

Determining strategies of *Acanthosicyos horridus* (!nara) to exploit alternative atmospheric moisture sources in the hyper-arid Namib Desert

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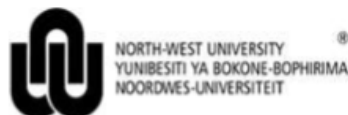
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ABSTRACT

The enigmatic melon species *Acanthosicyos horridus* Welw. ex Hook. f., locally known as Inara, is endemic to the hyper-arid Namib Desert where it occurs in sandy dune areas and dry river banks. The Namib Desert is a result of the cold Benguela current off the coast of Namibia. This results in extreme environmental conditions including high temperatures, rare pulse rainfall events and desiccating air. In this water-restricted environment, non-rainfall water inputs (NRWIs), including fog, dew and water vapour, may play an important role in ecosystem function and can influence organisms' behaviour to exploit alternative sources of moisture. Fog is considered to be the most important Non-Rainfall water inputs (NRWI) for most of the coastal Namib Desert, where *A. horridus* plants are common. It has been suggested that *A. horridus* is adapted to exploit fog as a moisture source. *Acanthosicyos horridus* shares many comparable adaptive features with other organisms that are known to exploit fog as a source of moisture. This study focused on *A. horridus*-fog interaction to determine whether *A. horridus* exploits fog, as it would illustrate strategies to benefit from NRWIs. The direct water uptake capacity of *A. horridus* shoots was investigated through absorption tests. Furthermore, the movement and behaviour of fluorescent water droplets on a *A. horridus* stem were investigated through time-lapse macrophotography. The shoot water potential was measured to investigate the effect of a fog on the water status of *A. horridus* stems. Chlorophyll *a* fluorescence was used to compare the photosynthetic potential of *A. horridus* plants on days with fog events to that on non-foggy days. Other environmental stressors were identified by comparing meteorological data with the photosynthetic potential of *A. horridus* stems. These tests advised on whether *A. horridus* has specific strategies to exploit NRWIs, i.e. through behaviour, habitat selection and habitat modification. *Acanthosicyos horridus* did exhibit the capacity for direct aerial absorption of fog water into the stems. Moreover, *A. horridus* did not exhibit visible signs of drought stress and this, together with the high shoot water potential, indicates that plants are reliant on permanent underground water sources as they are unlikely to survive on NRWIs alone, even within the zone of abundant fog in the Namib Desert.

Measurements of the photosynthetic potential indicated that temperature stress and wind were some of the main abiotic factors influencing the plant's overall vitality. Furthermore, the plants were able to recover their photosynthetic potential after exposure to air temperatures above 40°C.

Key words: Abiotic variables, Cucurbitaceae, chlorophyll a fluorescence, foliar absorption, fog harvesting, Namib Desert

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CHAPTER 1: INTRODUCTION

1.1 Background and rationale

Photosynthesis is a vital metabolic process in plants, but the function of the photosynthetic apparatus is sensitive to environmental stressors. Stressors include carbon dioxide (CO₂) availability, water deficit, radiation, temperature, pathological conditions, nutrient supply and pollutants (Kalaji *et al.*, 2016). In extreme environments such as deserts, some of these stressors (water deficit, radiation, temperature and nutrient supply) may be substantial and thus lead to specific adaptations to cope with predictable challenges. Measuring the rate of photosynthesis can be used as a tool to determine the overall vitality or 'health status' of a plant (Tòth *et al.*, 2005; Stirbet & Govindjee, 2011). Water deficit is one of the main environmental stressors under desert conditions. This may result in stomatal closure to reduce transpirational water loss as well as in a decrease in the potential, metabolic uncoupling and reduction in plant carbon balance (Campos *et al.*, 2014; Kalaji *et al.*, 2016; Mishra *et al.*, 2016). The decrease of stomatal conductance together with sustained irradiance results in an imbalance between intercellular energy relative to available CO₂ (Campos *et al.*, 2014).

Many different organisms have adapted to survive and even thrive in dry, hot desert environments (Gibson, 1998). Plants in these arid environments have evolved different strategies to endure, escape or evade desiccation (Gibson, 1998). Initially, biologists indicated that most of the observed structural characteristics in plants were adaptations to limit water loss, but recent evidence (Gibson, 1998) suggests that many of the physiological and structural adaptations maximise photosynthetic potential and regulate a plant's energy budget. In general, C₃ plants tend to have a temperature optimum around 20–25°C. Increased temperature stress together with water stress lead to stomatal closure. Stomatal closure results in a reduction in CO₂ uptake, thus leading to an overall reduction in photosynthesis (Hopkins & Hüner, 2008).

Acanthosicyos horridus Welw. ex Hook. f., locally known as !nara, is endemic to the Namib Desert, with distribution limited to sandy areas. In many populations, high levels of groundwater

are available (Klopatek & Stock, 1994). It has been suggested that the observed modifications of *A. horridus* enable it to cope with environmental stress caused by high temperatures, high light intensity and the desiccating desert air (Henschel *et al.*, 2004). Furthermore, this plant's resistance to water deficit and other environmental stressors may rely on physiological and morphological traits as observed elsewhere (Xu *et al.*, 2012).

Since *A. horridus* plants are endemic to one of the most extreme desert environments on earth, the question of what special adaptations have allowed them to survive and even flourish under the specific conditions that prevail in their habitat is pertinent. This may improve our understanding of how C₃ plants can adapt to such hot, dry environments and what structural characteristics are important against environmental stresses. Furthermore, the effect of non-rainfall water inputs (NRWIs) is one of the least studied hydrological components of ecosystems and could play a major role in ecosystem function and plant-soil relations, particularly in arid environments. An improved understanding of the ecological effect of NRWIs in water-scarce systems is also important to predict an ecosystem's response to environmental change.

The Namib Desert, where *A. horridus* occurs, is a cool, hyper-arid coastal desert situated in southwestern Africa (Goudie, 1972; Louw, 1972; Lancaster *et al.*, 1984), with low annual precipitation and high potential evaporation rates. Rainfall in the Namib Desert plays a vital role in maintaining ecosystem functions (Kaseke *et al.*, 2016), but rainfall is low and highly variable (Shanyengana *et al.*, 2002). However, NRWIs, like fog, exceed annual rainfall (Eckardt *et al.*, 2013) and may reduce the effect of water stress on a host of different organisms including lichens, plants and invertebrates (Wang *et al.*, 2016).

It has been suggested that *A. horridus* plants exploit fog precipitation (Berry, 1991; Hebel, 2000) as is the case with other Namib flora, such as *Stipagrostis sabulicola* (Louw & Seely, 1980) and *Trianthema hereroensis* (Seely *et al.*, 1977). The suggestion was mainly due to plant structural morphology (Berry, 1991; Henschel *et al.*, 2004; Kartusch & Kartusch, 2008) and distribution and population densities that seem to favour areas with frequent fog incidence (Berry, 1991). A stable

isotope study by Soderberg *et al.* (2014) was, however, unable to show that *A. horridus* utilises fog as a primary source of water.

This study used chlorophyll *a* fluorescence (ChlF) to compare the overall plant performance of *A. horridus* shoots on days when fog occurred to that during non-foggy days, to determine whether *A. horridus* plants utilise fog as a supplementary source of water. The physiological study was supplemented by an investigation of specific morphological traits that would allow the plant to efficiently harvest fog. If *A. horridus* plants were able to exploit the frequent fog events in parts of the Namib, then the same adaptations would allow it to exploit other non-rainfall moisture sources.

1.2 Layout and approach

This dissertation is divided into six chapters. The introduction (Chapter 1) provides brief background information on the study and explains the rationale. This chapter also states the principal aims and hypotheses of the study. The literature review (Chapter 2) provides background on the family Cucurbitaceae and plants that are known to exploit fog, while also outlining some of the methods frequently used to determine whether plants utilise fog. The study site (Chapter 3) is described in light of the different ecosystems of the Namib Desert in terms of geology, abiotic variables and fauna that utilise fog. Chapter 4 reports on how *A. horridus* plant may exploit non-rainfall moisture, including water movement along the stem, aerial fog absorption and xylem pressure. Chlorophyll *a* fluorescence (Chapter 5) provides information on the photosynthetic potential of *A. horridus* plants under the influence of fog and other abiotic variables. The synopsis and future prospects (Chapter 6) discuss the major findings and how the aims of the study were met.

1.3 Hypotheses and principal aims

The main objectives of this study were to:

- Analyse the relationship between *A. horridus* plant's overall vitality and abiotic variables.
- Assess and evaluate whether and how *A. horridus* plant is able to utilise non-rainfall moisture as a supplementary source of water.

The aims of the study were based on the following assumptions:

- i. *Acanthosicyos horridus* plants are C₃ plants and, in general, these plants tend to have a temperature optimum of 25–28°C. Exposure to environmental stress will result in a decrease in the photosynthetic potential and overall vitality *A. horridus* plants.
- ii. Hebeler (2000) examined the structural and ecophysiological shoot features of *A. horridus*. The rough structure of the hydrophobic wax layer should make the stem surface more wettable and, together with the trichomes, might retain non-rainfall moisture on the stem surface, e.g. after a fog event.
- iii. If *A. horridus* plants can absorb non-rainfall moisture directly through the stem, this will result in an increase in the photosynthetic potential of *A. horridus* plants after a fog event.

The approach to test these assumptions was to:

- Determine whether *A. horridus* plants utilise fog as a supplementary source of water.
- Determine how abiotic variables, particularly fog, will influence the photosynthetic potential
- Compare the water status of the plants between days with fog occurrence and non-foggy days.
- Test condensed water droplet uptake by *A. horridus* plant.
- Identify the structural characteristics that would contribute to efficient utilisation of NRWIs.

CHAPTER 2: LITERATURE REVIEW

2.1 Family Cucurbitaceae

The distribution and ecology of genera and species in the family Cucurbitaceae Juss., consisting of ca. 735 species in ca. 120 genera, vary enormously, but they are predominantly annual or perennial herbs or shrubs with trailing stems (climbers and creepers). Leaf axils may carry branched or lateral tendrils (Koekemoer *et al.*, 2014; Meeuse, 1962). The family usually has alternate leaves and is covered with rough trichomes. Most species that are native to temperate climates are seed-producing seasonal annuals or perennials that are susceptible to frost. Some genera that are aggressive climbers can flourish in humid environments, including *Sechium* (six species) that grows in the Neotropics. Other genera that are native to the Pleotropics include *Cucurbita* (13 species), *Momordica* (45 species), *Cucumis* (52 species) and *Citrullus* (four species) and occur mostly in Africa and South-eastern Asia.

There are ca. 16 genera and ca. 78 species of the family Cucurbitaceae in the flora of southern Africa. The family is best represented in the dry tropical and subtropical regions of the Highveld and KwaZulu-Natal as well as the Mpumalanga escarpment of South Africa (Robinson & Decker-Walters, 1997). Well-known southern African genera include *Citrullus*, *Coccinia*, *Gerrardanthus*, *Kedrostis*, *Momordica*, *Peponium*, *Trochomeria* and *Zehneria* (Koekemoer *et al.*, 2014). Six different genera (*Acanthosicyos*, *Citrullus*, *Cucumis*, *Cucurbita*, *Lagenaria* and *Momordica*) are found in Namibia, with only one being endemic (Meeuse, 1962; Mannheimer *et al.*, 2009).

According to Meeuse (1962), different southern African genera from the Cucurbitaceae family have been used extensively in medicine and some are economically important crops. These include the fruit-producing *Citrullus lanatus* Schrad. ex Eckl. and Zeyh., commonly known as watermelon, *Cucumis* L. (cucumbers, gherkins and melons) and *Cucurbita* L. (pumpkins, marrows and squashes). In the drier regions, indigenous inhabitants are known to use the fruit of *C. lanatus* (tsamma) and *Acanthosicyos horridus* (!nara) (Meeuse, 1962). Bitter substances (cucurbitacins) are widespread and common in the family (Mannheimer *et al.*, 2009).

The genus *Acanthosicyos* is a perennial shrub (*A. horridus*) or creeper (*A. naudinianus*), branched from the base, with spines paired at nodes and no tendrils. The plants are dioecious and female plants produce spine-tipped, conical fruits with many seeds. This genus only consists of two species, namely *A. naudinianus* (Sond. 1862) C. Jeffrey and *A. horridus* Welw. ex Hook. f. in Benth and Hook. f., Gen. Pl. 1: 824 (1867).

Acanthosicyos naudinianus is a geophyte (gemsbok cucumber, Fig. 1) that occurs in savanna bushveld habitats in Botswana, Namibia, Mozambique, South Africa, Zambia and Zimbabwe. The other species, *A. horridus*, is endemic to the Namib Desert (Berry, 1991; Meeuse, 1962; Müller, 2000) and is further discussed in the following section.



Figure 1: *Acanthosicyos naudinianus* (gemsbok cucumber) flower (credit: Eugene Marais)

2.1.1 *Acanthosicyos horridus*

2.1.1.1 Distribution and habitat

Acanthosicyos horridus is endemic to the Namib Desert (Fig. 2) and grows on aeolian sand dune substrates and the sandy banks of ephemeral rivers (Berry, 1991). *Acanthosicyos horridus* plants were proposed to mostly occur within the coastal fog belt, with only a few individuals scattered

more inland (Berry, 1991), but the distribution map of *A. horridus* (Fig. 2) indicates that numerous *A. horridus* plants are distributed outside of the fog belt. This map was compiled using herbaria specimen records and verified observation records from the Gobabeb Research and Training Centre (GRTC), National Botanical Research Institute (NBRI) in Windhoek, Namibia, and South African National Biodiversity Institute (SANBI). These records indicate that *A. horridus* plants are distributed along the length of the Namib, from Namibe in Angola to Port Nolloth in South Africa (Kutschera *et al.*, 1997; Van den Eynden *et al.*, 1992).



Figure 2: Distribution map of *Acanthosicyos horridus* in south-western Africa compiled using location records from the Gobabeb Research and Training Centre, National Botanical Research Institute and South African National Biodiversity Institute (credit: Campbell Nell)

Robinson and Seely (1980) recognised an *A. horridus* dune base community as one of four vegetation communities of the dune ecosystem and interdune valleys. It was suggested that plant community distribution along a dune elevation gradient is limited by sand stability and soil moisture availability (Robinson & Seely, 1980). The shallow soils of flat interdune valleys are unable to retain moisture for long periods, whereas the lower regions of dune slope communities have the greatest stability and soil moisture availability. Most of the perennial vegetation (including *A. horridus*) therefore prefers the dune base communities (Louw & Seely, 1980; Robinson & Seely, 1980).

Acanthosicyos horridus plants form tangled thickets that allow wind-blown sand and plant litter to accumulate in the spiny bush, resulting in hummock formation (Fig. 3). Hummock formation is a consequence of airborne sand and organic matter particles being trapped (Wallis & Raulings, 2011) and may also be a form of adaption to these harsh environments (Klopatek & Stock, 1994).



Figure 3: *Acanthosicyos horridus* hummocks that formed through accumulation of wind-blown sand and organic matter (credit: Monja Gerber)

However, *A. horridus* individuals are not restricted to dune base communities but are also typically found along dry river banks, with a large population density in the Kuiseb River Delta near Walvis Bay (Klopatek & Stock, 1994). The Kuiseb River is an ephemeral river and recharges

underground aquifers that maintain riparian vegetation along its course through the Namib, resulting in a linear oasis. This underground water source provides the water needed for *A. horridus* plants to survive and was found to influence the distribution of *A. horridus* plants (Müller, 2000). In the 1960s, a flood barrier was constructed on granite bedrock in the Kuiseb Delta to prevent flood water from inundating Walvis Bay. This barrier restricts the river's subterranean flow and resulted in the deterioration of *A. horridus* fields (Botelle & Kowalski, 1995). In addition, water pumped up from the Kuiseb River to supply various towns and mines with water is unsustainable and has resulted in decreased groundwater levels and further deterioration of *A. horridus* populations (Dausab *et al.*, 1994).

It was suggested that seedlings that germinate and grow in river ecosystems tend to have more nutrient and water provisions and can easily penetrate the moist substrate layers after the river has flooded (Moser, 2001). In contrast, seedlings found in the dune ecosystem depend on surface water such as rain and NRWIs to reach deep permanent underground water sources, estimated to be 30 m below the surface (Moser, 2001).

Acanthosicyos horridus thickets can form hummocks of 5–10 m in height and 10–40 m in diameter (Klopatek & Stock, 1994), with plant shoots projecting 0.1–1 m above the hummock surface (Henschel *et al.*, 2004). A study by Kartusch and Kartusch (2008) on the functional morphology of *A. horridus* stems showed that the greatest part of the plant is covered by sand. Transpiration is absent in the parts of the plant that are buried beneath the soil and only a small part can therefore photosynthesise. Male plants tend to flower (Fig. 4a) throughout the year (Budack, 1983), whereas the female plants flower (Fig. 4b) predominantly from September to April, with peaks in October and December (Klopatek & Stock, 1994).



Figure 4: a) Male *A. horridus* flowers and b) female flowers with small fruits (credit: Oliver Halsey)

Acanthosicyos horridus is a C_3 phreatophyte (Klopatek & Stock, 1994) with a root system that taps underground water (Gibson, 1998). The large root biomass is dominated by a tap root, with the total root system and biomass underneath the plant being proportional to the hummock size (Klopatek & Stock, 1994). The roots are estimated to reach up to 50 m down to reach underground water (Klopatek & Stock, 1994), but an illustration by Kutschera *et al.* (1997) indicated that the taproot may be over 100 m in length but only reaching depths of more than 5 m (Fig. 5). This figure illustrates the root structure of *A. horridus* on a flat surface, but the root structure within a hummock has never been investigated and remains unknown. The root xylem vessels are the broadest found in any plant and can retain up to 2 ml of water per root centimetre (Kutschera *et al.*, 1997). Klopatek *et al.* (1992) found that *A. horridus* plant is associated with vesicular arbuscular mycorrhizae, which aid in nutrient retrieval in nutrient-limited desert sand environments.

