

**Principal Author**

| Name of Principal Author (Candidate) | Dhafer A. Albakre |
| Contribution to the Paper | D.A.B conceived the ideas and methodology, analysed data, and wrote manuscripts and acted as corresponding author. |
| Overall percentage (%) | 80% |
| Certification: | This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper. |
| Signature | Date | 27 March 2019 |

**Co-Author Contributions**

By signing the Statement of Authorship, each author certifies that:

1. the candidate’s stated contribution to the publication is accurate (as detailed above);
2. permission is granted for the candidate in include the publication in the thesis; and
3. The sum of all co-author contributions is equal to 100% less the candidate’s stated contribution.

| Name of Co-Author | José M. Facelli |
| Contribution to the Paper | J.M.F conceived the ideas, interpreted data, critically assessed to drafts and issued final approval for publication. |
| Signature | Date |

Please cut and paste additional co-author panels here as required.
Chapter 6: Heavy mistletoe infection increases water and light stress in *Vachellia gerrardii* in dryland regions of the western Arabian Peninsula

Dhafer Albakre* and José M. Facelli
School of Biological Sciences, the University of Adelaide, Adelaide, SA 5005, Australia.
*Author for correspondence: Dhafer A. Albakre, Email: Dhafer.albakre@adelaide.edu.au

This chapter has been written following the style of *New Phytologist*.

**Summary**

- Three species of mistletoe, *Phragmanthera austroarabica*, *Plicosepalus curviflorus*, and *Viscum schimperi*, infect *Vachellia gerrardii* trees in the Arabian Peninsula. The physiological consequences of these infections are unknown. Here, we investigated the effects of mistletoe infection on the light utilisation capacity and water status of the four species (i.e., the three mistletoes and host plant) in trees classified as heavily, moderately, or slightly infected.

- Chlorophyll fluorescence and a Scholander pressure chamber were used to quantify the quantum yields and water potentials, respectively, of the mistletoes and the infected and uninfected branches of the hosts at predawn and midday. Thirty-six trees were analysed, 12 for each level of infection. Uninfected branches of the host were also analysed.

- Heavily infected hosts had lower water potentials and quantum yields than did moderately or slightly infected hosts. The predawn water potentials of *P. austroarabica* and *P. curviflorus* were lower than those of *V. schimperi* in heavy infections. Branches infected with *P. austroarabica*, but not with either of the other two species, were more
water stressed than uninfected branches. The midday quantum yield was higher for *P. austroarabica* and *P. curviflorus* infections than for *V. schimperi*.

- Heavy mistletoe infection increased the chronic photoinhibition and predawn water stress of the host. Our results suggest that the large leaves of *P. austroarabica* transpire, and hence require, more water than do those of the other two mistletoe species, and therefore that this species has a strong negative impact on host water status.

**Key words:**

Chlorophyll fluorescence, photoinhibition, quantum yields, water potentials, heavy mistletoe infection
Introduction
Mistletoes are parasitic plants mainly in the Viscaceae and Loranthaceae families (Mathiasen et al., 2008). These plants extract vast amounts of water from the vascular tissues of their host plants using penetrating structures known as haustoria (Press & Whittaker, 1993; Press & Graves, 1995). High transpiration rates ensure that the water potential of the mistletoe is lower than that of the host plant, resulting in a unidirectional flow of water and nutrients into the parasite (Strong & Bannister, 2002; Miller et al., 2003; Bowie & Ward, 2004). The photosynthetic capacity of mistletoes is either lower (Press et al., 1999; Johnson and Choinski, 1993) or equal to that of the host (Lüttge et al., 1998); thus, mistletoes can exhibit a significant dependency on the carbon content of the host (Marshall & Ehleringer, 1990; Marshall et al., 1994). As a result, mistletoe infection may cause numerous physiological responses in the host, such as reducing growth at the terminal branches or throughout the plant (Meinzer et al., 2004). However, the physiological response of a host to mistletoe infection varies. For instance, species of Loranthaceae succumb to high water stress before the host tree is influenced, and Amyema species, which are common in Australia, have a negligible impact on host growth, while others like Arceuthobium species (Viscaceae), which occur in North America, inhibit host growth and can even kill the host (Shaw et al., 2004). Scalon and Wright (2015) performed comprehensive surveys in various environments around the world and found that mistletoes belonging to Viscaceae had a more negative effect on host nitrogen content than those belonging to Loranthaceae, while the water relationship between mistletoes and their various hosts depended on water availability in the soil.

Photoinhibition normally occurs during long exposures to high light intensity. Usually leaves recover daily; however, the extension of this effect into the dark period, known as chronic
photoinhibition, indicates irreversible damage of the photosynthetic apparatus (Taiz & Zeiger, 2010). Environmental stresses, such as water limitation, can trigger or aggravate the damage caused by photoinhibition, thus reducing maximum quantum yield ($F_v/F_m$) in plants growing in arid regions (Liang et al., 1997). The measurement of this parameter is a useful indicator of the level of stress produced by mistletoes on their hosts.

In arid zones such as our study area, Wadi Alshafa in Saudi Arabia, the recent increase in mistletoe abundance and the combined infection by both viscaceous and loranthaceous mistletoes on individual hosts may stress or even kill the host plants. We aimed to ascertain the effects of mistletoe in Wadi Alshafa, which may help to determine the best management approaches for controlling mistletoes as ecologically important species while also conserving the remaining $V. gerrardii$ host trees. To the best of our knowledge, the direct effects of three species on a single individual are not documented.