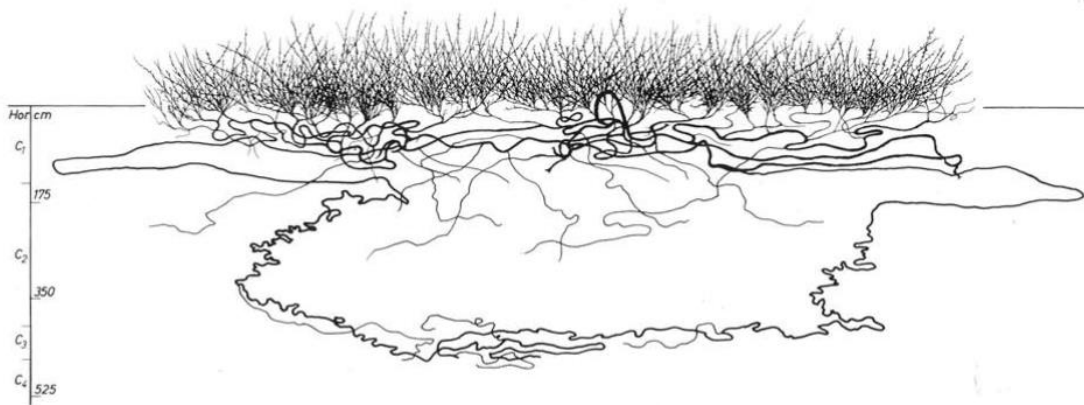


Figure 5: *Acanthosicyos horridus* underground root system that reaches a depth of more than 5 m (Kutschera *et al.*, 1997)

2.1.1.2 Desert adaptations in *Acanthosicyos horridus*

Acanthosicyos horridus exhibits typical xerophytic modifications to deal with environmental stress caused by high radiation, heat and drought. This includes the reduction of leaves to thorns (aphylly) and location of the photosynthetically active tissue in the thorns and stems (Fig. 6a). This results in reduced surface area, thereby limiting water loss through transpiration (Berry, 2001). Furthermore, *A. horridus* plants have stomata located on the stems, in furrows (Fig. 6b), with deeply sunken guard cells (Klopatek & Stock, 1994; Henschel *et al.*, 2004), which is a rare structural adaptation for desert plants (Gibson, 1998). The sunken stomata, together with the thick cuticle and trichomes, have also been suggested to reduce stem temperature and limit water loss (Henschel *et al.*, 2004). Another hypothesis is that sunken stomata protect the guard cells of desert plants from the hot, dry air to restrict stomatal closure and improve CO₂ uptake (Gibson, 1998).

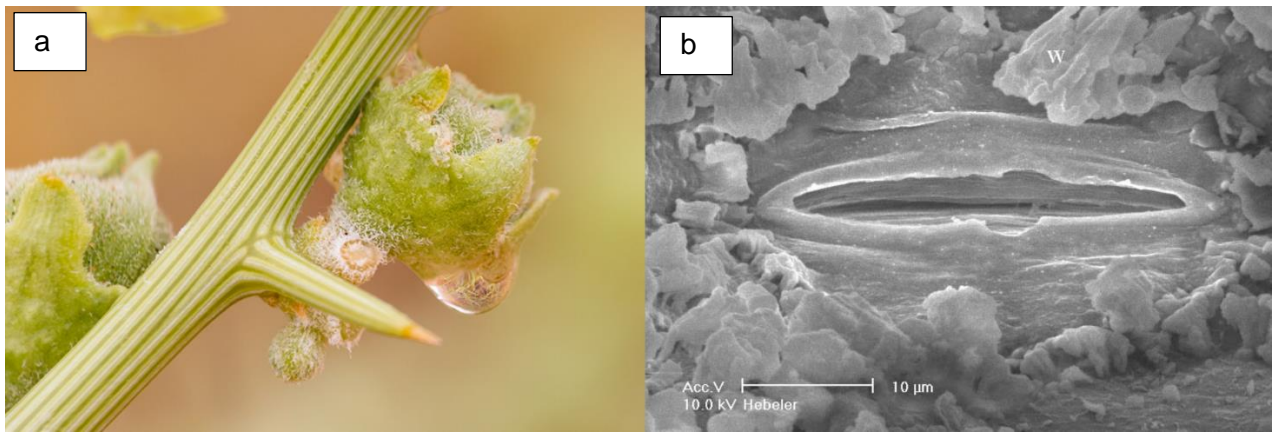


Figure 6: a) Xeromorphic modifications, including longitudinal grooves, leaves reduced to thorns and trichomes, to limit water loss and reduce stem temperature (credit: Oliver Halsey) and b) scanning electron microscope image of stomatal opening and wax (w) layer (Hebeler, 2000)

Acanthosicyos horridus plants' cylindrical stems (Fig. 6a) are hypothesised to be less efficient in intercepting sunlight than a planar leaf as only half of the stem is exposed to radiation. This results in a high degree of self-shading that protects the photosynthetic tissue against photodamage (Gibson, 1998). These adaptations also limit transpiration, which could be counterproductive as it results in a lower internal CO₂ concentration and overall lower net photosynthesis in some desert plants (Gibson, 1998). However, the furrowed stem may result in increased photosynthetic area of the cylindrical stem, consequently maximising net photosynthesis (Gibson, 1998). Furthermore, the multiseriate epidermis may act as a radiation filter to reflect ultraviolet (UV) light and protect the chlorenchyma against UV damage (Kartusch & Kartusch, 2008).

2.1.1.3 *Acanthosicyos horridus* ecology

Acanthosicyos horridus is a keystone species in parts of the Namib ecosystem and forms the basis of a complex food chain (Polis, 1991). It provides shelter and is a direct source of water and food for birds, insects and mammals and an indirect source of food for beetles. *Acanthosicyos horridus* plants also form an important ecological niche by stabilising the dune ecosystem and acting as a collection point for windblown organic litter (Klopatek & Stock, 1994). The plant also provides a moisture source for some organisms by bringing moisture to the dune surface through

its deep root system (Berry, 1991) and by intercepting non-rainfall moisture (Henschel *et al.*, 2004).

Burrowing gerbils (*Gerbillurus tytonis* and *G. paeba*) are secondary seed consumers and are responsible for seed dispersal (Klopatek & Stock, 1994; Müller, 2000) as they tend to bury *A. horridus* seeds found in faeces or around the hummock (Fig. 7a). Black-backed jackal (*Canis mesomelas*) are the main dispersers of the seeds as they tend to feed on the ripe melons of *A. horridus* plants (Fig. 7b). The jackals swallow whole seeds, which pass through their gut intact. The faeces provide nutrients for the seedlings to grow in the nutrient-deficient environment (Mayer, 2000; Müller, 2000).

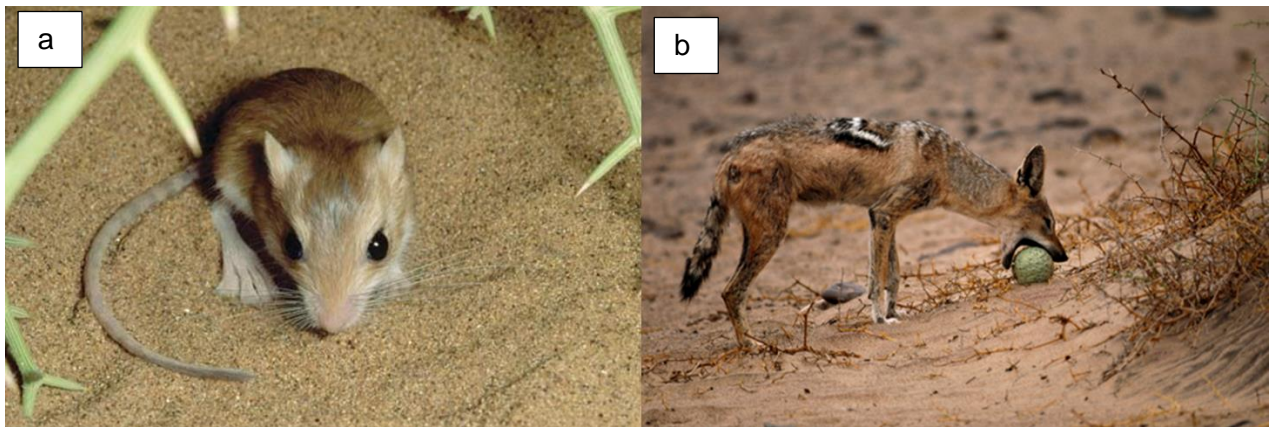


Figure 7: a) *Gerbillurus tytonis* under a *A. horridus* thicket (credit: Michael and Patricia Fogden) and b) *Canis mesomelas* eating an *A. horridus* fruit (credit: Des and Jen Bartlett/National Geographic Creative)

Pollinator vectors are essential to *A. horridus* plants because the plant has separate male and female plants and self-fertilisation is impossible (Mayer, 2000). In the immediate vicinity of GTRC, on the banks of the Kuiseb River, the blister beetle (*Mylabris zigzag*) and anthophorine bees, including *Anthophora auone* and *Amegilla velutina*, have been found to play a role in pollination (Fig. 8).

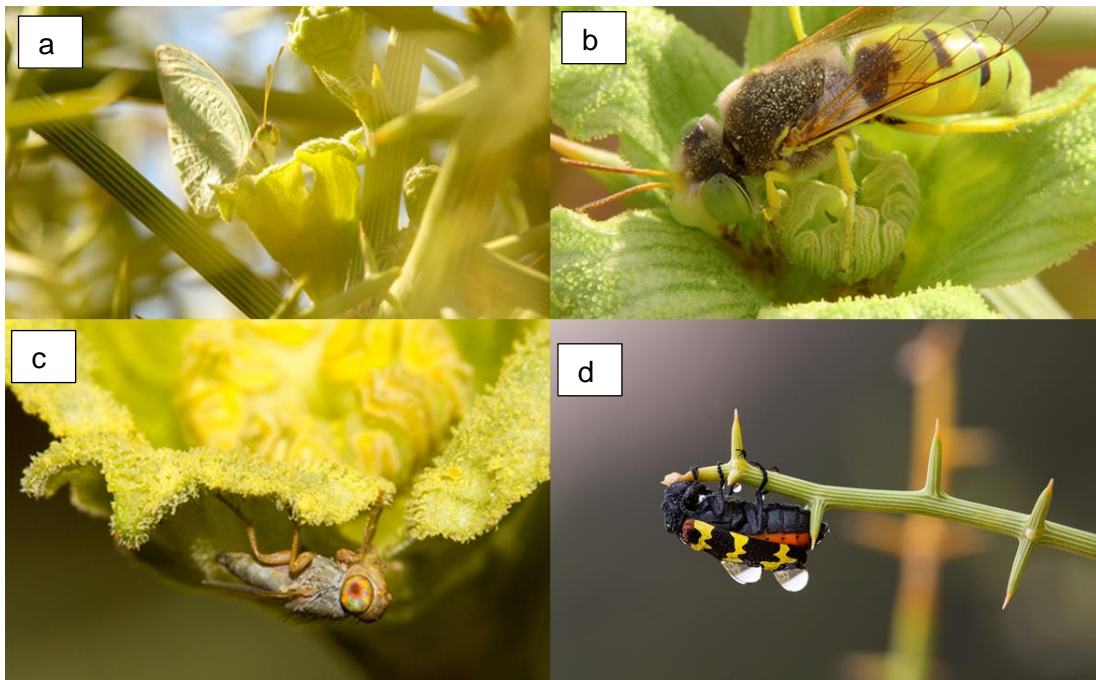


Figure 8: a) Butterfly on an *A. horridus* flower, b) sphecid wasp on *A. horridus* flower, c) Inara fly and d) blister beetle feeding on an *A. horridus* growth point (credit: Oliver Halsey)

2.1.1.4 *Acanthosicyos horridus* and the Topnaar

Acanthosicyos horridus plants have long been a valuable resource for the indigenous Topnaar (≠Aonin) community that lives along the banks of the Kuiseb River. The rural Topnaar tribe consisted of hunter-gatherers and pastoralists (Budack, 1983) who have adapted to this arid environment by exploiting local resources (Fig. 9). Their main sources of income are livestock, but they also harvest and sell *A. horridus* fruit and seeds, which form part of their tradition, culture, nutrition and economy (Dentlinger, 1977). Evidence found from Mirabib suggests that the use of *A. horridus* seeds dates back 8000 years (Sandelowsky, 1977).

Acanthosicyos horridus fruits are harvested when they are ripe and the fruits turn a yellow-green colour (Fig. 9), falling from the plant if probed with a stick (Schwartz & Burke, 1958; Arnold *et al.*, 1985). The *A. horridus* melons can be consumed immediately (Pfeifer, 1979) and have a rich and creamy taste when ripe (Botelle & Kowalski, 1995). More commonly, the flesh and seeds are cooked for hours (Fig. 9). After cooking, the seeds are separated from the flesh and sun-dried on

clay or a net. The seeds may be eaten raw or cold pressed to extract the oil, which may be used for cosmetic products (Botelle & Kowalski, 1995). The melon skins are dried for fuel or fed to the community's donkeys (Dentlinger, 1977).



Figure 9: Ripe *A. horridus* fruits are harvested by the local Topnaar community for consumption or the seeds are sold and provides a source of income (credit: Reyk Börner)

2.1.2 *Ecophysiological studies on Acanthosicyos horridus*

Hebeler (2000) examined the diurnal photosynthetic course on four individual *A. horridus* plants in the Gobabeb area. Hebeler (2000) measured transpiration and photosynthesis hourly over two 24- and 14-hour periods as well as photosynthetic active radiation (PAR), leaf temperature (T_{leaf}), ambient relative humidity (RH_a), ambient air temperature (T_a) and soil temperature (T_s). The study did not evaluate the effect of wind speed and wind direction. The results showed that the photosynthetic potential was diurnally inconsistent and varied on different shoots of the same plant. Hebeler (2000) concluded that factors influencing the maximal photosynthesis include plant damage, age, exposure to radiation, shoot water potential and wind. This study identified four

general photosynthetic patterns that represent the plant's response to different environmental stress factors. Firstly, under favourable conditions, there was an increase in net photosynthesis during the morning until 11:00, followed by a slow decline until sunset. Secondly, a maxima curve occurred at 11:00 and 17:00. This is possibly due to stomatal closure in response to temperature stress. Thirdly, a maxima curve occurred around 13:00, followed by a decrease in photosynthetic rate, which correlated to a decrease in PAR and temperature. Lastly, an overall low net photosynthesis was measured due to relatively low plant water content.

Hebeler (2000) observed an increase in the net photosynthesis and transpiration after the Kuiseb River flooded and groundwater sources were replenished. *Acanthosicyos horridus* plants exhibited an overall low net photosynthesis and high transpiration rate, which is consistent with the expected pattern for C₃ xerophytes in hot, arid environments. *Acanthosicyos horridus* plants are assumed to exhibit an opportunistic strategy that may trade high transpiration water loss for a steady carbon gain during drought or unfavourable conditions to maintain its large biomass. This would suggest that the plants are water wasters that occupy a specific niche in the harsh environment. Hebeler's (2000) study confirmed that *A. horridus* plants exploit groundwater as a water source and that these plants are opportunistic. Hebeler (2000) did not examine the effect of non-rainfall moisture events and how this would influence net photosynthesis.

A study by Moser (2001) measured ChlF on *A. horridus* seedlings to evaluate stress factors such as nutrient and water deficiency, which may induce photoinhibition. Moser (2001) concluded that the plants close to the river had higher biomass than those further away, as the loamy and silty riverbed has a higher water storage capacity and availability. Moser (2001) suspected that the calcareous substrate and coarse sand found in the dune and interdune areas cannot store precipitation.

2.1.2.1 Water potential measurements

Hebeler (2000) also investigated the course of diurnal water potential for *A. horridus* plants using a Scholander pressure bomb (Scholander *et al.*, 1965) during the morning, midday and evening as well as after dark. The values varied considerably, namely between -0.4 and -2.5 MPa, on

different shoots of the same plants during the day. However, these results are unreliable as Hebeler encountered some experimental problems during the investigation. These problems included difficulty distinguishing between phloem exuded sap and xylem water and some of the shoots had to be shortened and spines removed to fit the shoots into the chamber, resulting in an overall lower water potential value. Additionally, the anatomy of *A. horridus* plant caused air to be forced along the substomatal cavities in the pressure bomb, which resulted in heavy bubbling at the cut area, making it difficult to monitor the xylem pressure. To keep the cut area clean, an additional cut of 0.5 mm was made 3 mm below the cut surface. This allowed air from the stomatal cavities to exit through the secondary cut without affecting the central xylem vessels.

Hebeler's (2000) study indicated that *A. horridus* plants exhibited a remarkably high (less negative) water potential for desert plants. This was a further indication that *A. horridus* plants exploit groundwater as a main water source. This was confirmed by an increase in water potential after the Kuiseb River flooded during Hebeler's (2000) study.

2.2 Fog harvesters in the Namib

Where rainfall is sparse, non-rainfall moisture may be an important source of water and can aid in improving the water status of different plants (Limm *et al.*, 2008; Ebner *et al.*, 2011). Organisms may employ several strategies to harvest fog, including the precipitation of droplets on the surface and/or directly absorbing moisture through certain anatomical traits (Henschel & Seely, 2008).

2.2.1 *Stipagrostis sabulicola*

Ebner *et al.* (2011) investigated the fog-harvesting capabilities of the Namib endemic *Stipagrostis sabulicola* (De Winter) Pilg. (Poaceae, common C₄ dune grass) close to Gobabeb Research and Training Centre (Fig. 10). The leaves were harvested and the water potential measured with a portable PSYPRO™ water potential system on days with a fog event and non-foggy days. The results showed a significant difference between the stem water potential on foggy days and that on non-foggy days, suggesting that plant water status is improved by fog. Ebner *et al.* (2011)

concluded that the coalesced droplets that drip down to the soil are taken up by the fine root hairs of *S. sabulicola*.

This corresponds to observations by Vogel and Seely (1977) and Louw and Seely (1980) in that *S. sabulicola* exploits non-rainfall moisture from the wet sand surface through an extensive network of shallow root hairs. Nørgaard *et al.* (2012) suggested that the three-dimensional stem structure of *S. sabulicola* is more important in water collection than the surface properties. These authors compared the amount of water collected from a metal wire with that collected from *S. sabulicola* and found that it collected the same amount of fog water. Malik *et al.* (2014) argued that this could be due to the lower temperature of the metal wire, which resulted in dew formation, and that the type of material should be taken into consideration.

Roth-Nebelsick *et al.* (2012) studied how the leaf structure of *S. sabulicola* corresponds to fog-harvesting capabilities (Fig. 10). Droplet formation was directly observed and captured using a camera with a macro lens. These authors found that the leaf surface of *S. sabulicola* is covered in a highly irregular, hydrophilic wax layer with a slightly hydrophobic contact angle. Fog droplets were seen to coalesce on the stem and dripped down parallel to the longitudinal grooves on the stem to the root system (Fig. 10). The authors concluded that the rough surface structure of *S. sabulicola*, due to the silica hairs and irregular wax layer, makes this plant an efficient fog harvester.