Since high quality hosts, i.e., hosts with a greater ability to supply water and nutrients to the mistletoe, often have increased abundance of mistletoe or higher degrees of infection (Watson, 2008), we hypothesised that heavily infected host plants would be more stressed in terms of photosynthetic performance and water balance than moderately infected or slightly infected hosts. We investigated whether heavy mistletoe infection affects water status and light-use efficiency in the host. Prior to the study we identified a unique infection pattern involving three different species of mistletoe: $P. austroarabica$ and $P. curviflorus$ (both belonging to Loranthaceae), and $V. schimperi$ (Viscaceae). Variations in the leaf morphology of these species may reflect their performance and thus their effects on the host; therefore, we compared their physiological performances across different levels of infection and investigated whether they had different effects on the light utilisation and water status of the host.
Material and Methods

Plant species and sampling

The study site is located at Wadi Alshafa, southwest of Taif, Saudi Arabia (21° 12′ 11.66″ N, 40° 20′ 44.72″ E). The average annual rainfall at this site from 2003 to 2013 was 128.8 mm. Three different mistletoe species, which differ in leaf morphology, from leafless (\textit{V. schimperi}) (Collenette, 1999) to very broad leaves (\textit{P. austroarabica}) and small leaves (\textit{P. curviflorus}), occur in this area and exclusively infect \textit{V. gerrardii} trees, which are distributed along the wadi. The study was conducted along the wadi, at altitudes ranging from 2012 m (the highest location of the host distribution) to 1651 m (the lowest host distribution). Roads and agricultural edges were avoided (Norton & Smith, 1999). Six line transects of 100 m were placed at approximately 2 km intervals along the altitudinal gradient of the wadi, at 2012-1909 m, 1896–1976 m, and 1795–1651 m. All infected \textit{V. gerrardii} trees located on the transects were marked, and their mistletoe loads assessed: a total of 36 trees. We assessed mistletoe infection classes for each line as follow: 4 heavily infected trees, 4 moderately infected trees, and 4 slightly infected trees. We used trunk diameter as a proxy for larger and older trees > 100 cm, moderately infected trees were measured between (100- 50 cm) and slightly infected trees were <50 cm (Appendix. Fig. 1). Mistletoe load was based on the number and size of mistletoes.

Study design

The degree of infection (D) was determined by identifying 12 hosts with each level of infection, as per Sangüesa-Barreda \textit{et al.} (2013): low infection or slightly infected hosts had a mistletoe load occupying of less than a third of the canopy, with around 25% of host canopy being infected by a single species; medium infection or moderately infected hosts had mistletoe loads occupying up to two thirds of the host canopy, with around 50% of the host canopy being infected by one species;
and heavy infection or heavily infected host canopies were almost completely covered in mistletoe, with ≥75% of the host canopy being infected by one species. From heavily infected hosts, we chose samples of infected branches in the distal area relative to the haustoria, from uninfected branches, and from the parasitizing mistletoe species. We conducted the same procedures with moderately and slightly infected trees. Ecophysiological measurements, photosynthetic performance and water potential were performed respectively on uninfected and infected branches of the host (B) and on the mistletoes (M). Measurements were taken from leaf samples and shoots for all infection classes.

**Chlorophyll fluorescence measurement**

A portable, pulse-modulated chlorophyll fluorometer (Mini-PAM; Walz, Effeltrich, Germany) was used to measure the photochemical quantum yield of photosystem II (PSII) at predawn and midday (Genty *et al*., 1989). Photochemical quantum yield is a measure of light-use efficiency that can be used to detect symptoms of photoinhibition in PSII (Long *et al*., 1994). Leaf samples were taken from all three mistletoe species, as well as from infected and uninfected branches of hosts with different levels of infection. The leaves were fitted with a leaf-clip holder (2030-B; Walz) and oriented towards the sunlight to obtain the maximum photosynthetic photon flux density (PPFD), which was approximately 1200 µmol m⁻² s⁻¹ between 12:00 and 13:00 (midday quantum yield; Φ_{PSII}). The predawn fluorescence was measured in the same set of samples at 05:00 (PPFD=0), to calculate the maximum quantum yield of PSII (F_v/F_m).
Water potential measurement

Water stress status for *V. gerrardii* (the host) and the three mistletoe species was assessed using a Scholander pressure chamber (Scholander *et al.*, 1965). Freshly cut shoots of mistletoe and infected and uninfected host branches across different levels of infection were placed directly into the pressure chamber. Nitrogen gas was gradually added until the xylem sap started to leak from the cut shoots, and the balancing pressure was recorded for midday ($\Psi_{MDW}$) and predawn ($\Psi_{PDW}$) water potential gradients from the samples (KPa= $-\Psi$).

Data analysis

The variances of data were homogenous for $F_v/F_m$, $\Phi_{PSII}$, $\Psi_{PDW}$, and $\Psi_{MDW}$. We assessed the intensity of mistletoe infection on *V. gerrardii* and compared water potential and quantum yield at predawn and midday across degree of infection (Heavy, Medium, Low), the branch type (Infected, Uninfected, Mistletoe), and mistletoe species (*V. schimperi*, *P. austroarabica*, *P. curviflorus*). A standard least squares model was applied to the full factorial experiment (degree of infection (D) $\times$ mistletoe species (M) $\times$ branch type (B), followed by a pairwise comparisons test (Tukey-Kramer HSD post-hoc analysis). JMP ver. 4.0.4 (SAS Institute Inc., Cary, North Carolina, USA) was used to analyse all data at $\alpha=0.05$. 
Results

Water potential

A significant interactive effect between the degree of infection and the branch type was observed on $\Psi_{PDW}$ (Table 1, D*B). Mistletoes on heavily infected hosts had lower $\Psi_{PDW}$ than those on hosts with medium and low infections. The uninfected branches of heavily infected trees were more water stressed than those of trees with low infection rates (Fig. 1a). Infected branches of trees with heavy and medium infection levels had lower $\Psi_{PDW}$ values than those with a low infection rate (Fig. 1a). The $\Psi_{PDW}$ levels of the mistletoes were lower than those of the uninfected branches, and mistletoes from the heavily infected hosts had the lowest $\Psi_{PDW}$ of all the samples. While there were no differences in $\Psi_{PDW}$ between infected and uninfected branches of slightly infected host, infected branches of moderately and heavily infected hosts were more water stressed than were their uninfected branches (Fig. 1a).

There was also a significant statistical interaction between the effects of the degree of infection and the mistletoe species in the $\Psi_{PDW}$ values (Table 1, D*M). The $\Psi_{PDW}$ of all three species of mistletoe on slightly infected were similar. For moderately infected hosts, *P. austroarabica* had a more negative water potential than *V. schimperi*, while the $\Psi_{PDW}$ of *P. curviflorus* was not significantly different. For heavily infected host, the $\Psi_{PDW}$ of *P. curviflorus* was more negative than that of *V. schimperi*, while that of *P. austroarabica* was between these two values (Fig. 1b). Additionally, *V. schimperi* on heavily infected hosts was more water stressed than the individuals on the slightly infected hosts, whereas on moderately infected hosts this mistletoe had an intermediate $\Psi_{PDW}$. *P. curviflorus* growing on heavily infected hosts had a lower water potential than those on hosts with medium and low infection rates. For heavy and medium infection rates, the $\Psi_{PDW}$ values of *P. austroarabica* were more negative than those on hosts with low infection.
rates (Fig. 1b). A significant interaction was detected between the effects of mistletoe species and branch type on $\Psi_{PDW}$ (Table 1, M*B). $P. austroarabica$ had a more negative water potential than $V. schimperi$, but neither $P. austroarabica$ nor $V. schimperi$ had a difference in $\Psi_{PDW}$ when compared with $P. curviflorus$. Branches infected with $P. austroarabica$ and $P. curviflorus$ had a more negative $\Psi_{PDW}$ than those infected with $V. schimperi$; however, no difference in $\Psi_{PDW}$ was observed between all of the uninfected branches (Fig. 1c).

There were no significant differences in $\Psi_{PDW}$ for all combinations of infected and uninfected branches from hosts infected with $V. schimperi$, and although $P. curviflorus$ was more water stressed than was $V. schimperi$, no difference was found in the $\Psi_{PDW}$ of their infected and uninfected branches. The $\Psi_{PDW}$ of $P. austroarabica$ mistletoe was more negative than that of the uninfected branches, and the branches infected with this species were more water stressed than the uninfected branches (Fig. 1c).

There was a significant interaction between the effects of degree of infection and mistletoe species on the $\Psi_{MDW}$ values (Table 2, D*M). For mistletoes growing on slightly infected hosts, the $\Psi_{MDW}$ of $P. austroarabica$ was more negative than those of $V. schimperi$ and $P. curviflorus$. The Loranthus species $P. curviflorus$ and $P. austroarabica$ growing on a moderately infected host had a more negative $\Psi_{MDW}$ than did $V. schimperi$. There were no differences in the $\Psi_{MDW}$ values of the three mistletoe species growing on heavily infected hosts (Fig. 2a).

$P. curviflorus$ had a lower water potential on both heavily infected and moderately infected hosts than on slightly infected hosts. $V. schimperi$ had a more negative $\Psi_{MDW}$ on heavily infected hosts than on moderately and slightly infected hosts. No differences in $\Psi_{MDW}$ were found for $P. austroarabica$ across the different levels of infection (Fig. 2a).
A significant independent effect on $\Psi_{MDW}$ was determined for branch type (Table 2, B); the mean $\Psi_{MDW}$ of mistletoes was lower than that of the infected branches, but not of the uninfected branches, of the host (Fig. 2b).

**Light-use efficiency**

A significant interaction was detected for the effects of the degree of infection and branch type on the $F_v/F_m$ predawn quantum yield (Table 3, D*B). Mistletoes on heavily infected hosts had a higher value of $F_v/F_m$ than those on moderately and slightly infected hosts, whereas the $F_v/F_m$ of the infected branches of slightly infected hosts was higher than those of infected branches from hosts with medium and heavy infection.

The $F_v/F_m$ of uninfected branches of heavily infected hosts was lower than that of slightly infected hosts, whereas that of moderately infected hosts was not (Fig. 3a). The $F_v/F_m$ of mistletoes present in heavily infected hosts was higher than that of the respective infected branches; however, the $F_v/F_m$ of uninfected branches of these hosts was not significantly different from the mistletoe or the infected branches. Mistletoes on hosts with low and medium mistletoe infection had lower $F_v/F_m$ values than the infected and uninfected branches of their respective hosts (Fig. 3a). A Tukey test did not reveal any statistically significant differences in $F_v/F_m$ among the mistletoe species (Fig. 3b). The mistletoe species did differ significantly in their $\Phi_{PSII}$ midday quantum yield (Table 4), with a higher average $\Phi_{PSII}$ for both *P. austroarabica* and *P. curviflorus* than for *V. schimperi* (Fig. 4).
Discussion

Water potential

We have shown here that the infected and uninfected branches of heavily and moderately infected hosts were more water stressed than those of plants exhibiting low levels of infection, reflecting that the heavily and moderately infected trees experienced water stress for longer periods of time than trees with low levels of infection. Heavily and moderately infected plants may experience increased water loss due to the higher abundance of mistletoe; the high transpiration rates and low hydraulic conductivity of mistletoes enable them to maintain a lower water potential than the hosts, and continue to acquire water and nutrients even in darkness (Press & Graves, 1995; Strong & Bannister, 2002). Our data suggest that heavy mistletoe infections increase the water demand of the host plants, leading to severe water stress in the host. This is consistent with a previous study conducted in the arid zone of the USA, whereby the infestation intensity of the mistletoe Phoradendron californicum increased the predawn and midday water potentials of its host, Acacia gregii, relative to non-host trees (Lei, 1999).

We found that mistletoe growing on hosts that were heavily infected were more water stressed than mistletoe growing on plants with medium and low levels of infection. This may be due to the different sizes of hosts found in the wadi; as abundance of mistletoe generally increases with the tree size (Downey, Gill, & Banks, 1997; Smith & Reid, 2000). Larger A. gerrardii trees can compete and allocate much water from deeper soil than smaller trees through their longer root systems, reducing their negative water potential values. This explanation is consistent with Matula, Svátek, Pálková, Volařík, & Vrška (2015) who found that mistletoe abundance significantly increased with the host size and decreasing the competition by neighbouring trees.
Therefore, a high level of infection would exert a lower water potential, further promoting water uptake from the host. The predawn water potentials of hosts with low infection rates may be equivalent to those of their respective mistletoes. Presumably, smaller trees have lower overall transpiration, which accommodates their more limited requirements. We noted that *P. austroarabica* and *P. curviflorus* maintained lower water potentials than *V. schimperi* on medium and heavily infected hosts. Also, regardless of the degree of infection, *P. austroarabica* increased the water stress in infected host branches relative to their uninfected branches. *P. curviflorus* and *V. schimperi* had no influence on the Ψ_{PDW} of the host. These findings may be attributed to their different morphological traits, such as leaf area; transpiration is likely greater in *P. austroarabica* and *P. curviflorus* mistletoes, which have leaves, than in *V. schimperi*, which are leafless. Therefore, host water stress is influenced by the combined effects of the different water use strategies of mistletoes (from broad-leaved to leafless) and of total mistletoe loading, with *P. austroarabica* particularly aggravating host water stress. However, under severe drought, *P. austroarabica* could encounter chronic water stress that is more severe than that experienced by other species.