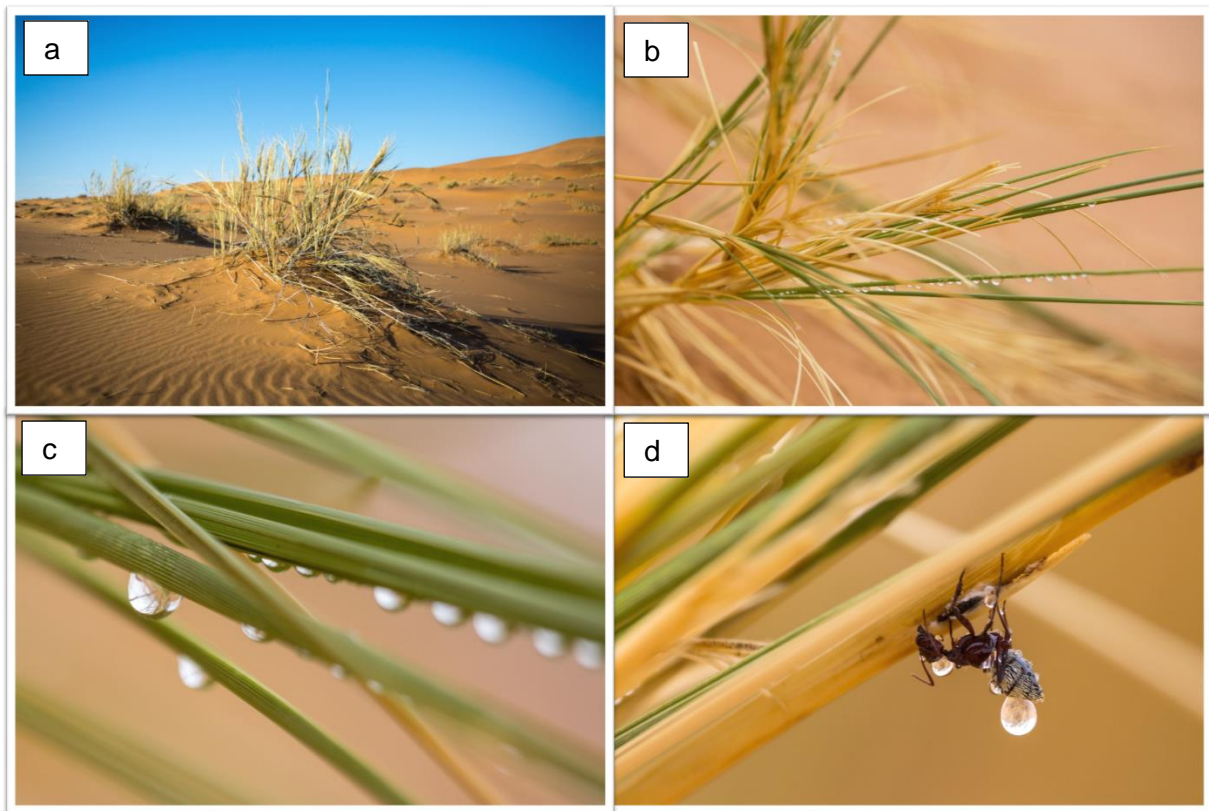


Figure 10: *Stipagrostis sabulicola* is known to form a) hummocks and b) droplets on young stems during a fog event; c) these fog droplets move along the stems and are d) consumed by dune ants

2.2.2 *Trianthema hereroensis*

Trianthema hereroensis Schinz. (Aizoaceae, succulent CAM dune shrub) is another species endemic to the Namib Desert (Fig. 11) and only grows within the fog belt (Seely *et al.*, 1977). These plants have an extensive root system that absorbs water that drips down from the aerial parts of the plant onto the soil. Further microscopic investigation of the leaves illustrated that the anatomy of this plant facilitates water absorption and storage. Seely *et al.* (1977) examined the foliar absorption and translocation of fog water by isolating 10 plants with a polyethylene sheet that was placed on the soil to prevent tritiated water from reaching the roots. Each individual plant was sprayed with 25 cm³ tritiated water before sunrise. These plants were subsequently excavated at different intervals after spraying. Tissue samples were removed from each plant at 6 mm intervals from root to tip and placed in sealed scintillation vials before freezing and laboratory analysis.

The results indicated that the plants can efficiently exploit fog water inputs, as labelled water was absorbed by the leaves just 2 h after being sprayed and significant amounts were found translocated to the roots. In addition, the position of the xylem outside of the phloem was suggested to aid in rapid translocation of tritiated water. This is a highly invasive method that would not be possible with *A. horridus* due to the size of *A. horridus* plants and the depth of their root systems. As only one *T. hereroensis* plant was investigated with a corresponding time after the tritiated water was sprayed, it could, therefore, have been subject to experimental error.



Figure 11: *Trianthema hereroensis* is an endemic succulent to the Namib Desert and exhibits foliar absorption of fog water (credit: Oliver Halsey)

2.3 Fog harvesters in other environments

2.3.1 *Sequoia sempervirens*

Redwood trees (*Sequoia sempervirens* (D.Don) Endl.) in Northern California are exposed to nocturnal fog events during summer months and exhibit foliar fog uptake through an increase in internal water content and water potential. Limm *et al.* (2009) compared direct water absorption in addition to leaf water potential, hydrogen isotope composition ($\delta^2\text{H}$) and nocturnal stomatal conductance when crowns were exposed on foggy and non-foggy days. These authors found a significant increase in leaf mass, water content and water potential during a fog event. This was further confirmed by the isotope analysis of the photosynthetic active tissue (stems and leaves), as fog water is more isotopically enriched (i.e. has more of the heavier isotopes ^2H and ^{18}O) than

rainwater does. A review article by Malik *et al.* (2014) confirmed that isotope analysis is a valuable tool when investigating a plant's ability to harvest non-rainfall moisture. (Isotope analysis of *A. horridus* by Soderberg *et al.* (2014) is discussed in Section 2.4.)

2.3.2 *Opuntia microdasys*

Ju *et al.* (2012) investigated the structural characteristics of *Opuntia microdasys* (Lehm.) Pfeiff. (Cactaceae, CAM) in the Chihuahua Desert that help facilitate fog harvesting. The Cactaceae family has adaptive characteristics to withstand desiccation and some species are known to have spines that are beneficial in fog interception. Scanning electron microscope details (Fig. 12) showed that a spine consists of three structurally different parts, namely the spine tip that consists of conical barbs, the middle section with multi-layered grooves and the base with clustered trichomes. The integration of these structures may contribute to the plant's fog-harvesting capabilities (Fig. 12). Ju *et al.* (2012) investigated the impact of a spine's growth direction on the behaviour of coalesced water in a laboratory setting using time-lapse macrophotography and saturated fog flow. The results showed that gravitational force did not influence the directional water harvesting abilities. The authors also found that fog water is slowly directed to the base by the conical tip and gradient grooves and is subsequently rapidly absorbed when the coalesced drops encounter the trichomes at the base. Lastly, the gradient of surface-free energy is due to the roughness of the spine's surface caused by the micro-grooves. The gradient of Laplace pressure is due to the conical shape of the spine tip, which is further enhanced by the roughness of the spine. Malik *et al.* (2014) mentioned that it would have been useful if Ju *et al.* (2012) had reported on the presence of wax areas on the spine for inter-species comparisons.

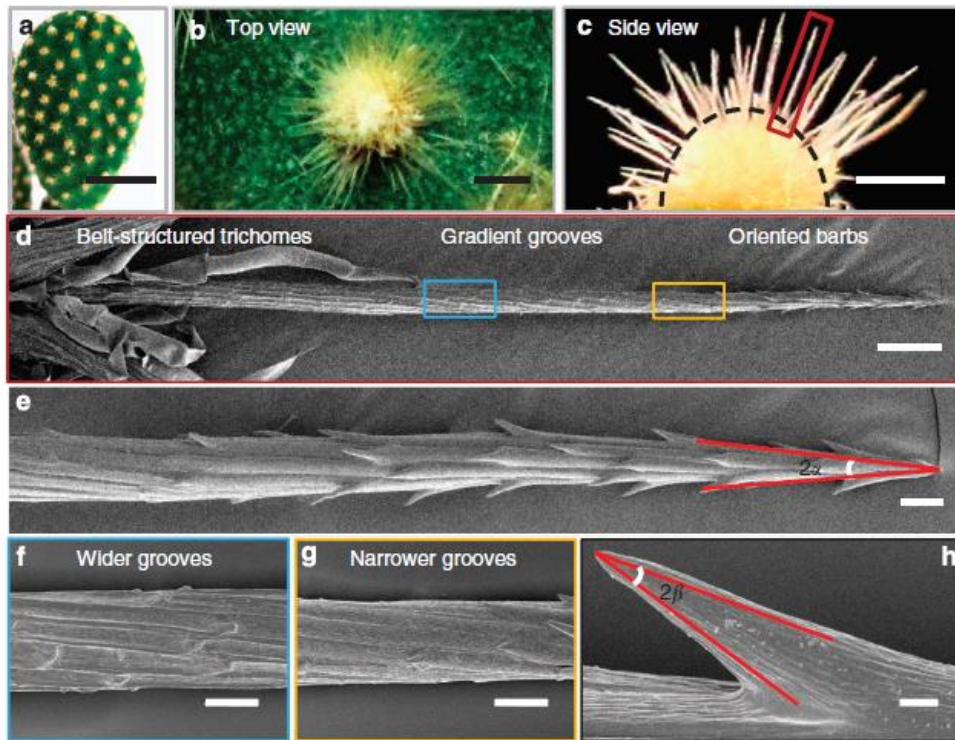


Figure 12: Surface structures and appearance of *Opuntia microdasys*. a) stem covered in clusters of trichomes, b) magnified top view of trichomes, c) side view of trichomes, d) SEM image of a spine divided into three distinct regions, the tip e) with oriented barbs, the centre with f,g) wide and narrow gradient grooves and h) an oriented barb with (Ju *et al.*, 2012).

2.4 *Acanthosicyos horridus* and fog

There has been some debate on the subject of *A. horridus* plants and fog, as some of the flora of the Namib Desert can exploit moisture inputs from fog events. This plant does occur in, but is not restricted to, the fog belt (Berry, 1991). Furthermore, Hebeler (2000) indicated the plant's reliance on groundwater and an opportunistic increase in biomass production after the Kuseb River flooded. Non-rainfall water inputs are known to be a more reliable source of moisture than rain (Shanyengana *et al.*, 2002). This raises the question of whether the structural characteristics of *A. horridus* would enable the plant to effectively exploit fog and how *A. horridus* plants may use fog.

Müller (2000) compared the distribution of *A. horridus* plants in the dunes near Gobabeb with groundwater distribution, using information from the Namibian Department of Water Affairs, by plotting all *A. horridus* plants in relation to groundwater with GIS mapping software. The study concluded that *A. horridus* requires shallow groundwater to become established and is therefore not fog-dependent. In contrast, Berry (1991) stated that *A. horridus* plants occur mostly within the coastal fog belt with only a few individuals scattered inland. However, the distribution map compiled from herbaria specimens indicated that *A. horridus* plants are distributed outside of the fog belt. Klopatek and Stock (1994) contended that *A. horridus* distribution is not limited to areas with fog and that flowering and fruit formation is dependent on rainfall.

Soderberg *et al.* (2014) used stable isotopic analysis to determine whether *Welwitschia mirabilis*, *A. horridus* and tree species around the Kuiseb River at Gobabeb utilise fog as a source of water. Soderberg *et al.* (2014) used stable hydrogen and oxygen isotopes to describe fog occurrence, uptake and volume, measured as ratios between heavy and light isotopes ($^{18}\text{O}/^{16}\text{O}$, $^2\text{H}/^1\text{H}$). Water movement through evaporation and condensation in the environment leads to a change in isotopic compositions because of fractionations. The analysis showed that the isotopic stem water corresponds with groundwater in *W. mirabilis*, *A. horridus* and *Acacia erioloba*.

A study by Hebelers (2000) on the structure of *A. horridus* shoot features revealed that even though the wax surface is hydrophobic, the rough structure might make the surface easily wettable. In combination with the trichomes, this might retain or absorb fog precipitation of the stem surface (Fig. 13).



Figure 13: Droplet formation on *A. horridus* stem in the early morning during a fog event (credit: Monja Gerber)

Kartusch and Kartusch (2008) suggested that the large amounts of stem-borne adventitious roots found in *A. horridus* hummocks may be an indication that water is taken up by precipitated fog. However, this has not been further investigated and there remains much to learn about the root structure within *A. horridus* hummocks.

Malik *et al.* (2014) reviewed the converged mechanisms that different fauna and flora use to harvest non-rainfall moisture such as dew and fog. These structures could have several other functions and some are present on *A. horridus* (Henschel *et al.*, 2004; Berry, 2001; Kartusch & Kartusch, 2008) and could, therefore, play an important role in exploiting non-rainfall moisture:

- a) Groove-like structures as on *A. horridus* shoots are thought to enable directional water flow and are usually longitudinal parallel grooves. Directional water flow reduces water loss through evaporation and enhances water capture by directing water away from the harvesting site, thus allowing more water collection.
- b) Cone-like structures, such as *A. horridus* spikes, are one of the driving forces behind directional water flow. It also creates a Laplace pressure gradient. *Opuntia microdasys* and *S. sabulicola* have cone-like structures.
- c) Directional water flow involves coalesced drops moving in a fixed direction due to the roughness gradient. Water moves from a lower surface roughness gradient to an area with a higher roughness gradient. Water therefore moves from a more hydrophobic surface with lower surface energy, less wettability and rougher area, to a more hydrophilic area with higher surface energy. This was the main contributing factor in directional water flow on *S. sabulicola* stems. *Acanthosicyos horridus* plant has a hydrophobic wax layer, but the roughness of this layer is suggested to make the surface more wettable (Hebeler, 2000).

Some studies have suggested that *A. horridus* plant does not directly utilise fog as a source of water, but the plant may be influenced by other environmental factors associated with fog events, which may indirectly improve the plant's water status. For example, a study by Berry (1991) suggested that when a seedling appears, windblown sand accumulates around it. Fog then condenses on the plant's stem and the droplets that fall on the sand allow the sand to be compacted. As the plant grows, more sand accumulates and is compacted to form a hummock, which then provides ideal conditions for organisms like gerbils to make burrows (Fig. 14).



Figure 14: Burrow on an *A. horridus* hummock

CHAPTER 3: STUDY SITE

3.1 Geography

The study area is situated in the Namib dune ecosystem at the GTRC, located 60 km inland from the coast in the Namib Desert (Fig. 15). The Namib Desert is situated along the coast of southwestern Africa, stretching south from the region of Namibe in Angola to the Orange River in South Africa, and extends 130 km inland between the Atlantic Ocean and the Great Western Escarpment. This long and narrow desert is approximately 2000 km in length (Louw, 1972) and covers about 270000 km² (Koch, 1962; Robinson & Seely, 1980). At GTRC, the Namib Desert is divided by the Kuiseb River into the northern gravel plains and the southern dune ecosystem, known as the Namib Sand Sea (Fig. 15) (Goudie, 1972; Louw, 1972; Louw & Seely, 1980; Robinson & Seely, 1980).

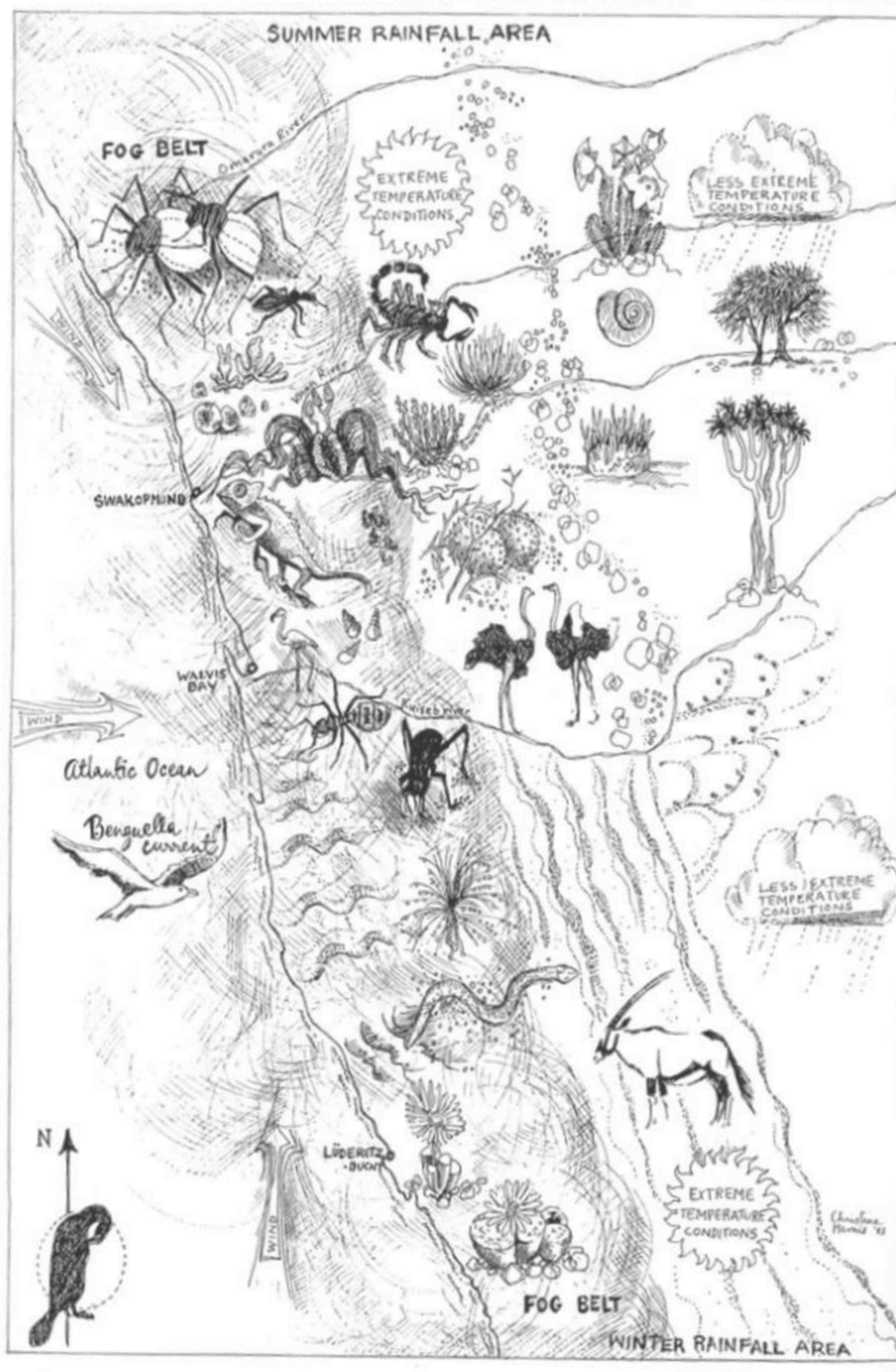


Figure 15: The Namib Desert is divided into several climatic zones, which are preferred by different organisms (Seely, 2004)

3.2 Abiotic environment

Desert ecosystems world-wide are located within two arid belts (Evans & Thames, 1981) and arid areas occupy more than 30% of the earth's land surface (Fig. 16). From a hydrological perspective, these systems are open high-pressure systems with no clearly defined boundaries resulting from global air circulation patterns (Evans & Thames, 1981).