Our investigation of Ψ_{MDW} showed that all species, but particularly *P. austroarabica*, suffered from water stress in trees with a heavy infection load. In addition to the effects of host size and the excessive requirement of water at predawn for trees with heavy mistletoe infections, we expected that the warmer air temperatures at noon would increase the rate of transpiration in all species, thus increasing water stress in combination with thermal stress. This explanation may be most plausible for *P. austroarabica*, since the leaves of this species are wider and larger than those of the other species.
We found an independent effect on midday water potential, where the ΨMDW of mistletoe was more negative than that of the infected branch, whereas the water balance between the infected and uninfected branches of the host was not different, suggesting that mistletoes always maintain a lower water potential than the host plant. This finding is consistent with research conducted in the Negev Desert by Bowie and Ward (2004) that showed that *Plicosepalus acacia* had a more negative water potential than the *Acacia raddiana* host but did not induce water stress or mortality in the host.

**Quantum yield**

Heavily infected hosts had a lower $F_v/F_m$ than those with a low infection load during the recovery period. Therefore, heavily infected plants are more prone to long-term chronic photoinhibition, increasing their probability of light stress. This reduction in light-use efficiency is most likely caused by their increased water stress, which is determined by their predawn water potential. Conversely, mistletoes on heavily infected hosts showed a higher photosynthetic performance than those with medium and low infections. This may be due to the host size, with larger trees being better able to supply reliable resources that enhance mistletoe growth. We also found that, in particularly heavy infections, the mistletoes did affect the $F_v/F_m$ of the infected branches, whose maximum quantum yields were significantly lower than their respective uninfected branches. This is supported by our finding that mistletoes induced a higher level of water stress in infected branches than in uninfected branches. In addition, all of the mistletoe species had a similar $F_v/F_m$, likely because of the absence of daylight stress before dawn, which mitigates the water stress of both the host and the mistletoe species.
In full sunlight, the average midday quantum yield (Φ_{PSII}) of *P. austroarabica* and *P. curviflorus* was higher than that of *V. schimperi*. The variation in light-use efficiency may reflect the light requirement for each species, which could be explained based on the morphological differentiation in loranthaceous and viscaceous mistletoes; the larger leaf areas of *P. austroarabica* and *P. curviflorus* may better capture photons, while the slender leafless stems of *V. schimperi* are likely less efficient at this process. This explanation requires further investigation, including the quantification of photosynthetic pigments in the three species, which act to dissipate excessive light (Matsubara *et al.*, 2003).

In conclusion, neither the degree of mistletoe infection nor the branch type influenced the midday quantum yield of the host plant. This result was expected, as ΦPSII is affected by lengthy exposure to high levels of light, leading to a dynamic photoinhibition of the PSII reaction, which can recover before dawn. The optimal values of $F_v/F_m$ are approximately $\geq 0.83$; however, a decrease in quantum yield at predawn results in chronic photoinhibition (Maxwell & Johnson, 2000; Demmig-Adams *et al.*, 2006). This is consistent with our finding of maximum quantum yield, especially in plants exhibiting medium and heavy levels of infection, in which the $F_v/F_m$ values ranged from 0.61 to 0.83, demonstrating that the host plants and the mistletoes were photoinhibited. This suggests that another unknown effect may influence the water status and light-use efficiency of both the host and the mistletoes. The reduction in light harvesting efficiency due to increased water stress in both the mistletoes and the host, especially in heavily infected trees, could point to another factor. In the field, we observed that water wells are abundant throughout the wadi, and these might reduce the groundwater supply to the host, thus increasing the water stress of the host and the mistletoes. This study highlights the need to further examine the effect of human activities on the wellbeing of acacia trees.
Overall, differences in leaf morphophysiology of Viscaceae and Loranthaceae mistletoes and pattern of mistletoe abundance is shaped by the host status along with altitude gradient in the Wadi. Scalon, Rossatto, Domingos, & Franco (2016) reported that mistletoe *Passovia ovata* parasiting evergreen and deciduous hosts, had a difference in photosynthetic parameters, and pre-midday water potentials. Mistletoe showed a degree of plasticity in their ability (anatomical and physiological) to cope with differences in intrinsic host functions, enabling the use of a greater range of the hosts.
References


Tables

Table 1 Three-way ANOVA results of predawn water potential model ($\Psi_{PDW}$) for degree of infection (D), mistletoe species (M), and branch type (B). Significant effects are in bold.

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Table 2 Three-way ANOVA results of midday water potential model ($\Psi_{MDW}$) for degree of infection (D), mistletoe species (M), and branch type (B). Significant effects are in bold.

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<td>2</td>
<td>2</td>
<td>16509816</td>
<td>19.2192</td>
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</tr>
<tr>
<td>M</td>
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<td>2</td>
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</tr>
<tr>
<td>D*M</td>
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<td>4</td>
<td>9614144</td>
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</tr>
<tr>
<td>B</td>
<td>2</td>
<td>2</td>
<td>6614098</td>
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<td>0.0009</td>
</tr>
<tr>
<td>D*B</td>
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<td>4</td>
<td>1539236</td>
<td>0.8959</td>
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</tr>
<tr>
<td>M*B</td>
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<td>4</td>
<td>2499823</td>
<td>1.4550</td>
<td>0.2236</td>
</tr>
<tr>
<td>D<em>M</em>B</td>
<td>8</td>
<td>8</td>
<td>3696861</td>
<td>1.0759</td>
<td>0.3883</td>
</tr>
</tbody>
</table>

Table 3 Three-way ANOVA results of maximum quantum yield model ($F_v/F_m$) for degree of infection (D), mistletoe species (M), and branch type (B). Significant effects are in bold.

<table>
<thead>
<tr>
<th>Source</th>
<th>Nparm</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>F Ratio</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
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<td>2</td>
<td>0.06146424</td>
<td>13.8282</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>M</td>
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<td>2</td>
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<td>0.0170</td>
</tr>
<tr>
<td>D*M</td>
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<td>4</td>
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<td>2.2554</td>
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</tr>
<tr>
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<td>0.13094791</td>
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</tr>
<tr>
<td>D<em>M</em>B</td>
<td>8</td>
<td>8</td>
<td>0.02005991</td>
<td>1.1283</td>
<td>0.3555</td>
</tr>
</tbody>
</table>
Table 4 Three-way ANOVA results of minimum quantum yield model ($\Phi_{PSII}$) for degree of infection (D), mistletoe species (M), and branch type (B). Significant effects are in bold.

<table>
<thead>
<tr>
<th>Source</th>
<th>Nparm</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>F Ratio</th>
<th>Prob &gt; F</th>
</tr>
</thead>
<tbody>
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<tr>
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</tr>
<tr>
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<td>1.5742</td>
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</tr>
<tr>
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<td>0.021</td>
<td>0.7936</td>
<td>0.6097</td>
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</tbody>
</table>
Figures:

Fig. 1 Factors influencing predawn water potential ($\Psi_{PDW}$). The interactive effect of (a) degree of infection x branch type (b) degree of infection x mistletoe species and (c) mistletoe species x branch type on $\Psi_{PDW}$. In (a), white, light grey, and dark grey represent light, medium, and heavy infection, respectively. In (b) and (c), white, light grey, and dark grey fills represent $P.\ austroarabica$, $P.\ curviflorus$, and $V.\ schimperi$, respectively. N=36, mean± SD. The dashed line separates the mistletoe from the host tree. Different letters denote a significance level of $\alpha < 0.05$. 

175
Fig. 2 Factors influencing midday water potential. (a) The interactive effect of degree of infection (D) x branch type (B) and (b) the effect of branch type alone. In (a), white, light grey, and dark grey fills represent *P. austroarabica*, *P. curviflorus*, and *V. schimperi*, respectively. In (b), white, light grey, and dark grey fills represent uninfected branch, infected branch, and mistletoe. N=36, mean± SD. The dashed line separates the mistletoe from the host tree. Different letters denote a significance level of $\alpha < 0.05$. 
Fig. 3 Factors influencing maximum quantum yield ($F_{v}/F_{m}$). (a) The interactive effect of degree of infection x branch type and (b) the marginally independent effect of mistletoe species alone. In (a), white, light grey, and dark grey fills represent low infection, medium infection, and heavy infection, respectively. In (b), white, light grey, and dark grey fills represent *P. austroarabica*, *P. curviflorus*, and *V. schimperi*, respectively. N=36, mean± SD. The dotted line separates the mistletoe from the host tree. Different letters denote a significance level of $\alpha < 0.05$. 
Fig. 4 Independent effect of midday quantum yield $\Phi_{PSII}$ of mistletoe species. White, light grey, and dark grey fills represent *P. austroarabica*, *P. curviflorus*, and *V. schimperi*, respectively. N=36, mean± SD. Different letters denote a significance level of $\alpha < 0.05$. 
Fig. 1 Host trunk diameter as a proxy for larger and older *V. gerrardii* trees in Wadi Alshafa. (a) Heavily infected host trees had a larger trunk diameter than moderately infected or slightly infected trees; data are means ± SD, n=12 for each infection level. Different letters denote a significance level of \( \alpha < 0.05 \). (b) Linear relationships are significant between the trunk diameter of trees and either the predawn or midday negative water potential. The red line correlation represents predawn \(-\Psi\) \((Y = 6.175*X + 1445, P<0.0001)\); the black line correlation represents midday \(-\Psi\) \((Y = 5.99*X + 3948, P=0.0006)\); the dashed lines represents 95% confidence intervals.
Chapter 7: Conclusions

The expansion of mistletoe in the western region of the Arabian Peninsula is perceived as a serious ecological problem affecting several plants, including some economically and ecologically significant species of *Senegalia* and *Vachellia*. The main focus of this study was the effect of mistletoe on its host in different ecosystems in western Saudi Arabia. While the overall impact of mistletoe has been well-documented in many areas, mistletoes have different impacts on their various hosts. One possible reason for this is that the mistletoe–host association is driven by environmental conditions. Mistletoe can have an aggressive negative impact on the growth of its host, and hence is often considered a pest in the USA (Geils & Hawksworth, 2002). However, mistletoes can also have negligible or no effect on some critical physiological aspects of the host, such as nitrogen nutrition. To date, no study exists regarding the incidence and abundance of mistletoe, or the effects of mistletoe on host plants and plant communities under different environmental conditions in the western region of the Arabian Peninsula. In this thesis, I investigated mistletoe infection in two different systems: mistletoes parasitising several host species throughout different geographical locations, and three species of mistletoe coexisting on a single host. Hence, my work on mistletoe infection aims to fill a substantial knowledge gap in the field of dryland ecology. This information is crucial to determining whether management of mistletoe is required, and, if so, to assist an evaluation of the management options available in the infested areas.

Chapter 1

This study determined that, overall, the morphological characteristics of the host (i.e., height, trunk diameter) seemed to affect the prevalence of mistletoe in both Taif National Park and Wadi Alshafa. However, there were exceptions to this. In Taif National Park, the morphological
characteristics did not explain the incidence of mistletoe *Plicosepalus curviflorus* on three species (*V. flava*, *V. gerrardi*, and *V. tortilis*). These species grow in low-lying areas, such as valleys and bench lands. In these lower areas, water and finer sediments accumulate, making growing conditions more suitable for both uninfected and infected trees. Therefore, topographic factors are closely related to mistletoe prevalence and override the effect of the host size. Topographic slope also appeared to be related when the host characteristics—i.e., the large canopy area of *Senegalia asak* seemed to favour *P. curviflorus* incidence. I suggest that *P. curviflorus* persists better on larger, more vigorous *S. asak* hosts because this tree species grows on rocky slopes, conditions under which smaller hosts cannot supply enough resources. I conclude that the presence of *P. curviflorus* may be indirectly influenced by topographical factors that most likely control the distribution of resources and thus the growth of the host trees.

I modelled both the incidence and abundance of the mistletoe species and explained their relationship to site altitude, host canopy volume, and the other mistletoe species growing in Wadi Alshafa. Mistletoe species responded differentially to environmental conditions. The incidence and abundance of *Phragmanthera austroarabica* increased with both altitude and host canopy volume, while those of *Viscum schimperi* increased with altitude only, and those of *P. curviflorus* decreased with altitude and increased with host canopy volume. Moreover, the mistletoe species seemed to affect each other’s presence. *P. curviflorus* and *V. schimperi* seemed to compete with each other, whereas *V. schimperi* seemed to facilitate the prevalence of *P. austroarabica*. The pattern of mistletoe species prevalence may be largely explained by their morphological factors, most simply by their leaf size; *P. austroarabica* has large leaves, *P. curviflorus* has small leaves, and *V. schimperi* is leafless.
Leaf size affects the physiological response of mistletoe to varying temperatures at the growth site and water availability in the host tree. Temperature and water availability, in turn, are largely controlled by topographic factors, especially altitude. Therefore, I conclude that topographic factors largely explain the prevalence of mistletoe in the desert landscapes of our sites, but biotic interactions amongst mistletoe also affects their prevalence.