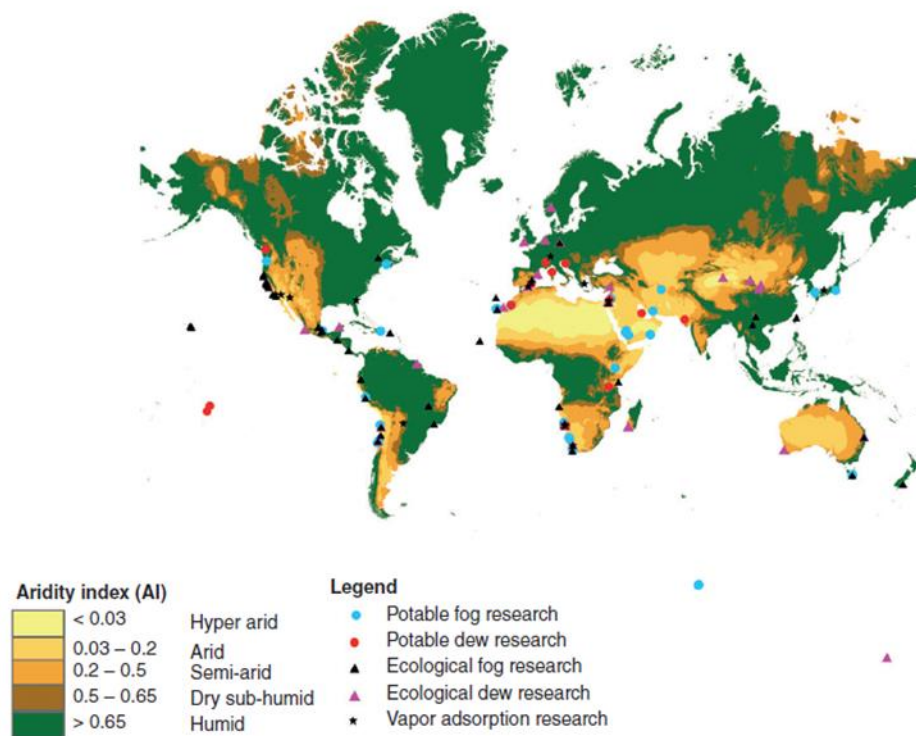


Figure 16: Global distribution map of non-polar arid lands based on Meigs' (1953) classification of warm arid surface regions based on the aridity index, defined as the ratio between precipitation and potential evapotranspiration (Wang *et al.*, 2016)

An arid region can be defined as a region where the potential evapotranspiration (ET) exceeds the annual precipitation (P) (Breckle *et al.*, 2001). Arid climatic conditions are accompanied by high radiation and high evaporation due to water being a limiting factor (Fig. 16). The water delivery in each desert system depends on global air circulation patterns and may be influenced on a local and regional scale (Evans & Thames, 1981). Furthermore, water distribution is dependent on the nature of pulse rain events, geology, soil type and the transformation of

precipitation into runoff due to the topography. The geomorphological processes of erosion, weathering and accumulation of substrate results in typical landscape patterns (Breckle *et al.*, 2001).

Most desert organisms share comparable adaptive strategies to survive in this water-stressed environment (Evans & Thames, 1981). The combination of high radiation loads, high temperatures and extreme climatic variations in non-polar deserts results in morphological, symbiotic and physiological strategies of desert biota to moderate, evade or avoid the effects of environmental stressors (Evans & Thames, 1981).

3.2.1 Wind

Wind is an ecologically significant factor in the Namib Desert (Fig. 17). The wind regime determines the texture and structure of the substrate, humidity, temperature and distribution of detritus (Holm & Scholtz, 1980). The annual wind regime consists of predictable intraseasonal wind patterns (Holm & Scholtz, 1980). Schulze *et al.*, (1976) found that during the winter months, from May to August, there is a dominant easterly ('Berg' wind) with velocities of around 21 km/h. The strong and desiccating 'berg' wind (Fig. 17) is associated with a low relative humidity (0–5%) and high temperature (Holm & Scholtz, 1980). During winter, easterly wind storms usually start before dawn (Holm & Scholtz, 1980) and maximum wind speeds are reached at midday, subsiding at around sunset (Schulze *et al.*, 1976).



Figure 17: Rare winter rainfall at Gobabeb Research and Training Centre (July 2016) accompanied by a strong easterly wind (credit: Oliver Halsey)

In the summer months, from December to February, the wind is relatively stable, with low velocities and a south-westerly direction (Schulze *et al.*, 1976; Tyson & Seely, 1980; Henschel & Seely, 2000). The westerly wind is a moist and cool sea breeze that results in more frequent fog events. However, the occasional northerly winds may be dry and hot (Holm & Scholtz, 1980). Furthermore, the study from Schulze *et al.*, (1976) shows that maximum wind speeds during summer occur around 18:00 and minimum wind speeds at 06:00.

3.2.2 Temperature

Temperatures in the central Namib are highly dependent on the dominant wind direction (Fig. 18d). Temperatures at Gobabeb (1962–1972) measured by a Stevenson screen had an absolute minimum of 2.1°C and a maximum of 42.3°C, with daily maximums around 32°C in the summer and 27°C during winter, and an annual a periodic range of 17.3°C (Schulze, 1976; Holm & Scholtz, 1980; Theron *et al.*, 1980; Henschel & Seely, 2000). The macro-climate temperature conditions are not as extreme as those in the Kalahari (Fig. 18d), but temperatures are known to change

rapidly with the wind direction. In addition, there is a negative correlation between temperature and relative humidity (Figs. 18b & d) (Holm & Scholtz, 1980).

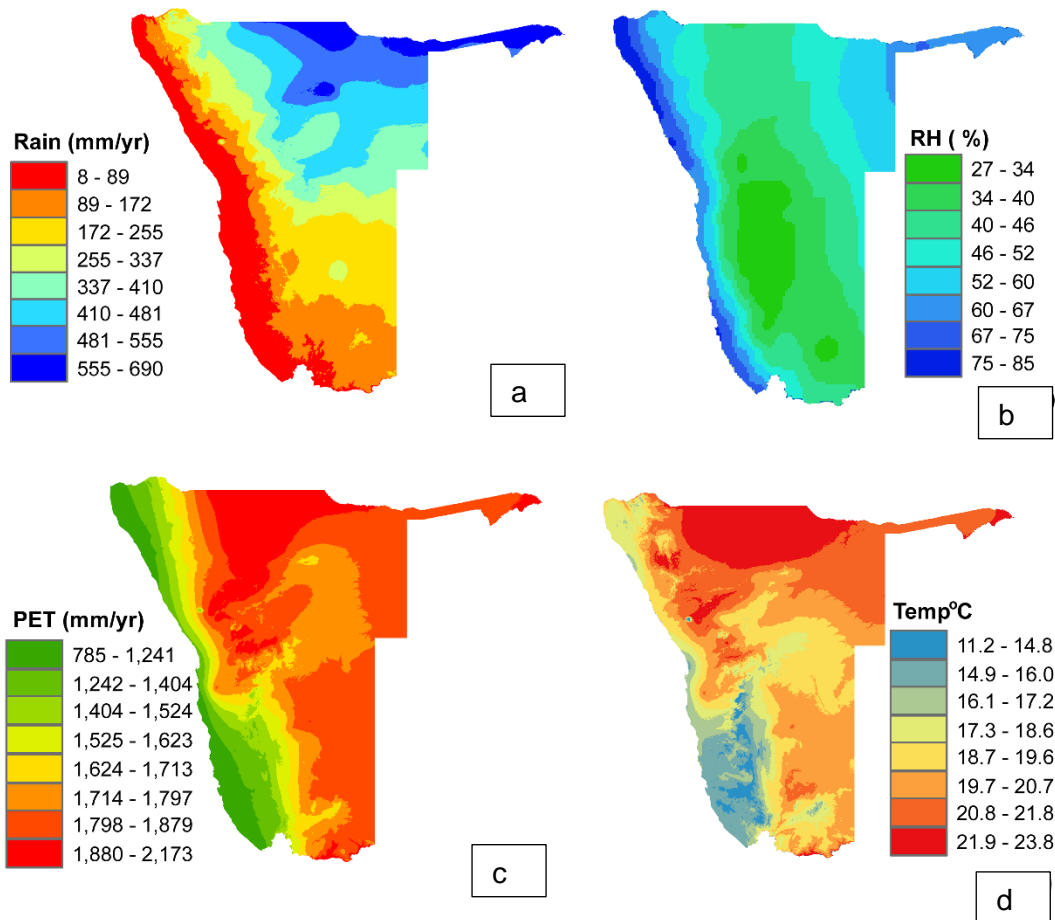


Figure 18: Annual meteorological data across Namibia: a) mean rainfall, b) mean relative humidity (RH%), c) mean potential evapotranspiration (PET) and d) mean temperature (°C) (Kaseke *et al.*, 2016)

3.2.3 Rain

Rainfall in the Namib Desert is temporally and spatially highly variable (Fig. 18a) and decreases along an east-to-west gradient (Louw, 1972; Warren-Rhodes *et al.*, 2013). Convective rainfall is limited by the dry descending air of the Global Hadley Circulation (Eckardt *et al.*, 2013). Rainfall in the western (coastal) region is low and the area receives 0–18 mm of rain annually (Fig. 18a) (Louw, 1972; Shanyengana *et al.*, 2002; Henschel & Seely, 2008). This results in sparse vegetation with a limited number of specialised species (Louw, 1972). The eastern (interior) region receives annual rainfall between 23 mm (Goudie, 1972; Eckardt *et al.*, 2013) and 56 mm (Louw,

1972). Rainfall varies intra- and interannually and occurs as localised and erratic convective summer rain (Eckardt *et al.*, 2013; Warren-Rhodes *et al.*, 2013). Despite the low annual rainfall and high variability, rain remains an important factor for different organisms (Louw, 1972).

3.2.4 Non-rainfall water inputs

Non-rainfall water inputs in the Namib Desert consists of three different vectors, namely dew, water vapour and fog (Kaseke *et al.*, 2016), and depends on variations in the sea surface temperatures beyond the cold Benguela upwelling (Lancaster *et al.*, 1984; Henschel & Seely, 2000; Eckardt *et al.*, 2013). These quasi-permanent cold upwelling cells of the Benguela system, in association with the inversion layers, restrict convective rain and result in sustainable coastal fog, dew associated with relatively high humidity (Fig. 18b) and a cool south-westerly wind (sea breeze) (Louw, 1972; Eckardt *et al.*, 2013).

NRWI vectors have different formation mechanisms and are influenced by surface conditions and the abiotic environment (Kaseke *et al.*, 2016). The dew point temperature is described as the temperature to which water vapour and air must be cooled to result in saturation and dew precipitation (Tyson & Preston-Whyte, 2012). Dew formation occurs when water vapour in the air condenses on a surface that has a temperature equal to or lower than the dew point temperature (Kaseke *et al.*, 2016). Water vapour absorption into the soil occurs when the temperature of the surface is greater than that of the dew point and the relative humidity (%RH) in the atmosphere is greater than that in the soil pores (Kaseke *et al.*, 2016).

Fog contains traces of dissolved solids and dust particles (Eckardt *et al.*, 2011). These particles form the nuclei needed for initial condensation that results in water droplet formation. This process was first described by Aitken in the nineteenth century and occurs when an unsaturated air parcel is cooled and attains additional moisture, resulting in air saturation. The water vapour then condenses onto aerosol nuclei and increases in size to form cloud droplets (Tyson & Preston-Whyte, 2012).

Fog events in the Namib Desert (Fig.19) are most frequent along the coast (Fig. 18b) and decrease towards the interior (Louw, 1972; Warren-Rhodes *et al.*, 2013). Coastal stratus clouds (fog) penetrate approximately 40 km inland (Theron *et al.*, 1980) and the effects are felt up to 100–120 km inland (Louw, 1972 Lancaster *et al.*, 1984; Warren-Rhodes *et al.*, 2013). This precipitation mostly occurs in summer, from January to March (Schulze *et al.*, 1976).



Figure 19: Fog events in the central Namib Desert (credit: Oliver Halsey)

According to Eckardt *et al.* (2013) and Henschel and Seely (2000), there are four types of fog: advective coastal fog (low stratus), high stratus clouds, radiation fog and fog drizzle. Advective fog enters the Namib Desert via the south-westerly wind at elevations below 200 m. High stratus clouds are capped by the Namib inversion layers and are more common in the central Namib Desert (Eckardt *et al.*, 2013). These stratus clouds enter from the north-west to fuse with the lower easterly airflow, which originates from the desert interior, with the consequence of a south to south-southwest vector, resulting in fog events at GTRC, 56 km east of the coast (Louw, 1972; Henschel & Seely, 2000; Eckardt *et al.*, 2013). Radiation fog develops when moist, clear coastal air combines with the easterly mountain-plain wind to form clouds restricted to low ground-level topography (Henschel & Seely, 2000), such as interdunes and dry river valleys (Eckardt *et al.*, 2013). Radiation fog is associated with the lowest atmospheric layers, where a mass of humid, warm air is cooled over the ground surface through irradiation and results in heat emission. Lastly, fog drizzle is a rare occurrence associated with cold winter fronts (Henschel & Seely, 2000; Eckardt *et al.*, 2013).

Shanyengana *et al.* (2002) considered NRWIs as reliable water sources for fauna and flora. This has resulted in organisms that are adapted for fog harvesting and usage, including beetles, grass, succulent shrubs and lichens (Louw, 1972; Lancaster *et al.*, 1984; Warren Rhodes *et al.*, 2013). NRWI also plays an important role in decomposition, nutrient cycling and possibly groundwater recharge within the ecosystem (Kaseke *et al.*, 2016)

3.3 Biotic environment

There are other organisms in Namib ecosystems that are known to utilise NRWI as a moisture source. These include the biological soil crust, which forms an important component in dryland ecosystems and influences rain interception and infiltration, water storage capacity, soil evaporation and soil stability that prevents soil erosion (Lange *et al.*, 1994; Wang *et al.*, 2016). This crust consists of complex communities of microphytes, including lichens, mosses, fungi and green and cyanobacteria (Lange *et al.*, 1994).

The distinct lichen communities (Fig. 20) of the Namib Desert (Seely & Pallet, 2008) are formed by a beneficial association between algae and fungi. The algal component is responsible for photosynthesis, while the fungi form the body (thallus) and attach to the substrate (Lange *et al.*, 1994; Seely & Pallet, 2008). Lichens require sufficient light for photosynthesis, high humidity and NRWIs as water sources (Seely & Pallet, 2008; Wirth, 2010). Lichens are opportunistic and can increase their water content by 150% during a fog event (Wirth, 2010) This hydrated lichen can photosynthesise after sunrise until dehydration occurs and the lichen enters a state of 'latent life' (Lange *et al.*, 1994; Seely & Pallet, 2008). Known lichen species include *Caloplaca elegantissima* (Fig. 20), *Xanthoparmelia walteri*, *Teloschistes capensis*, *Xanthorea* sp. and *Xanthomaculina convolute* (Seely & Pallet, 2008).



Figure 20: *Caloplaca elegantissima* (Nyl.) Zahlbr., also known as the Namib sun (credit: Oliver Halsey)

Furthermore, there are a host of reptiles and invertebrates that utilise NRWIs due to the erratic nature of rainfall and limited amount of surface water in the Namib Desert (Henschel & Seely, 2008; Nørgaard *et al.*, 2012; Wang *et al.*, 2016). Some organisms drink fog and dew precipitation on surfaces as has been observed in *Meroles anchietae* (shovel-snouted lizard), commonly found around *A. horridus* hummocks, and *Parabuthus villosus* (black hairy thick-tailed scorpion, Fig. 21a) (Polis & Seely, 1990). Some organisms have special behaviours to harvest fog and dew. The Namib beetle, *Lepidichora* spp. (Fig. 21b), is known to construct trenches on dune crest surfaces perpendicular to the wind during a fog event, thus concentrating moisture (Seely & Hamilton, 1976). *Bitis peringueyi* (side-winding adder) has been observed to flatten its body against the cool soil surface, thereby increasing the exposed surface area and allowing increased fog deposition, which it then licks up (Fig. 22a).

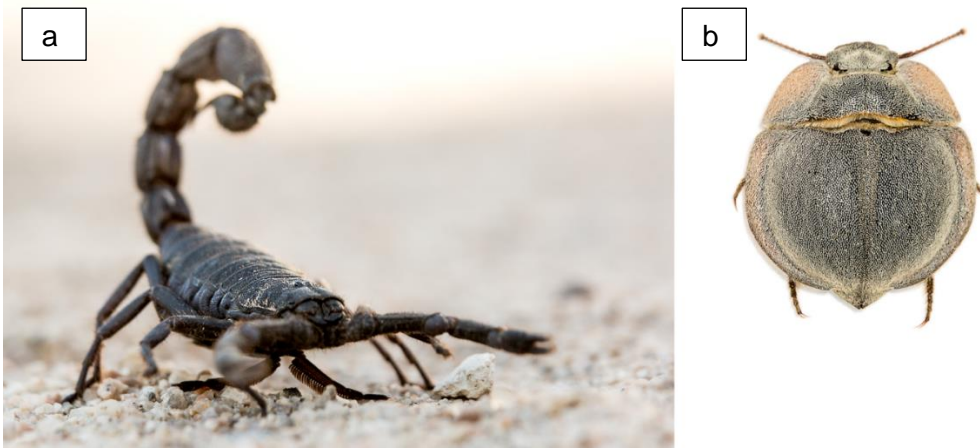


Figure 21: Organisms that exploit fog precipitation: a) *Parabuthus villosus* is known to drink fog precipitation on surfaces and b) *Lepidichora* spp. (trench beetle) is known to construct a trench in the sand surface to intercept fog water (credit: Oliver Halsey)

One of the best-known behaviours to directly harvest fog has been observed in *Onymacris unguicularis* (Fig. 22c), also known as the fog-basking beetle, and a close relative, *O. bicolor* (Fig. 22b). These beetles have been observed (Seely, 1979) to position themselves in a head-down stance with their abdomens elevated on a dune crest during a fog event (Fig. 22c). This allows fog deposition on their hydrophobic elytra to run down towards the mouth (Seely, 1979).

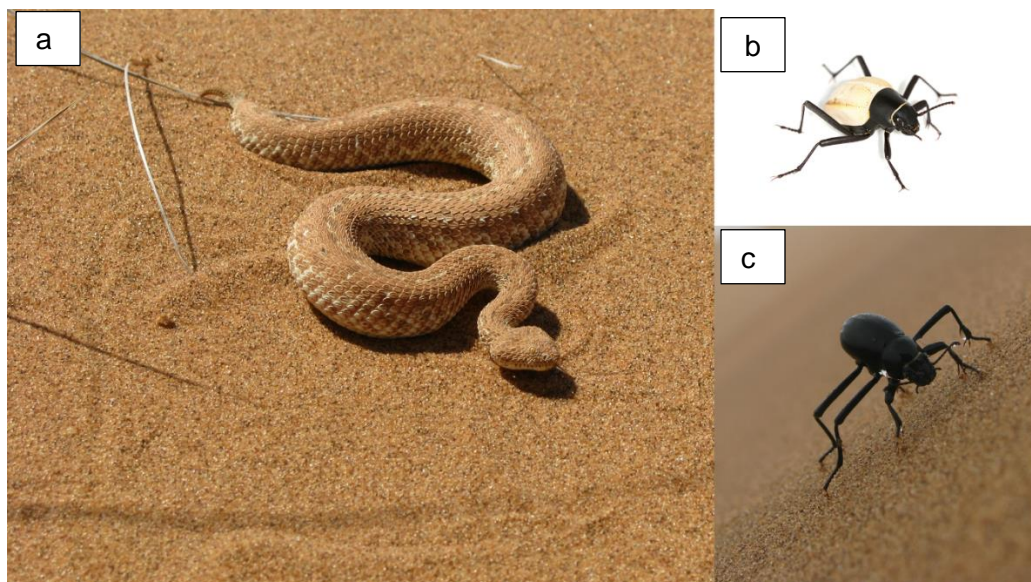


Figure 22: Fauna that can intercept fog water directly on living surfaces: a) *Bitis peringueyi* (side-winding adder), b) *Onymacris bicolor* and c) *O. unguicularis* (Credit: Oliver Halsey)

CHAPTER 4: STRATEGIES OF THE *A. HORRIDUS* TO EXPLOIT NON-RAINFALL MOISTURE

4.1 Introduction

Increasing evidence suggests that NRWIs such as fog and other components play an essential role in soil-vegetation interaction in deserts. These components can potentially provide a significant input in maintaining vegetation dynamics and biogeochemical processes (Wang *et al.*, 2016). These NRWI components include water vapour, dew and fog (Wang *et al.*, 2016) and are known to exceed annual rainfall in the central Namib Desert (Shanengana *et al.*, 2002). NRWIs are known to indirectly contribute to the water status of plants by modifying the plant's energy balance, increasing stomatal conductance, increasing CO₂ uptake and decreasing transpiration (Wang *et al.*, 2016). Fog may also directly improve the water status of the plant through foliar (aerial) absorption or water uptake through the roots from the surrounding wet soil. (Wang *et al.*, 2016). Fog may therefore improve the plant's water status, resulting in increased photosynthesis and plant biomass (Eller *et al.*, 2013).

Plants in this environment frequently dominated by fog have developed different adaptive strategies to directly utilise this NRWI (Wang *et al.*, 2016). Fog precipitation sometimes only occur when it is intercepted by an object. Plants located in a moist microenvironment have certain traits to increase its fog-harvesting efficiency on aerial surfaces (Ebner *et al.*, 2011; Henschel & Seely, 2008; Limm *et al.*, 2009). The potential flux of fog interception, according to Yates and Huntley (1995) as first described by Herwitz (1986), is influenced by different biotic and abiotic variables.

Biotic influences include the anatomical characteristics of aerial plant parts and the wettability of these plant structures (Yates & Huntley, 1995). Anatomical characteristics include the height and size of the canopy and the size and arrangement of the leaves and stems (Ebner *et al.*, 2011). Fog interception efficiency is known to increase in relation to certain biotic mechanisms, including

a decrease in stem diameter and an increase in vegetation density (Ebner *et al.*, 2011). Abiotic variables include the total amount of radiation, wind speed and the density and duration of fog events (Yates & Huntley, 1995; Ebner *et al.*, 2011). Fog interception increases in response to an increase in wind velocity, an increase in fog density and fog droplet size and, lastly, a decrease in the thickness of the boundary layer between the aerial surface and the atmosphere (Ebner *et al.*, 2011).

Intercepted fog results in droplet formation on the aerial surfaces of plants due to the high surface tension of water and hydrophobic plant structures (Yates & Huntley, 1995; Henschel & Seely, 2008). Coalesced droplets may drip directly towards the surrounding surface or root zone as throughfall (Yates & Huntley, 1995) or, at a critical size, the droplets flow along the stems and leaves as stem-flow (Yates & Huntley, 1995), otherwise known as occult precipitation (Ebner *et al.*, 2011). Throughfall drips down to the surrounding environment where it may be taken up by surface root hairs, as seen in *S. sabulicola* (Ebner *et al.*, 2011). These roots have a layer of soil particles around them that are cemented together by mucopolysaccharides to form a rhizosheath (Price, 1911). This rhizosheath plays a role in limiting water loss from the roots to the surrounding soil by producing air gaps in the drying soil (Ebner *et al.*, 2011). The rhizosheath is also capable of storing small amounts of water and may improve water supply to plants by 25% (Ebner *et al.*, 2011).

The aerial parts of the plant may directly absorb NRWIs (Vogel & Müller-Doblies, 2011), e.g. *T. hereroensis* absorbs fog water through the leaves, which is then transported to the roots to alleviate drought stress (Seely *et al.*, 1977). In addition, this succulent has an extensive root system, which enables it to absorb any fog that drips to the surrounding soil (Seely *et al.*, 1977). Foliar absorption is driven by different variables of the soil-plant-atmosphere-continuum (SPAC), based on the principle that water moves from high water potential (ψ) to a lower water potential (Goldsmith, 2013). This was first described by Philip (1966) and is used as the framework for water movement through plants.

Thus, foliar absorption depends on sustainable atmospheric conditions to establish a driving gradient, where the water potential of the leaves and stems needs to be lower than that of the surrounding boundary layer (Goldsmith, 2013). Water may be absorbed into the stem or leaves through the hydrophilic cuticle, together with interior hydrophilic leaf tissue (Goldsmith, 2013). It is also possible for stems or leaves to absorb water through other pathways including trichomes, hydathodes (Yates & Huntley, 1995; Limm *et al.*, 2009) and the stomata (Goldsmith, 2013). Aerial absorption results in an increase in the water potential inside the leaves and stems and it may be possible that water is transported from the shoots to the roots, while water is simultaneously transported in the opposite direction, from the roots towards the stems (Goldsmith, 2013). Hydraulic redistribution is the driving gradient that allows water movement from the plant into the soil and is the movement of water from wetter (higher ψ) to drier (lower ψ) soil layers (Goldsmith, 2013). This may be beneficial for plant and biotic communities in the surrounding area and may also impact the biochemical cycles in the ecosystem (Goldsmith, 2013).

Fog may further contribute to litter decomposition, increased microbial activity and animal water usage and some evidence suggests that it may contribute to groundwater recharge (Wang *et al.*, 2016). Many *A. horridus* individuals occur within the fog belt (Berry, 1991) and, as observed, fog water droplets coalesce on the stems of *A. horridus* and stay suspended on the stem or drip towards the surrounding soil. It is therefore hypothesised that the dense thickets of *A. horridus* plants may allow efficient fog harvesting. Thus, the question is 'How does the *A. horridus* plant interact and respond to fog and what strategies may the plant employ to utilise fog as a supplementary source of moisture?'

4.2 Material and methods

4.2.1 Study site

Field experiments were conducted in the Gobabeb Valley, 3 km south of the GRTC in the central Namib Desert, Namibia (Fig. 23).



Figure 23: Distribution map of three male *A. horridus* hummocks investigated in this study (credit: Campbell Nell)

Three male *A. horridus* hummocks of different sizes were selected to monitor the plants' physiological response to fog events (Table 1). The height and length of each hummock were determined using drone photography and ImageJ software. A Canon PowerShot D30 camera was attached to the bottom of a commercial 3DR drone (IRIS+) and programmed to take aerial photographs in 10 s intervals of the *A. horridus* hummocks and a pole (known distance) in the

picture for scale. ImageJ software was used to measure the height of each hummock and the length from north to south and east to west (Table 1).

Table 1: Location and size of three *A. horridus* hummocks used in this study

Hummock	Location	Location	Height (cm)	Length (cm) (E-W)	Length (cm) (N-S)
A	S 23.59265 E 015.05116	Dune base	222.31	1595	807
B	S 23.59038 E 015.05200	Between dune base and dune slope	322.86	3966	2675.82
C	S 23.59016 E 025.05147	Interdune area	387.55	3226.45	2932.01

Each hummock was divided into eight triangular transects based on the cardinal and ordinate direction (Fig. 24) with the 'wagon wheel transect' method. One mature stem per triangular transect was selected and marked with an aluminium tag to represent the corresponding cardinal and ordinate direction. This method allowed the research to focus on one direction and all abiotic influences in the corresponding direction. The eight transects were subjected to different abiotic factors during the day, due to the variable nature of wind, radiation and fog (Fig. 24).

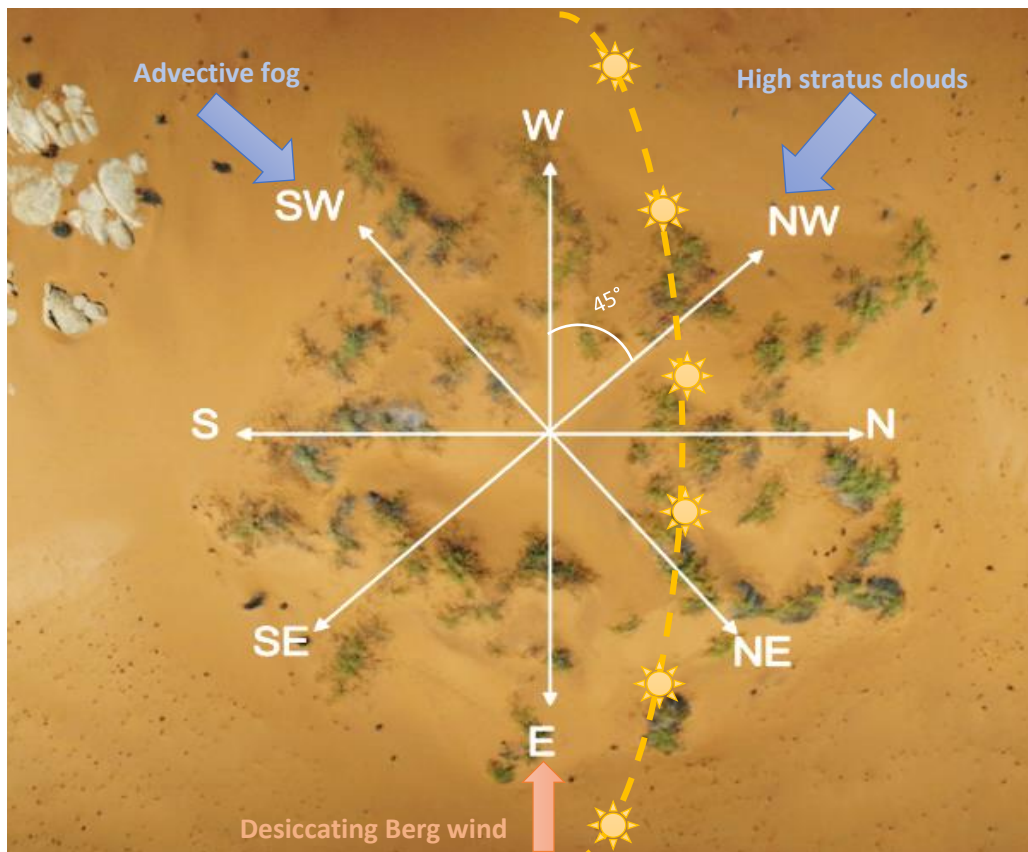


Figure 24: Aerial photograph of an *A. horridus* hummock with a schematic of the wagon-wheel transect method to divide the hummock into eight triangular transects based on the cardinal and ordinate directions

According to Shulze (1976), the dominant wind direction during summer is south-west to north-east. In the winter, the wind is dominated by a desiccating easterly 'berg' wind (Fig. 24). The northern transect of the hummock is also exposed to increased light intensity when compared to the southern transect and this is due to the orientation of the sun during the day.

High stratus clouds are blown inland from a north-westerly direction (Fig. 24) and penetrates 56 km inland at Gobabeb. Furthermore, advective fog originates from a south-westerly (coastal) direction and moves across linear dunes towards Gobabeb. There are still limitations in our understanding of fog behaviour in the dune ecosystem. The dune orientation, perpendicular to the dominant wind direction associated with advective fog, could possibly have a significant interaction with fog movement and flow.

The *A. horridus* hummocks focused on in this study are situated in the dune ecosystem about 3 km south-west from the Gobabeb FogNet meteorological station. This station, situated on gravel plains, was used for wind-speed and -direction data. It should be noted that the wind regime may vary within the dune ecosystem.

The wind-direction data were used along with the Wingscape camera to determine the dominant wind direction during the duration of a fog event, thus identifying the direction on an *A. horridus* hummock that is directly influenced by fog or other environmental parameters. These meteorological data were compared to photosynthetic potential in Chapter 5.

4.2.2 Fog monitoring

Fog density and incidence was measured using a waterproof Wingscape time-lapse camera and based on the method designed by Marais (2015). The camera was set to Central African Time and programmed to take a photograph at 5 min intervals during the day and night from August 2016 to July 2017.

The Wingscape camera was installed facing a south-westerly direction, away from direct sunlight, to reduce lens flare and minimise shadows, and the tripod was therefore positioned at the north-east margin. The camera was powered by an external 12 V battery, which was mounted in a waterproof battery box. The cables from the battery to the camera were sealed using PVC pipes, which prevented animals from chewing on the wires and sealed it off from moisture. The battery was exchanged with a recharged battery every second week. The photographs were downloaded from the 16 GB SD card weekly.

The image background area included an *A. horridus* hummock about 60 m from the camera and this was used together with checkerboard targets to measure the decrease in visibility during a fog event during the day (Fig. 25a). Spotlights were used to determine the decrease in visibility at night (Fig. 25b). The first target was installed at a 10 m distance from the camera together with a spotlight facing towards the camera (Fig. 25a). This checkerboard was painted with 5 x 5 cm squares. The second checkerboard marker was placed 20 m away from the camera, together

with a spotlight with a slight offset to ensure that it was not blocked by the 10 m target. The checkerboard was painted with 7.5 x 7.5 cm squares. It was experimentally determined by Marais (2015) that 5 cm squares at 10 m have the same pixel size as 7.5 x 7.5 cm squares at 20 m. Spotlights were further installed at 40 and 50 m distances. Lastly, fog density was categorised on a scale from 0 to 4 (Table 2).



Figure 25: a) Photograph from Wingscape time-lapse camera with checkerboard markers set up at 10 m and 20 m distances from the camera to measure visibility during a daytime fog event and b) spotlights set up at various distances to determine the visibility during a night-time fog event

Table 2: Qualitative measure of fog density using a fog scale from 0 to 4

Fog scale	Description
0	Fog density is 0 on non-foggy days
1	Light fog event: slight haze in picture and horizon not visible. Hummock (60 m) and both checkerboard markers still visible.
2	Hummock not visible, but both checkerboard markers still visible
3	Checkerboard marker at 20 m not visible
4	Dense fog – checkerboard marker at 10 m distance not visible

A Lascar, EL-USB-2-LCD, Easy Log data logger was mounted on a stake next to the camera tripod to measure T_a (°C) and RH (%) at the study site. The Lascar data logger was suspended inside a small radiation screen to screen the data logger from direct solar radiation, dust and precipitation. The mini-Stevenson screen was constructed using an empty 1 l milk carton (Tetra Pak®). Small louvers were cut on each side and bent at a 30° angle to allow air circulation within the container. The bottom of the container was covered with a perforated base plate to avoid any direct influence from surface radiation (Marais, 2015). The data logger was programmed to log data at 5 min intervals, from August 2016 to July 2017.

In addition, surface wetting through non-rainfall moisture was measured using a leaf wetness sensor (LWS) (Decagon Devices, 2007). This device measures the change in resistance across a grid of electrodes that mimics a leaf surface. The LWS measured the duration of surface wetness and a CR10X Campbell Scientific data logger was programmed using LoggerNet 4.2 software to record and log the leaf wetness at 30 min intervals. The sensor was mounted on the time-lapse camera tripod facing a south-westerly direction and the leaf wetness was measured from April to July 2017. The LWS was used together with the Wingscape camera data to distinguish between NRWI sources. The LWS measured any form of wetness (fog or dew), but the Wingscape camera was used to visually distinguish between fog and dew.

Wind-speed and -direction data were obtained from the Gobabeb FogNet meteorological station (GBB-Met FogNet) 3 km from the study area (http://www.sasscalweathernet.org/weatherstat_hourly_we.php?loggerid_crit=8893).