Chapter 2

I investigated the effects of *P. curviflorus* on the nutrient status of three host species by comparing N, P, K, Ca, Na, Mg, Fe, Mn, and Zn contents between parasitised and unparasitised trees. Furthermore, I examined the nutrient uptake mechanisms of *P. curviflorus* using the nitrogen:calcium hypothesis. Since N is considered a highly mobile element and Ca is considered immobile, when the N:Ca ratio in the mistletoe was > 1, I inferred that the mistletoe used active uptake (i.e. tapped nutrients from the host’s phloem), and when the N:Ca ratio was < 1, I inferred that the mistletoe used passive uptake (tapped nutrients from the host’s xylem). *P. curviflorus* reduced the content of N and Mn from *S. asak* and P and Zn from *V. flava*, but it did not affect nutrient levels in *V. tortilis*. Moreover, mistletoe seemingly acquired nutrients actively from *S. asak* (N:Ca > 1), but passively from *V. flava* and *V. tortilis* (N:Ca < 1). This diversity in mistletoe behaviour to exploit nutrients may reflect the environmental conditions under which the hosts grow and also the needs of this parasitic plant to utilise specific nutrient elements.

Three species of mistletoe infect *V. gerrardii* in Wadi Alshafa. I investigated the effects of different levels of mistletoe load on nutrient status in the host *V. gerrardii*. Levels of N, P, Na, and Ca were higher in host plants heavily infected by *P. austroarabica* and *P. curviflorus*, whereas Mg and Ca levels were higher in hosts heavily infected by *V. schimperi*. 
The varying physiological responses to mistletoe might be due to different morphological factors, such as the morphology of photosynthetic organs, which may lead to variable requirements for nutrients. Regardless of the mistletoe species, the degree of infection is indeed driven by host characteristics; most of the heavily infected hosts were larger and possibly older than the moderately infected, slightly infected, or uninfected hosts, and these bigger host plants can produce a sustained supply of nutrients and water for a large number of mistletoe individuals.

Chapter 3

I tested whether the three species of mistletoe differed in nutrient status and whether host branches infected by different species varied in nutrient status. I took the samples from a single host *V. gerrardii* that was infected by two or three species, and performed chemical analysis of N, P, K, Na, Ca, and Mg. We found that the three species had fairly different mineral nutrient patterns, accumulating nutrients at different levels. I found that *P. austroarabica* extracted more N and Na and *V. schimperi* extracted more P, compared to *P. curviflorus*. Moreover, *V. schimperi* derived more Mg from the host than other mistletoe species. The effects of mistletoe on the nutrient status of their host branches were slight, except for *P. austroarabica*, which had a stronger effect on the levels of N and P of the host. Additionally, all mistletoe individuals reached a higher concentration of K than their host branches, while Ca was higher in branches infected by *V. schimperi* than in those infected by *P. austroarabica* or *P. curviflorus*. It is likely that the variability in nutrient accumulation is a result of the functional variation of morphological factors, such as photosynthetic organ morphology, which can shape nutrient acquisition.
Chapter 4

I hypothesised that *P. curviflorus* infection adds more physiological stress to the host when there are intra-seasonal fluctuations and erratic rainfall patterns. Water potential and photosynthetic performance in mistletoe and the four host species occupying four different topographic positions were investigated. For mistletoe growing on different species of host, a lower quantum yield ($F_v/F_m$) and a more negative water potential was observed on *S. asak*, *V. flava*, and *V. tortilis* in the dry period than in the wet period; however, mistletoe performance did not vary on *V. gerrardii* between the dry and wet periods. For mistletoe and infected branch stress levels, mistletoe had a more negative water potential, and either a lower or equal pre-dawn or midday quantum yield, compared to those of the infected branches of host species, except for *S. asak*, in which no difference in water potential was found between the mistletoe and the infected branch in the dry or wet periods. Moreover, the $F_v/F_m$ of *P. curviflorus* was higher than that of the infected branches and this difference was more pronounced in the wet period compared to the dry period. For host and non-host stress levels, a significant interaction between effects of infection and effects of period was found for *S. asak*, in which infected branches had a greater reduction in $F_v/F_m$ compared to uninfected branches or uninfected hosts in the wet period compared to the dry period. Although the period had an influence on the midday water potential of infected and uninfected hosts, the effect of infection was significant in the shrub-like *S. asak*, *V. flava*, and *V. tortilis*, in which infected branches had lower pre- dawn water potentials than uninfected branches or uninfected hosts. My findings suggest that mistletoe performance and its effect on different host species are driven by periodic effects, which influence the water balance of each species growing in different parts of the landscape. Mistletoe performance varied on the three shrub-like species *S. asak*, *V. flava*, and *V. tortilis*, but it did not vary on the tree *V. gerrardii*.
This suggests that mistletoe may perform better on *V. gerrardii* since this species is a large tree, and therefore might have better access to water and nutrients through its long root system in drought conditions than shrub-like species with shorter root systems. Because the recovery time reflects long-term effects, infected branches were more water stressed than uninfected branches or uninfected hosts in shrub-like species in the dry period. Therefore, it can be inferred that mistletoe infection greatly enhances water stress in shrub-like hosts. Additionally, mistletoe infection induces chronic photoinhibition in *S. asak* in the wet period, suggesting that mistletoe demands large amounts of water and nutrients to avoid host exclusion, especially in drier sloped conditions.

**Chapter 5**

In Wadi Alshafa, where *V. gerrardii* was infected at different levels by three species of mistletoe, we investigated the water status and light utilisation of hosts at pre-dawn and midday and whether they were negatively affected by increased mistletoe infection. Furthermore, the fact that the changes in altitude affected mistletoe prevalence suggests that there are differences in their responses to temperature and moisture, which creates different levels of physiological stress for mistletoe species (Ch. 1). Thus, I compared the physiological performance of these species, and how it influenced the host. Heavily infected hosts suffered from higher levels of water stress and were more prone to photoinhibition than moderately and slightly infected hosts. In daylight, there was a clear difference in light use efficiency, where Loranthaceae mistletoe species *P. austroarabica*, and *P. curviflorus* utilised the light better than the Viscaceae mistletoe *V. schimperi*. Moreover, the water stress level was significantly higher in the *P. austroarabica*-infected branches than in the infected branches of the other two species.
In conclusion, an overabundance of mistletoe increased water and light stress in the host. Mistletoe performance and effects on the host varied, which can most likely be attributed to morphological variation amongst species, and the host’s compatibility to fulfil the water and nutrient requirements of the mistletoe.