4.2.3 Tracing water movement on stems

Water movement on the surface of *A. horridus* shoots was investigated by exposing the stems to fluorescent water in a humid environment. A warm steam vaporiser (Kaz, 2100, Hudson, New York) was used to increase the RH in a dark room, to mimic the environmental conditions that occur during fog events. During these experiments, room temperature and RH were measured as 20–23°C and 45–50%, respectively. The stems were placed at a vertical orientation angle and fluorescent water was dripped on the stems. Fluorescent water, which emits a bright green light

when observed in a dark room under UV light was created by mixing a 1:200 dilution of fluorescein dye and deionised water. The behaviour of fog water on the stems was documented using a Canon EOS 5D Mark III camera with a F4IS and 500 D macro extension lens (shutter speed: 1.3 s; aperture: F/8, ISO-100) mounted on a tripod. The time-lapse photographs were taken at 2 s intervals for 30 min and compiled by Oliver Halsey (photographer and filmmaker at Gobabeb Research and Training Centre). This method was modified from that of Roth-Nebelsick *et al.* (2012).

4.2.4 Fog absorption through the stems

The first method followed the gravimetric procedure of Vogel and Müller-Doblies (2011). A total of nine young stems with growth tips and seven mature stems were collected throughout the year (19 July 2016, 16 August 2016, 13 September 2016, 08 November 2016 and 14 December 2016) and investigated. The cut area was immediately sealed with Vaseline™ and Parafilm 'M'® to avoid water loss (Fig. 26). The samples were collected in the morning and left at room temperature in the open air for 24 h before being weighed. The clippings were then placed in a sealed glass jar on a filter paper and sprayed with fog water collected from the Coastal MET FogNet Station (latitude: -23.05631001, longitude: 14.62594704) The samples were exposed to simulated fog for 24 and 48 h and the excess water was removed using a paper towel, after which they were weighed again (Vogel & Müller-Doblies, 2011). Fog water was collected from the Coast MET FogNet station, because this station is situated near the coast, close to Walvis Bay, and receives more frequent fog events that yield more fog precipitation than at GTRC.



Figure 26: Stem clippings of *Acanthosicyos horridus* collected to test direct water absorption through the shoot. Various clippings were used, including a growth tip (left), mature stem (middle) and fasciated stem (right)

Another method to determine water absorption through the stem has been described by Limm *et al.* (2009). *Acanthosicyos horridus* stem clippings were collected before sunrise to simulate nocturnal conditions for fog exposure. A total of eight young stems with growth tips and eight mature stems was collected on two different dates (9 April 2017 and 11 July 2017) and investigated. The cut surface of the stems was sealed using Vaseline™ and Parafilm 'M'® to avoid water loss through evaporation. The initial mass (Mass 1) was then measured before submerging the stems in deionised water for 180 min in complete darkness. The stems were submerged, with the sealed end protruding above the waterline, to standardise the water availability. After 180 min, the clippings were removed from the deionised water and excess water on the surface was removed by patting the stems dry with paper towels and then reweighing the stems (Mass 2).

To measure whether any residual water was left on the surface after patting the stems dry, the stems were left to air dry for a few minutes to allow surface water to evaporate. The stems were

reweighed (Mass 3) before submerging them in deionised water for 1 s, after which they were towelled dried and reweighed (Mass 4). This allowed potential measurement errors due to towel drying to be considered. The brief wetting would not result in any absorption through the stem and the mass after wetting was, therefore, an indication of the residual water left on the stem surface. The amount of water absorption through the stem was then calculated using the following equation:

$$\text{Water uptake} = (\text{Mass 2} - \text{Mass 1}) - (\text{Mass 4} - \text{Mass 3})$$

4.2.5 Water potential measurements

The diurnal water potential was measured using a Model 1000 pressure chamber instrument (PMS Instrument Company, Oregon, USA) and compressed nitrogen gas (AFROX Namibia) from cylinders. Three growth tips and three mature stem clippings were collected before dawn (06:00), at midday (12:00) and in the afternoon (16:00) for each of the four cardinal directions on Hummocks A and C on two days with fog and one without fog. The cut ends were sealed using Vaseline™ and Parafilm 'M' ® to prevent moisture loss through the cut area. The clippings were taken to the laboratory and ends cut again before placed in a pressure chamber instrument to measure the xylem tension on non-foggy days (11 July 2017) and foggy days (14 and 25 July 2017). The thorns were not removed and stem clippings of approximately 4 cm in length were used. The sample size consisted of 48 growth tips and 48 mature stems for foggy days and 24 growth tips and 24 mature stems for non-foggy days. The *A. horridus* stems exuded 'bleeding sap' from the phloem when subjected to pressure, but this could easily be distinguished from the xylem sap, which has a whiter colour and is exuded from the centre of the stem. Measuring the xylem tension afterwards in a lab resulted in inaccurate values due to the water being pulled back into the stem through microscopic thin columns in the xylem vessels due to the dominating negative pressure inside the xylem vessels. Water loss through the cuticle before measuring the xylem tension will result in increased values. Furthermore, the stem was cut again before measuring the tension and resulted in experimental error and values that are too low. In conclusion this experimental procedure should be repeated in the field and xylem tension should

immediately be measured. The sample size should also be increased but was not possible in the scope of this study due to research equipment not being available. The results are displayed to give a qualitative result rather than quantitative.

4.2.6 Statistical analysis

Statistical analysis of the data was performed using the software programme SigmaPlot Version 12.0. One-way repeated-measures analysis of variance was used to detect any difference between related means. The Holm-Šídák test was used to determine significant differences in stem mass after the absorption tests. This was further used to determine significant differences in the water potential of *A. horridus* shoots on days with a fog event compared to days with no fog. Differences were considered significant at $p < 0.05$.

4.3 Results

4.3.1 Tracing water movement on *A. horridus* shoots

During the observation of *A. horridus* shoots in the morning during fog events, it was noted that coalesced fog water moved along the grooves on the shoots and dripped down to the surrounding soil. The time-lapse investigation revealed that the fluorescent water preferentially flowed down along the grooves on the stem of *A. horridus* (Fig. 27). The droplets coalesced and increased until they reached a critical size, then rolled down towards the shoot base. The smaller fluorescent droplets remained suspended in the grooves and exhibited a fluorescent decay (Fig. 27). This may indicate shoot water absorption, but the maximum RH was only 50% and it was therefore not possible to exclude evaporation as the cause of fluorescent decay.

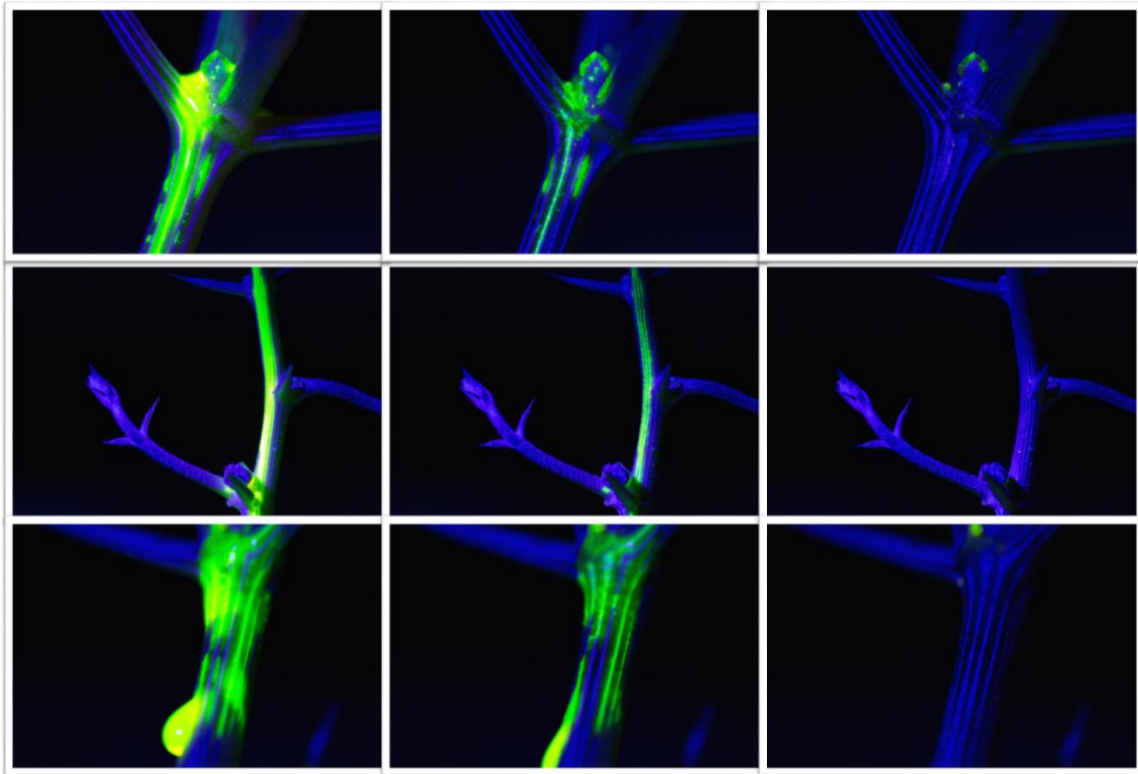


Figure 27: Tracing water movement on *A. horridus* shoots using fluorescein dye and macro time-lapse photography (credit: Oliver Halsey)

4.3.2 Fog water absorption through the stems

4.3.2.1 Absorption test after-Vogel and Müller-Doblies (2011)

Preliminary results from evaluating fog water absorption through the stems of five *A. horridus* growth tips and five mature stems were promising as they indicated absorption after 24 and 48 h. The sample size was increased (Fig. 28). Statistically (according to the Holm-Šidák test), there was no significant difference between initial stem mass and mass after 24 h of simulated fog exposure in either the growth points ($n = 9$) ($p = 0.954$) or the mature stems ($n = 7$) ($p = 0.960$). Furthermore, no significant difference was found after 48 h in the stem mass of the growth points ($p = 0.937$) or mature stems ($p = 0.979$), when compared to initial stem mass (Fig. 28). This was believed to be due to the variation between the initial stem mass of each replicate in a sample.

To exclude this variability within a sample and to normalise the data, the difference in mass was determined after 24 h and 48 h (Fig. 29)

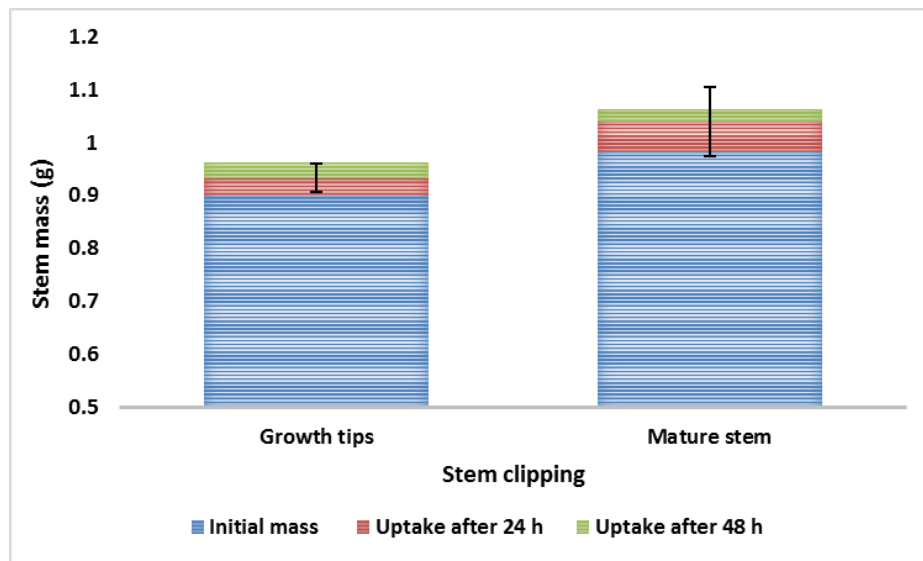


Figure 28: Water absorption by photosynthetic shoots of *Acanthosicyos horridus* after 24 and 48 h indicated no significant increase in mass for either growth points (n = 9) or mature stems (n = 7)

The shoots of *A. horridus* did exhibit some increase in stem mass (Fig. 29) after 24 and 48 h of simulated fog exposure. The difference in stem mass or 'water uptake' was found to be significant for the growth points after 24 h and ($p = 0.042^*$) and 48 h ($p < 0.001^{**}$). In comparison, the results for the mature stem clippings indicated that there was a significant fog 'water uptake' after 24 h ($p < 0.001^*$) and 48 h ($p < 0.001^{**}$). This difference in stem mass might not be the result of water uptake alone because of the possibility that residual water was left on the stem surface after towel drying.

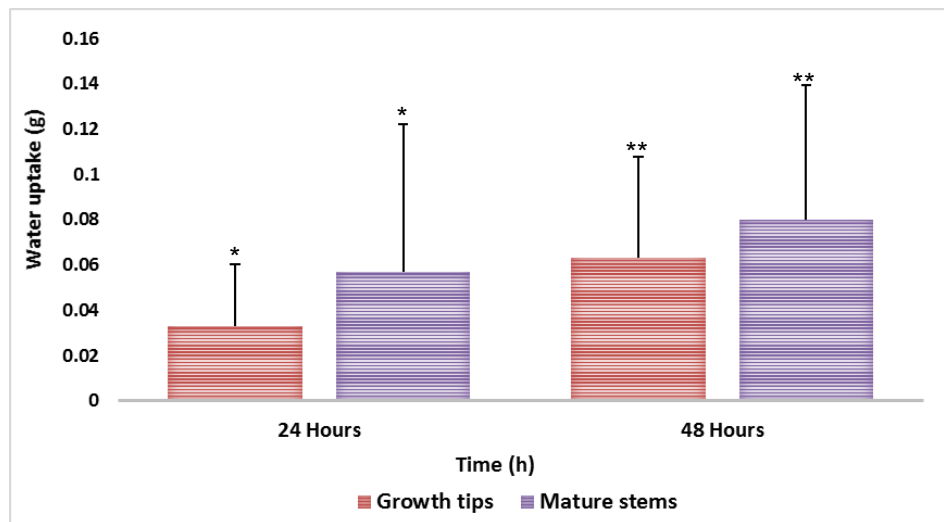


Figure 29: Water uptake by photosynthetic shoots of *Acanthosicyos horridus* after 24 and 48 h. Results from the one-way analysis of variance (Holm-Šidák test) showed a significant ‘water uptake’ after 24 and 48 h for the growth tips and significant difference in stem mass was found after 24 and 48 h for the older stems

The next method (Limm *et al.*, 2009) considered and removed possible errors due to residual water left on the stem surface (Fig. 30). The results show that there was no significant difference between the initial stem mass and the mass of the growth tips ($p = 0.864$) or that of the mature stems ($p = 0.951$) after 180 min submergence in deionised water (Fig. 30). The initial mass of the stem clippings of one sample group varied considerably and the data were normalised by determining the difference in stem mass ($Mass_{180min} - Mass_{initial}$), which may be considered as water uptake. Results show that the water uptake was significant for the growth tips ($p < 0.001$) and mature stems ($p = 0.028$) after 180 min submergence.

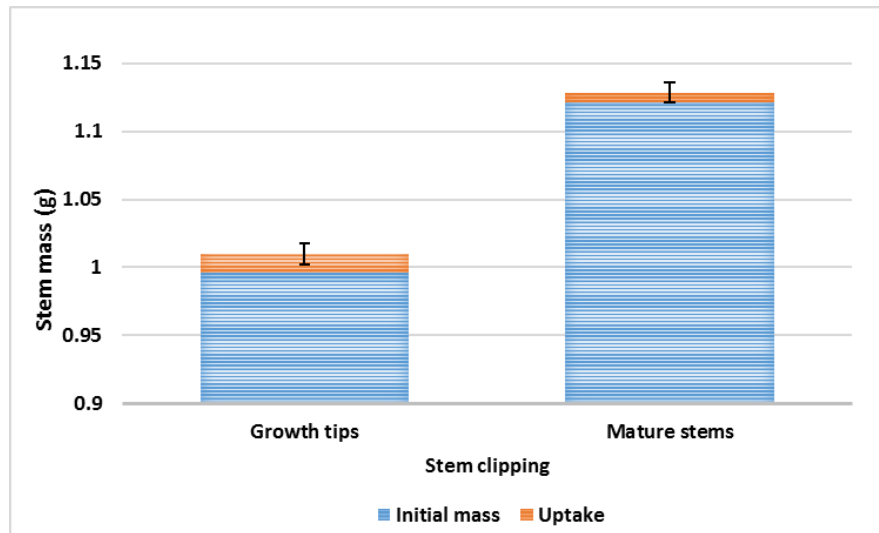


Figure 30: Water uptake capacity of photosynthetic shoots of *Acanthosicyos horridus* after 180 min submergence in deionised water under dark conditions (n = 8)

4.3.3 Water potential measurements

Examining xylem tension of *A. horridus* shoots with a pressure chamber instrument during the course of the day showed that the xylem tension was not uniform throughout a single *A. horridus* plant (Fig. 31). This might possibly be the result of different abiotic factors influencing the plant's xylem tension in various directions within the plant (Table 3). For example, the water potential for Hummock C varied throughout the day of 11 July when no fog occurred (Fig. 31). The water potential was the lowest in the morning for the growth tips in the corresponding southern and eastern transects. A possible explanation is the influence of the dominating south-easterly (122.6°) wind before 06:00 on 11 July (Fig. 31). This wind continued until 12:00 and is reflected by the lower shoot water potential from the samples collected from the southern and eastern transects (Fig. 31).

These southern and eastern shoots had a higher water potential at midday than in the morning and this could be due to a decrease in wind speed. Alternatively, it could indicate stomatal closure to prevent water loss through transpiration and, therefore, increasing the water potential of the *A. horridus* shoots. A similar increase in the water potential of the shoots representing the northerly, easterly and southerly directions were measured until the afternoon (Fig. 31), whereas the

western transects had a lower water potential in the afternoon than at midday. This increase in water potential during the day is highly unlikely and should be further investigated. During this period there was also a decrease in the humidity from 63% to 23% and would result increased xylem tensions resulting in more negative values. The decrease in the water potential of *A. horridus* shoots in the westerly direction corresponded to a change in wind direction after 12:00 in a dominantly south-westerly direction.

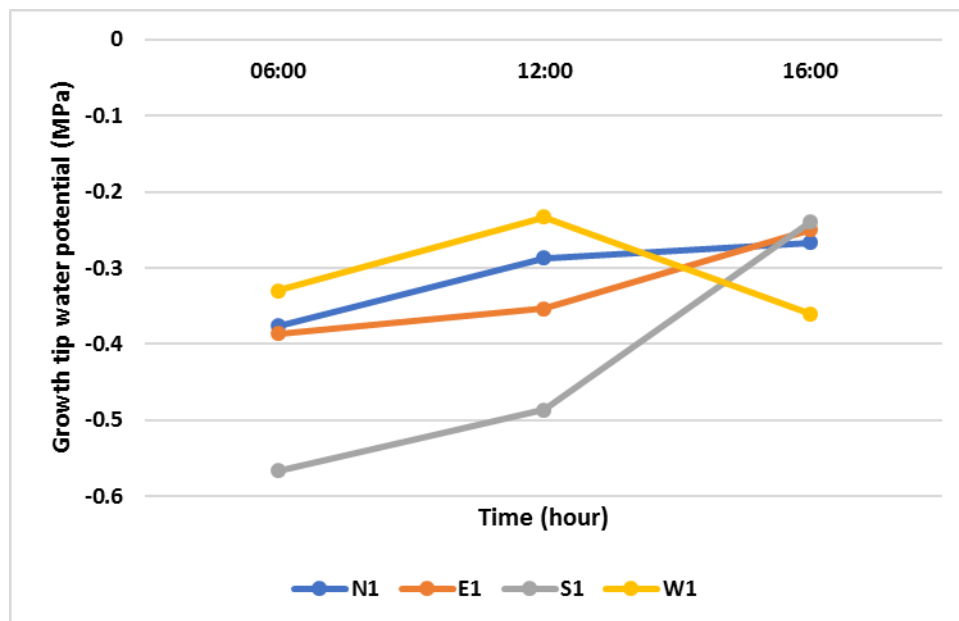


Figure 31: Diurnal water potential measurement for *Acanthosicyos horridus* growth tips of Hummock C on 11 July 2017, a non-foggy day, with N1, E1, S1 and W1 representing the *A. horridus* stems in the corresponding direction.

Table 3: Meteorological data collected from the Gobabeb FogNet station on 11 July 2017, a non-foggy day

		06:00	12:00	16:00
Air temperature	°C	11.17	17.636	26.74
Soil temperature (10 cm)	°C	15.58333333	17.11	28.68
Wind speed	m/s	2.14	1.185	3.09675

Wind direction	°	122.6 (SE)	114.038 (ESE)	230.1 (SW)
Wind speed (max)	m/s	4.246666667	2.7952	5.326
Humidity	%	63.92833333	43.63	22.965

The water potential measurements varied between -0.1 and -1.1 MPa in a single plant throughout the day (Fig. 32). These are values that corresponds with the values of well-watered plants when measured before sunrise and indicates the absence of any drought stress and is highly unlikely in this harsh environment. The water potential was strong for a desert plant and could possibly indicate that *A. horridus* are dependent on a permanent water source (groundwater). *Acanthosicyos horridus* plants had a significantly stronger ($p = 0.007$) water potential on the morning (07:00) and midday ($p = 0.012$) of a fog event (14 July 2017) when compared (Fig. 32) to that on a non-foggy day (11 July 2017). The results indicated an increase in the water potential on 11 July 2017 throughout the day and could imply water potential recovery. In contrast, the water potential decreased throughout the day on 14 July, when fog was present, and this could indicate that the stomatal conductance was high during the entire day. There was no significant difference ($p = 0.947$) in the water potential in the late afternoon when comparing the shoots on foggy and non-foggy days (Fig. 32).

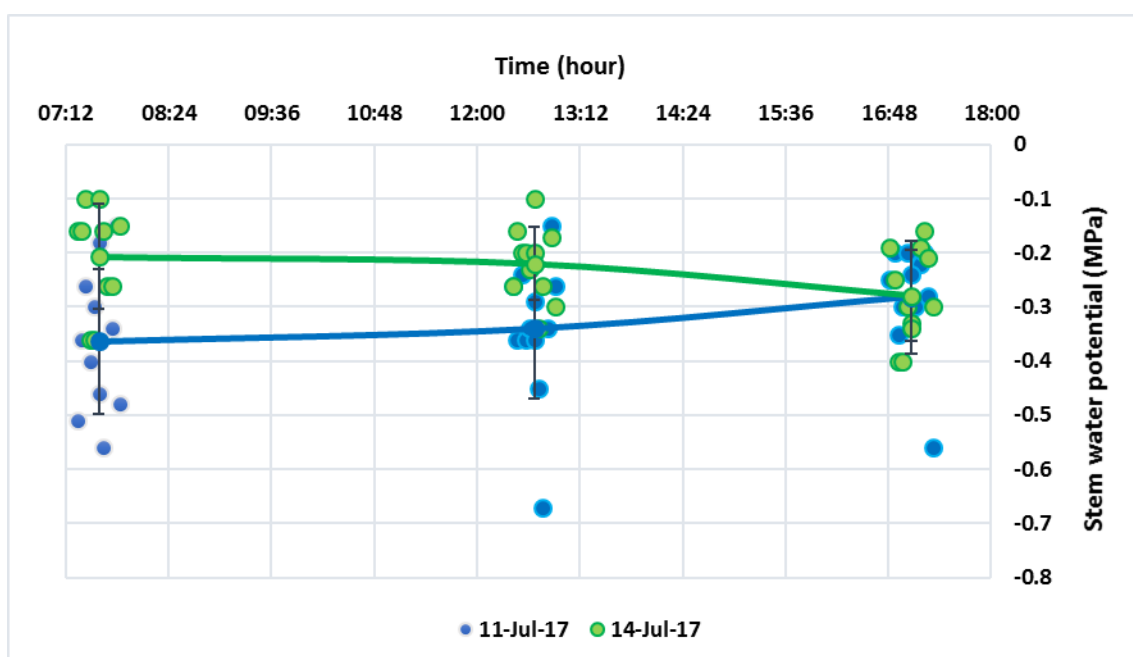


Figure 32: Diurnal course of growth point water potential of Hummock C, Gobabeb valley, before a fog event (11 July 2017) and on the day of a fog event (14 July 2017)

4.4 Discussion

Species that grow in arid environments develop several adaptations to deal with environmental stress. In desert organisms, strategies to exploit NRWIs could potentially aid in improving the water status of the plant through direct absorption of fog precipitation, either through aerial structures or surface root hairs. The presence of fog could also indirectly benefit *A. horridus* plants by influencing their water status in response to changes in certain environmental parameters associated with fog events.

Acanthosicyos horridus has several xerophytic modifications to deal with various environmental stressors in the Namib Desert. This includes large cylindrical epidermis cells, arranged like palisades, and a thick cuticle. This arrangement of the epidermis reduces the risk of cell dehydration by only exposing a small fraction of the cells to the desiccating air outside (Hebeler, 2000). It also provides structural support and elasticity to the stems against torsional strain. Additionally, the UV-absorbing phenolics might protect the chlorenchyma against photoinhibitory damage (Kartusch & Kartusch, 2008). Furthermore, the thick cuticle minimises transpirational water loss by limiting cuticular conductance for water vapour (Gibson, 1998; Hebeler, 2000).

The stomatal grooves have been found to be covered with hydrophobic wax protrusions (Hebeler, 2000). This may reflect radiation and decrease the shoot operative temperature and may also maintain a higher RH around the stomata (Hebeler, 2000). Furthermore, the grooves in photosynthetic stems increase the boundary layer resistance between the plant and the atmosphere (Gibson, 1983). In addition, the presence of trichomes and wax may further increase this resistance. The rough structure of the hydrophobic cuticle, together with the trichomes, might make the surface more wettable and might retain or even absorb NRWI on the shoot surface (Hebeler, 2000). Longitudinal grooves also enable directional water flow and enhance fog capture by directing water away from the harvesting site (Malik *et al.*, 2014).

Such water retention in the grooves was seen with the time-lapse photography when investigating the water movement along an *A. horridus* stem. The larger coalesced droplets rolled down towards the base via the grooves and is thought to be the result of the hydrophobic wax cuticle. The *A. horridus* plant therefore exhibits stemflow and some of these coalesced droplets drip to the surrounding soil. Other smaller fluorescent droplets remained suspended in the grooves, where most of the trichomes are located. Trichomes make the surface more wettable and seem to aid in water retention on the surface. The smaller droplets showed fluorescent decay over time. This might indicate possible absorption of the fluorescent water into the *A. horridus* stems, but because the RH was only 50% it is not possible to exclude evaporation. Moreover, the absorption test indicated direct water uptake through the stems of *A. horridus*. The first absorption test (Vogel & Müller-Doblies, 2011) showed that there was a significant difference in the growth tip mass after 24 and 48 h of fog exposure, but this test did not consider the possible error due to residual water. The second absorption test (Limm *et al.*, 2009) did consider the possible error due to towel drying of the stems. These results show that there was a significant increase in the stem mass after a 3-h dark submergence in deionised water. This further indicated absorption of water directly into the stem of *A. horridus*. This method only considered dark conditions for fog exposure. In contrast, the Wingscape camera showed that the duration of a fog event was usually from around 01:00 until 08:00 in the morning. Therefore, *A. horridus* is also exposed to diurnal fog. The diurnal water fog uptake should be further investigated because of the possible interaction of the stomata.

Acanthosicyos horridus plants are C₃ plants that have been shown to exhibit diurnal stomatal conductance (Hebeler, 2000). It is possible that fog water may be absorbed during the day through the stomata (Goldsmith, 2013), but this needs to be further studied and could not be done during the scope of study due to equipment not being available. A possible pathway for water absorption might be the trichomes (Yates & Huntley, 1995). The second absorption test (Limm *et al.*, 2009) only considered nocturnal fog exposure and, in general, the stomata of C₃ plants are closed at night and CO₂ assimilation takes place during the day, thus excluding stomata as a

possible absorption pathway; however, this can only be confirmed through future gas exchange measurements.

The absorption of fog precipitation through the stem was shown to directly increase the water potential of the stems. *Acanthosicyos horridus* had a stronger water potential on days when fog occurred than on non-foggy days. It is difficult to compare the effect of fog alone on the water potential due to other environmental parameters that were also shown to influence the water potential values. The water potential of *A. horridus* seems to be sensitive to other environmental conditions, especially wind speed and direction. The water potential was the lowest in the dominating wind direction. This could be due to the increased wind speed decreasing the boundary layer between the atmosphere and the stem and therefore increasing transpiration, resulting in a lower water potential of the stems. This decrease in the diffusion pathway may be beneficial for the plant by increasing CO₂ uptake and decreasing the stem temperature.

Franco-Vizcaíno (1994), found that the water supply in sandy soils exerts control on stomatal conductance in C₃ succulents. The succulents, *F. columnaris* exhibited strong diurnal fluctuations (-1 to -2 MPa) in the leaf water potential, in soil with abundant moisture, and is the result of active daytime transpiration. These fluctuations are observed in the water potential of *A. horridus* (Franco-Vizcaíno, 1994), however, the water potential values for *A. horridus* does not correlate with these values and *A. horridus* has an overall higher water potential is observed. Monson and Smith (1982) found that to desert plant species, *Baccharis* and *Oliveya* had relatively high seasonal water potential (≈ -1.0 – -2.9 MPa and ≈ 1.5 – 4.0 MPa) and might be the result of a deep taproot system that can reach underground water. These species exhibited drought-avoiding attributes and water wasting characteristics (Monson and Smith, 1982). Plants with these attributes avoid drought stress through rapid water absorption.

The values for *A. horridus* are extremely high when compared to the water potential values of these two desert plants. These water potential values obtained, for this study, should be re-evaluated due to the high degree of experimental error. The variability in the data presented in figure 31 and figure 32 probably resulted from random timing of sampling, water loss through

cuticle and a secondary cut made to the stem clippings before measuring the xylem tension. Water potential measurements should be taken immediately after the stem was cut to avoid water loss through the cuticle.

Fog precipitation on the soil may also enable nocturnal re-equilibration with soil water, thus resulting in a stronger pre-dawn water potential (Limm *et al.*, 2009; Ebner *et al.*, 2011). The time-lapse photography showed that fog water can move along the grooves to the base of *A. horridus* as stemflow. This water may drip down towards the surrounding soil where it can be absorbed by surface roots as in found with *S. sabulicola* (Section 2.3.1.1). However, *S. sabulicola* has an extensive shallow root system (Vogel & Seely, 1977; Louw & Seely, 1980; Ebner *et al.*, 2011; Roth-Nebelsick *et al.*, 2012), whereas *A. horridus* is believed to not possess surface root hairs but has an extensive taproot system that reaches underground water (Kutschera *et al.*, 1997). In contrast, Kartusch and Kartusch (2008) indicated that the *A. horridus* plant has adventitious roots with mycorrhizal associations (Klopatek *et al.*, 1992). However, there are still limitations in knowledge about the root structure of *A. horridus* and it is not possible to conclude that fog precipitation can be absorbed through the roots.

An *A. horridus* seedling that germinated in the active channel of the Kuiseb River at Gobabeb, after more than 20 mm of rain in July 2016, was dug up to investigate its root system (Fig. 33). The *A. horridus* seedling had a taproot system 7 cm in length, two months after the rain pulse. The roots consisted of very fine surface root hairs that possessed a rhizosheath (Fig. 33). This rhizosheath was also found around the roots of *S. sabulicola* (Ebner *et al.*, 2011). Due to the slow growing rate of the taproot, these seedlings growing in the Kuiseb River or dune ecosystem would not be able to acquire water from the underground aquifer system. This could illustrate the dependence of *A. horridus* seedlings on NRWIs to establish and survive. There have been efforts to cultivate *A. horridus* as a commercial crop in Namibia to provide the local Topnaar community with a secure source of income from *A. horridus* fruit harvesting. Germination of the *A. horridus* seeds is possible, but there has been no success in establishing these seedlings (Maggs-Kölling, in press).

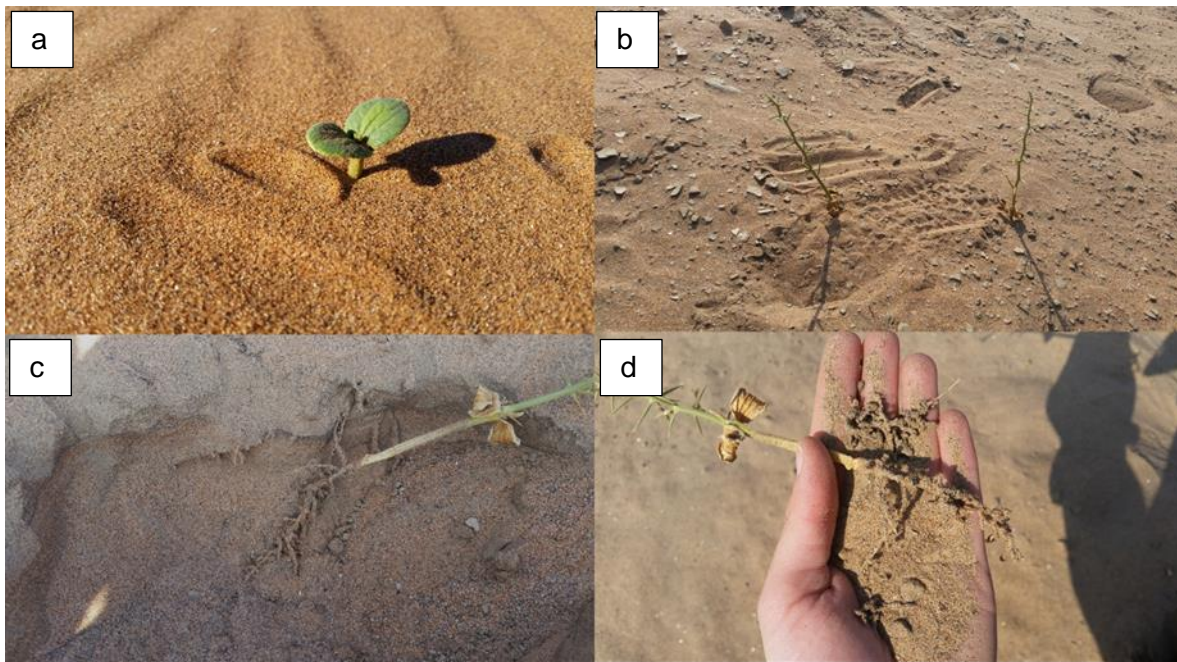


Figure 33: a) *Acanthosicyos horridus* seedling that germinated after 20 mm of rain in July 2016 located at the dune base near Gobabeb, b) *A. horridus* seedlings growing in the Kuiseb River channel, c) root structure of a *A. horridus* seedling, consisting of surface root hairs, and d) fine surface root hairs possess a rhizosheath to help prevent water loss and to retain moisture (credit: Monja Gerber)

There are still some limitations in the understanding of the biochemical and physiological responses of C_3 desert plants to water stress (Van Heerden *et al.*, 2007). In general, the stomata of C_3 plants are open during the day when transpiration rates are high. At the onset of water deficit, these plants use stomatal closure as an important survival mechanism (Van Heerden *et al.*, 2007). These stomatal restrictions are considered the primary cause of a reduction in the photosynthetic potential during drought conditions and are coupled with a decline in mesophyll conduction (Van Heerden *et al.*, 2007). However, *A. horridus* plants have deeply sunken stomata arranged in grooves along the stem. The deeply sunken stomata result in higher humidity around the guard cells and a smaller vapour diffusion gradient between the air in the grooves and the substomatal cavities. Thus, *A. horridus* plants can reduce transpirational water loss without limiting CO_2 diffusion (Gibson, 1998; Hebel, 2000). In addition, the higher water potential of *A. horridus* shoots indicates the reliance of the *A. horridus* plant on a permanent underground water source and further indicates that *A. horridus* is not water stressed.

The water potential measurements illustrated that the water potential was higher on days with fog events and slowly decreased throughout the day. This direct increase of the water status of *A. horridus* through aerial absorption of NRWIs may allow increased stomatal conductance throughout the day, resulting in increased carbon gain and, ultimately, an increase in the plant's biomass. It is also possible that NRWIs indirectly improve the stem water status by increasing the RH and thus decreasing the water vapour diffusion gradient between the stem interior and the atmosphere (Ebner *et al.*, 2011). This possibility can be investigated in the future, using gas exchange measurements to compare the net assimilation rate of *A. horridus* during a fog event to that during non-foggy days

When comparing the daily abiotic variables of days with a fog event to those of non-foggy days, results indicate that there was no significant difference between the average daily temperature or RH. These results correspond with those of Ebner *et al.* (2011), who indicated that fog occurrence did not have a significant impact on most daily parameters but did increase the atmospheric saturation vapour pressure, an important parameter not measured during their investigation.