**Integration of findings**

Two sites with different environmental conditions were used to answer different sets of questions. I have linked different questions to give a comprehensive perception of the mistletoe effects at each site.

**Taif National Park**

Across distinct topographic surfaces and erratic rainfall patterns, I determined the differences in host and non-host characteristics (Ch. 1). The incidence of *P. curviflorus* was not significantly different between the three species *V. flava*, *V. tortilis*, and *V. gerrardii*. However, the host plants of one species, *S. asak*, had wider canopy areas than the non-host plants. An examination of nutrient status was carried out on the shrub-like species (Ch. 2), and only *S. asak* showed lower N levels in both the infected and uninfected branches of the host than in uninfected hosts. Consistently, mistletoe infection had a stronger negative effect on *S. asak*, and infected branches in particular showed a higher pre-dawn water stress level and suffered from photoinhibition (Ch. 4). These results suggest that there are negative effects on the physiological performance of *S. asak* as a result of long-term mistletoe infection. The observed geographic distribution of host species is considered the most obvious explanation of the differences mentioned in this system. I found that prolonged drought had large effects on the performances of both the host and mistletoe; both the host and mistletoe had a lower photosynthetic performance in the dry period than in the wet period, which is in agreement with the findings of Bowie and Ward (2004).
However, in the wet period, when resources were more readily available for both the host and mistletoe, it was likely that the observed decreased light use efficiency resulted from increased water stress and nitrogen limitation, as these parameters were different between hosts and non-hosts. Interestingly, the reduced nitrogen balance in both infected and uninfected branches of the hosts compared to the non-hosts may have been a sign of nitrogen deficiency.

*P. curviflorus* growing on *S. asak* had an active nutrient uptake, and seemed to have a connection with the host phloem (N:Ca > 1); however, it had a passive nutrient uptake and seemed to have a connection with the host xylem on *V. flava* and *V. tortilis* (N:Ca < 1) (Ch. 2). *P. curviflorus* possesses a specific haustorium in its endophytic system (Waly & Jrais, 2013), and the sinkers in its endophytic system might be influenced by the nutrient and water statuses of the host in different environments. Mistletoes have different haustorial endophytic structures that allow for different levels of penetration and resource acquisition (Waly & Jrais, 2013). The variation in nutrient exploitation may result from differences in the sinkers, which derive nutrients from the host. *P. curviflorus* growing on *A. asak* might increase the reach of its sinkers into the host tissues to reach the host phloem as a response to drier slope conditions, while *P. curviflorus* growing on other host species in low-lying areas, such as valleys and bench lands, might not face stress conditions, and can easily obtain water and nutrients from the host’s xylem without increasing the reach of their sinkers. In these lower areas, water and finer sediments accumulate, making the growing conditions more suitable for the host plant and possibly also for the mistletoe. Further research on our above estimations is necessary to provide evidence and reach a conclusion.
**Wadi Alshafa**

Along the dry valley, three mistletoe species infected the dominant tree *V. gerrardii* growing different between altitudes and host canopy volumes (Ch. 1). *P. austroarabica* prevalence increased with a larger host canopy volume and also with a higher altitude at sites in Wadi, whereas *V. schimperi* increased with a higher altitude but not larger canopy volume, and *P. curviflorus* increased with a larger canopy volume, but decreased with a higher altitude. In this ecosystem, the temperature during summer is a very important factor, as it decreases with higher altitude. Additionally, there was an inverse relationship between the host canopy volume and altitude gradient. This suggests that the altitude of a site can affect the temperature and water balance, and, in turn, largely affect the host canopy volume and the prevalence of mistletoe species.

An important difference between *P. austroarabica* and *P. curviflorus* is that these mistletoe species differ in haustorium and endophytic system morphologies, representing uptake functions that have adapted to the particular Wadi Alshafa microclimate. *P. curviflorus* possesses a sinker endophyte, while *P. austroarabica* possesses a flanging endophyte (Waly & Jrais, 2013). Therefore, it was expected that a sinker endophyte may have a greater ability to penetrate the host tissues, and access reliable water and nutrients from the host phloem, enabling *P. curviflorus* to survive on the host in the harsh and hot summer temperatures at the lower sites in Wadi. In contrast, *P. austroarabica* might establish a flanged haustorium on the host at high altitudes, where the influence of thermal stress is decreased. However, the morphology of the haustorium and endophytic system of *V. schimperi* is unknown, and further investigations are required to identify its effects on the nutrient uptake of the plant.
Another ecological aspect observed in the Wadi ecosystem was the coexistence of three species of mistletoe at different levels of infestation on single hosts. Notably, a negative relationship was found between *P. curviflorus* and *V. schimperi*, as well as between *P. curviflorus* and *P. austroarabica*. On the other hand, the abundance of *P. austroarabica* increased along with that of *V. schimperi*. Potentially, competitive and facilitative relationships (Queijeiro-Bolaños et al., 2017) can take place between the species of mistletoe in the Wadi. However, these mistletoe interactions need further investigation to be fully described; it would be interesting to germinate mistletoe seeds on the same branch of hosts across different altitudes to assess the competition among mistletoe species.

Although some studies (Bowie & Ward, 2004; Ward et al., 2006) have summarised that mistletoe did not cause water stress in the host, the present study showed that an overabundance of mistletoe increased water stress and caused chronic photoinhibition in the host (Ch. 5). The Wadi Alshafa system differs substantially from the environmental systems investigated in previous studies because it has a single dominant tree species hosting different species of Loranthaceae and Viscaceae. I performed a more detailed examination of the coexistence pattern in the Wadi ecosystem, in which the nutritional relationships and physiological performances of the three species of mistletoe and their host’s infected branches (Ch. 3 and Ch. 5, respectively) were analysed. *P. austroarabica* had a more negative impact on the host’s infected branches than the other two species; it gained more nutrients (especially nitrogen), displayed better light use efficiency, and resulted in more negative water potentials in the infected branches. The morphological variations between the mistletoe species, such as the morphology of the photosynthetic organs, may result in varying physiological responses, resulting in differential effects on the host.
An anatomical study by Jrais (2013) elucidated that leaf cuticle thickness was different among Loranthaceae mistletoe species; *P. curviflorus* had a thicker cuticle than *P. austroarabica*. It is likely that cuticle thickness influences the water permeability of the mistletoe species *P. austroarabica* and *P. curviflorus*; therefore, it is inferred that the thin cuticle seen in *P. austroarabica* contributes to an increased rate of water loss. This supports our finding, in which *P. austroarabica* showed a more negative water potentials (greater water stress). Previous physiological indicators may justify the ecological inference of our finding, in which a high abundance of *P. austroarabica* existed at higher altitudes to avoid thermal stress, especially in the summer period. In contrast, the thick cuticles of *P. curviflorus* may reflect their adaptation to water stress conditions; I found this mistletoe had a less negative water potential, and possibly avoided chronic water stress in the hot summer temperatures of the low altitude sites due to this adaptation. Unfortunately, there are no anatomical studies of the leafless *V. schimperi* (Jrais, 2013; Waly & Jrais, 2013). Further research on the haustorial junctions and gas exchanges of these mistletoe species is necessary to draw conclusions from these observations.

**Conclusion and future directions**

This project is a significant contribution to the field of desert mistletoe ecology. This study aimed to provide an understanding of the effect of mistletoe on *Senegalia* and *Vachellia* species in two settings in the arid western Arabian Peninsula. My findings showed differences in mistletoe effects on their hosts. Consequently, management practices may be not essential for mistletoes in Taif National Park, where there were no significant impacts on their hosts. However, a management strategy seems to be worthwhile in Wadi Alshafa. Notably, heavy and medium mistletoe infection depleted micronutrients such as N, P, and K particularly by *P. austroarabica*. This led to nutrient and water stress and inhibited the photosynthetic performance of hosts.
Therefore, I suggest a disinfection method for moderately and heavily infected hosts. This method consists of mistletoe removal by cutting their haustorium; the point where mistletoe attaches to the host branch. Interestingly, this study revealed that Loranthaceae had more negative effects on nutrient and water status of their hosts compared to Viscaceae. Consequently, applying the removal method for accumulation of Loranthaceous mistletoes is highly recommended.

In an ecological and physiological context, this research provides evidence of biotic and abiotic interactions. In Taif National Park, abiotic effects (i.e., topographic variation and prolonged drought) controlled the mistletoe–host association, resulting in varying effects of mistletoe on the host. There is a marked point when the effect of altitude was a major driver for a combination of biotic and abiotic factors in the Wadi ecosystem. As the altitude of a site can affect the temperature and water balance, it can greatly affect the host canopy volume, and thus the prevalence of mistletoe species. Here, logistic regression models suggested a fascinating biotic interaction, with competitive and facilitative relationships possibly occurring when the three mistletoe species coexisted on a single host.

In addition, in this study we have considered the physiological and ecological aspects of the mistletoe–host relationship and evaluated the differences in these aspects that are performed by a range of parameters, including water status, nutrient levels, and light utilisation, in mistletoe families Viscaceae and Loranthaceae. In the two different systems evaluated, desert mistletoe species, whether in their prevalence or their effects on the host, seem to be greatly driven by water demand in arid environments.

This research demonstrates mistletoe effects on native trees. It could also be possible to apply physiological measurements on fruit trees, thereby addressing mistletoe infection on farmlands and its economic impacts. The comparisons between the effects of mistletoe infection on native
and non-native plants are worth further investigation, as this helps formulate solutions to mistletoe problems and also provides irrefutable evidence in case local people complain about mistletoes management.

In addition to the recommended further research, it is also suggested that various environmental factors such as light, temperature, salinity and drought, have effects via changes in intercellular CO₂, so I recommend using carbon isotopes as a comprehensive tool for assessing mistletoe-host relationships under combined ecological factors. The water use efficiency inferred from ¹³C has been widely related to long-term trends in the internal regulation of carbon uptake and water loss in plants, therefore detecting mistletoe effects on photosynthesis machinery, and hence the entire host growth (Ehleringer et. al., 1986).

Mistletoe seeds are dispersed by birds, and the variation in incidence and abundance of mistletoe species can result from the preference of birds for the fruit of certain mistletoe species (Montaño-Centellas, 2013). Notably, heavy mistletoe infection is often related to the frequent visiting of birds when the host trees produce their fruits. Monitoring bird behaviour helps us to understand the underlying reason for mistletoe prevalence on a broad scale. Therefore, the time of mistletoe removal is important, and it would be advantageous if mistletoe removal could be done before they produce fruit, limiting bird visits to the disinfected trees and encouraging them to search for fruit in other areas.

Even though this study suggests a traditional method (e.g., pruning infected trees) to address mistletoe infection with a short-term solution in the dry environment. Management must address the underlying causes of the problems leading to mistletoe expansion. This requires an ecosystem approach in order to investigate both the direct and indirect causes of the current status of mistletoes.
Although this research is the first study to show the relationship between mistletoe and hosts in the Arabian Peninsula, and it has provided considerable morphological and physiological assessments based upon empirical work, there are still critical knowledge gaps that should be addressed. I have provided two different infection patterns in examples of arid lands, but there are massive mistletoe infections in many areas of the Arabian Peninsula, especially those that have wildwoods and farmlands, which need considerable work to provide background information such as morphological and physiological data. The problem resulting from the expansion of mistletoes documented here is unusual because of the complexity of ecological interactions involved. While a change in one ecological factor influences other ecological factors, affecting the relationship of host and mistletoe, the relative roles of facilitation and competition mechanisms of mistletoes are also complex. In the present study, mistletoe-host relationships exhibited different outcomes for the prevalence of mistletoe infection, and their physiological effects. My suggestions have relied on variation in topographic traits (i.e. slopes, valleys, etc.), which result in disparity of water and nutritional relationships for mistletoe and host species. However, as observed during field work, human activities such as drilling water wells and road construction can also cause heterogeneity in water and nutrient distribution and preclude access to reliable water and nutrients for the trees. Therefore, host-mistletoe relationships also require further investigation with respect to habitat quality in low productivity systems (e.g. water availability and nutrient distribution). The spatial distribution of the mistletoe species, their abundance, and other ecological interactions are not yet fully understood in the Arabian Peninsula.
References