An increase in the vapour pressure deficit gradient (VDP) between the leaf and surrounding air results in lower stomatal conductance and transpiration rates (Bunce, 1997). The stomatal closure may occur in patches with high VDP, which results in a lower assimilation rate with no reduction in the internal CO₂ concentration of the photosynthetic tissue. This was not investigated during this study and could provide useful information on the indirect influence of parameters associated with fog events on *A. horridus*. The transpirational water loss is a function of stem temperature and as stem temperature rises, the stomatal conductance increases accordingly up to stomatal closure. This increase in stomatal conductance is due to increased transpiration and evaporative cooling as VDP increases (Gibson, 1998; Hebel, 2000; Lawson *et al.*, 2013).

It is possible that the intercepted fog and other NRWI components contribute to the nutrient cycling and litter decomposition of dead *A. horridus* stems. Evidence suggests that NRWIs and especially water vapour absorption have a significant effect on CO₂ fluxes due to litter decomposition (Wang *et al.*, 2016). *Acanthosicyos horridus* is found in nutrient-poor dune

ecosystems and the increase in litter decomposition due to NRWIs benefits the *A. horridus* plant by providing the needed nutrients for photosynthesis, growth and fruit and flower production.

Various studies have observed that organic matter may be degraded due to associated microbial activity (Agam & Berliner, 2006; Jacobson *et al.*, 2015). Jacobson *et al.* (2015) found that a mesophilic ascomycete community associated with *S. sabulicola* is responsible for the decomposition of organic matter and has a fast response to water inputs. *Acanthosicyos horridus* was also suggested to possess vesicular arbuscular mycorrhizal association in the surface roots, which assists in nutrient composition (Klopatek *et al.*, 1992). The fog precipitation that drips from the stem of *A. horridus* to the surrounding soils could possibly provide the water needed for microbial activity and, therefore, litter composition. In contrast, NRWIs may have a negative impact on plant health by creating a microfilm on the plant surface, allowing pathogen spores to germinate (Agam & Berliner, 2006). This may be the cause of stem fasciation, which is observed in some *A. horridus* plants, that results in a flattened belt shape of the stem (Mannheimer *et al.*, 2008). Microbial involvement has not yet been studied on fasciated *A. horridus* stems and there are still limitations in the knowledge on microbial communities associated with these plants.

A recent study compared the nematode abundance and diversity around *A. horridus* thickets to that in bare soil. Results indicated that nematode abundance was almost 400 times greater inside a *A. horridus* thicket when compared to that in bare sand (Steinberger *et al.*, in prep.). Nematodes require a film of water for activity among the soil particles and their ecology is dominated by surface tension, porosity, water percolation, viscosity and humidity. Hosier (1992) found that the dune ecosystem had low numbers of nematodes and was dominated by bacterivores. Fog precipitation on plants may provide enough moisture for nematode activity, indicating that fog may also provide a source of water for other organisms associated with *A. horridus* plants.

It can also provide the moisture input needed to stabilise the sand around *A. horridus* thickets, resulting in hummock formation (Agam & Berlinger, 2006; Berry, 1991). Hummock-forming plants may be considered 'physical ecosystem engineers'. These are organisms that alter the physical state of biotic and abiotic materials in an environment through physical maintenance, creation or

modification of habitats (Jones *et al.*, 1997). An autogenic ecosystem engineer directly transforms an environment by altering the structure, while remaining part of the new, engineered environment. These alterations may have a positive or negative ecological consequence on other dependent species (Jones *et al.*, 1997). This stabilising effect can also benefit burrowing organisms associated with the *A. horridus* plant.

4.5 Conclusion

In conclusion, *A. horridus* did exhibit significant fog absorption capacity through the shoots, indicating that fog water directly provides a source of moisture for *A. horridus* plants and increased the shoot water potential. It is also possible that fog or other NRWIs (dew and water vapour) can indirectly benefit the plants by influencing the associated environmental parameters, thereby influencing gas exchange through stomatal conductance, and should be considered for future studies. This decrease in water potential due to water deficit results in stomatal closure to limit water loss through transpiration. The decreased stomatal conductance, together with sustained irradiance, might result in an imbalance in the intercellular energy relative to the CO₂ available. This imbalance might further result in photorespiration to protect the photosystems against photodamage.

Other NRWI components, including dew and water vapour, were not investigated during this study and growing evidence suggests that these sources could play an important role in ecosystem functions. Further investigation is required to identify organisms associated with the *A. horridus* plant and their interaction with NRWIs.

CHAPTER 5: CHLOROPHYLL A FLUORESCENCE OF *A. HORRIDUS*

HEADING 1 WON'T PRINT. DON'T DELETE – DOING SO WILL LEAD TO INCORRECT NUMBERING.

4.6 Introduction

Photosynthesis is a vital metabolic process in plants and measuring the photosynthetic potential can therefore be used as a tool to determine the overall 'health status' of a plant (Tòth *et al.*, 2005; Stirbet & Govindjee, 2011). The rate of photosynthesis in plants may be limited by environmental factors including soil water, excessive PAR, high temperature, nutrient supplies, CO₂ availability, pathological conditions and pollutants (Kalaji *et al.*, 2016; Hopkins & Hüner, 2008), of which the first four are particularly important in arid areas such as the Namib Desert. A reduction in photosynthesis may lead to an accumulation of excess energy and result in the synthesis of reactive oxygen species (ROS) that disrupt the photosynthetic apparatus, causing a decrease in plant productivity (Kalaji *et al.*, 2016; Li *et al.*, 2012). Photosystem II (PSII) is the most sensitive to environmental changes and can be an indication of changes in physiological processes and the photosynthetic electron transport chain (ETC) (Kalaji *et al.*, 2016; Li *et al.*, 2016).

Chlorophyll *a* fluorescence measurement is a sensitive, rapid, non-invasive tool used in ecophysiology and basic photosynthetic studies to monitor abiotic and biotic plant stressors (Tòth *et al.*, 2005; Oukarroum *et al.*, 2007; Hopkins & Hüner, 2008; Mishra *et al.*, 2016). Fluorescence can be defined, according to Hopkins and Hüner (2008), as an excited molecule that emits long light wavelengths (680–740 nm) as the molecule returns to its original ground state. Measuring ChlF of photosynthetic organisms after dark adaptation allows the primary quinone electron acceptor (Q_A) of the PSII reaction centre (RC) to be fully oxidised and enables the measurement of minimal fluorescence (F₀) (Li *et al.*, 2016). The open RC [P680 Pheo Q_A] can absorb light energy and plants exposed to continuous illumination exhibit a characteristic fast polyphasic fluorescence induction curve (OJIP transient) with certain inflexion points that relate to different steps in the reduction of the photosynthetic ETC (Stirbet & Govindjee, 2011; Mishra *et al.*, 2016). This is also

known as the Kautsky effect, named after Hans Kautsky (1891–1966), who together with Hirsh first reported on the variable nature of ChlF (Stirbet & Govindjee, 2011; Hopkins & Hüner, 2008).

In a measured OJIP transient, or OJIP curve, the O-J phase corresponds to the reduction of the acceptor side of PSII. The J-I phase (2–30 ms) of the induction curve parallels the reduction of the secondary quinone electron acceptor (Q_B), plastoquinone (PQ) and the cytochrome b_6f complex (Cyt b_6f). The I-P phase (30–300 ms) of the induction curve corresponds to the reduction of electron carriers, including ferredoxin, and NADP on the photosystem I (PSI) side (Kalaji *et al.*, 2016). The maximal fluorescence (P or F_M) is reached when the RCs are closed and Q_A and all the electron carriers are in a reduced state [$P680^+$ Pheo Q_A^-] (Hopkins & Hüner, 2008; Tóth *et al.*, 2005; Mishra *et al.*, 2016).

Analysis of the OJIP transients can provide information on the function and structure of the different pigment proteins, with focus on PSII (Oukarroum, 2007; Li *et al.*, 2016). The OJIP transients can reflect changes in the PSII antenna complex, RC, the reduction-oxidation of the PQ pool and electron flow (Li *et al.*, 2016; Stirbet & Govindjee, 2011). All photosynthetic organisms need to balance the energy budget between photochemically absorbed energy, energy used for growth and metabolism or energy dissipated non-photochemically as heat (Strasser *et al.*, 2000).

Plants exposed to severe drought may reduce transpirational water loss through stomatal closure. This results in a decrease in the intracellular CO_2 concentration and water potential and ultimately impairs the photosynthetic activity (Mishra *et al.*, 2016).

Acanthosicyos horridus plants in the Namib Desert, which are at some distance away from a shallow aquifer, such as the Kuiseb River, are under considerable water stress. It is then likely that if *A. horridus* NRWIs such as fog, those plants that are already under hydrological stress will most clearly respond to NRWIs, such as fog events. By measuring the ChlF before and after fog events, it should be feasible to determine changes in photosynthetic activity that would indicate the plant's physiological response to NRWIs. This chapter reports on such a study carried out near GTRC in the central Namib Sand Sea.

4.7 Materials and methods

4.7.1 *Chlorophyll a fluorescence*

A non-invasive Handy PEA-fluorimeter (Plant Efficiency Analyser, Hansatech Ltd, UK) was used to measure the fast polyphasic fluorescence transients for a duration of 1 s (maximum light intensity of $\sim 3000 \mu\text{mol photon m}^{-2} \text{s}^{-1}$, and 01.0 gain) on three *A. horridus* male plants near Gobabeb (Section 4.2.1, Table 2 and Fig. 23). A mature stem was selected to represent each eight triangular transects of a *A. horridus* hummock. Stems with a large diameter were selected due to the size of the leafclip shutter. The thorns diameters were too small to fill the leafclip shutter, resulting in light leakage and inaccurate measurements. and marked with an aluminium tag. Five measurements were taken per stem. The measured increases are represented by the OJIP induction curves (Strasser *et al.*, 1995; Tsimilli-Michael & Strasser, 2008) for each of the measurements.

Fog at Gobabeb is most frequent from August to March (Schulze *et al.*, 1976). Measurements were therefore taken for seven consecutive nights at the beginning of every second week from August 2016 to March 2017. As the fog incidence decreased during the winter months from March to July 2017, measurements were only taken once a week and after a fog event. One *A. horridus* stem was selected and marked to represent each of the eight triangular transects (Fig. 24) for each of the three hummocks. Five ChIF replications were measured per stem at night (1 h after sunset) to ensure dark-adapted leaves.

PeaPlus (Version 1.10) was used for the calculation of photosynthetic parameters (Table 4) from variable fluorescence (Strasser *et al.*, 2007). These parameters were categorised into four groups: (1) the basic measured and calculated values, including fluorescence (F_t) and variable fluorescence (V_t), (2) the quantum probability and yield, (3) the energy fluxes, including the (ABS/RC) parameter at the start of fluorescence induction as the specific energy flux per RC for absorption (ABS) (Strasser *et al.*, 2007), and (4) the vitality indices, including the performance index (PI) parameters (Kalaji *et al.*, 2016). PI is an indicator of the vitality of the plant and

represents the product of several parameters, including the density of the RC, quantum efficiency of photochemistry and the conversion of energy in the ETC (Kalaji *et al.*, 2016).

An attempt was made to measure the chlorophyll content of *A. horridus* stems using a CCM-series chlorophyll content meter used but the stems of *A. horridus* were too dense to measure the chlorophyll content. This was the only instrumentation available at this remote Research Centre and it should be considered using UV-spectrophotometry to investigate chlorophyll content in the future.

Table 4: Photosynthetic parameters calculated from measured OJIP induction curves (adapted from Tóth *et al.*, 2005; Oukarroum *et al.*, 2007; Strasser *et al.*, 2004)

	Parameters	Description
Step 1	RC/ABS	Amount of light energy (E_{light}) absorbed per active RC, representing the effective Chl antenna size of the active RC
Step 2	$\phi_o/(1-\phi_o)$	Amount of $E_{\text{excitation}}$ trapped per RC
Step 3	$\psi_o/(1-\psi_o)$	The efficiency that an electron will move further than Q_a^- . The conversion of $E_{\text{excitation}}$ to the e- transport chain.
Step 4	$\delta_o/(1-\delta_o)$	The efficiency with which an electron from the intersystem electron carriers moves to reduce end electron acceptors at the PS1 acceptor side. Or it can refer to the reduction of NADP^+ as end e- acceptors.
The first three steps contributing to photosynthesis.	PI_{ABS}	Performance index absorbance, an indicator of a plant's vitality, describing the plant's ability to absorb light energy and convert this energy to chemical energy: $PI_{\text{ABS}} = \text{RC/ABS} \times \phi_{\text{Po}} / (1 - \phi_{\text{Po}}) \times \psi_o / (1 - \psi_o)$

A multi-parametric expression of all four steps.	PI_{total}	<p>Performance index for energy conservation from exciton to the reduction of PSI end acceptors, giving a good indication of the plant's overall vitality, and includes the ability of the plant to use this available energy:</p> $PI_{total} = (RC/ABS) \times [\phi_o / (1 - \phi_o)] \times [\psi_o / (1 - \psi_o)] \times [\delta_o / (1 - \delta_o)]$
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Table 5: The following equations were used in assessing the JIP test for the analysis of chlorophyll a fluorescence and the relevant photosynthetic parameters.

Parameters	Definition and equation
F_o	Initial fluorescence measured when the photosystem II (PSII) reaction centres (RCs) are open at the O-step (t = 0.05 ms), representing the absorption of energy
F_J	Chlorophyll (Chl) <i>a</i> fluorescence intensity measured at the J-step (t = 2 ms), representing the trapping of energy in the form of Q _A ⁻
F_I	Chl <i>a</i> fluorescence intensity measured at the I-step (t = 30 ms), representing the electron transport between PSII and PSI
F_{P/M}	Maximal fluorescence measured at the P-step (t ≈ 300 ms), when all PSII RCs are closed, representing the reduction of end electron acceptors
F_v	Variable fluorescence: $F_v = (F_M - F_0) \text{ or } (F_t - F_{0.05}) / (F_{300} - F_{0.05})$
V_T	Relative variable fluorescence of O-J-I-P induction curve: $V_T = (F_T - F_0) / (F_M - F_0)$
Δ_v	Difference in relative variable fluorescence: $\Delta V = (V_{treatment} - V_{control})$

F_v/F_0	Efficiency of electron donation to PSII RCs and the rate of photosynthetic electron conversion at the PSII RCs
F_v/F_M	Related to the maximum quantum yield (ϕ_{Po}) of PSII
$\delta = (1-V_i) / (1-V_i) (1-V_j)$	The reduction of NADP+ as end e- acceptors

4.7.2 *Abiotic variables and statistical analysis*

A Wingscape time-lapse camera was used to determine when a fog event occurred as well as its density and duration (see Section 4.2). Daily variations in T_a ($^{\circ}\text{C}$) and RH (%) were recorded by a Lascar temperature humidity logger (see Section 4.2). Contemporaneous data on wind speed and direction were obtained from the Gobabeb FogNet meteorological station, 3 km away at the GTRC.

Statistical analysis of the data was performed using the programme SigmaPlot Version 12.0. One-way repeated measures analysis of variance was used to detect any difference between related means. The Holm-Šídák test was used to determine the significant difference at the O(K)JIP-steps of ChlF when comparing days when fog occurred to days with no fog. Differences were considered significant at $p < 0.05$.

4.8 Results

4.8.1 *Wind direction*

A typical progression of wind direction during a fog event (Fig. 34a) illustrates that fog entered the desert interior from the north-west (plain mountain) on 6 February 2017 and fused with easterly airflow in the desert interior, resulting in a south to south-westerly vector around 02:00 and a dominant north-westerly vector from 04:00. On 17 December 2016 (Fig. 34b), fog also entered the desert interior from a north-westerly direction and airflow maintained a dominant north-westerly to westerly vector.

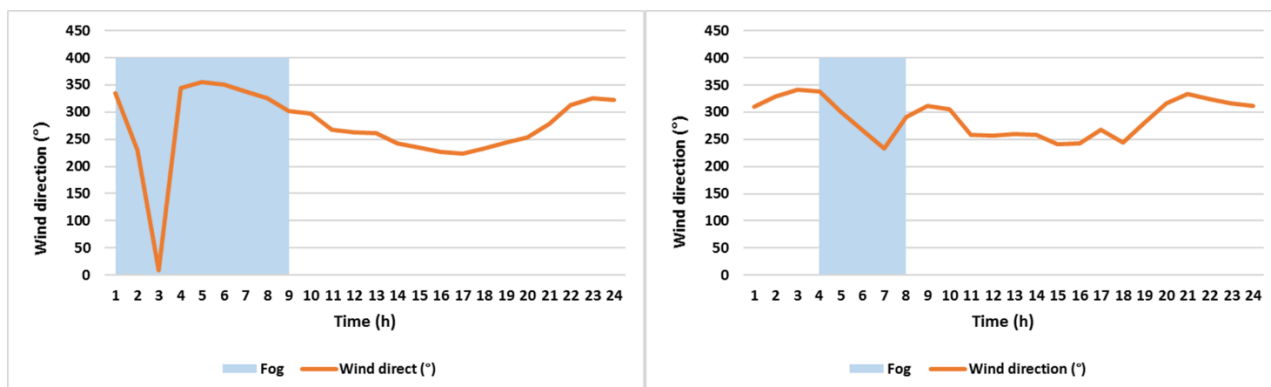


Figure 34: Time progression of the wind direction during fog events, recorded by the Gobabeb FogNet Meteorological Weather Station on (a) 6 February 2017 and (b) 16 December 2016. The wind progression was compared to the duration of fog, determined by Wingscape time-lapse photography

Based on the wind progression data during a fog event and the large number of ChIF data, the results for ChIF focused on the transect that corresponded with the dominant wind direction during a fog event to determine the influence of fog on photosynthetic potential.

4.8.2 Fog occurrence

The occurrence and duration of a fog event on 6 February 2017 showed fog from 02:00 to 08:30 (Fig. 35). The 10 m checkerboard marker was not visible, indicating a fog density of 4 (Fig. 35a), on a scale from 0 to 4. Time-lapse photographs on 7 February 2017 showed fog occurrence from 02:00 to 08:30. Both checkerboard markers were visible, but the visibility was less than 60 m and the horizon was not visible, indicating a fog density of 2 (Fig. 35b). No ground-level fog occurred on 8 February 2017 and the horizon was visible (Fig. 35c). The time-lapse fog monitoring did not record any fog on 15 and 17 December 2016 (Figs. 35d & f), but fog occurred on 16 December 2016 from 01:00 to 06:00 with a fog density of 2 (Fig. 35e).

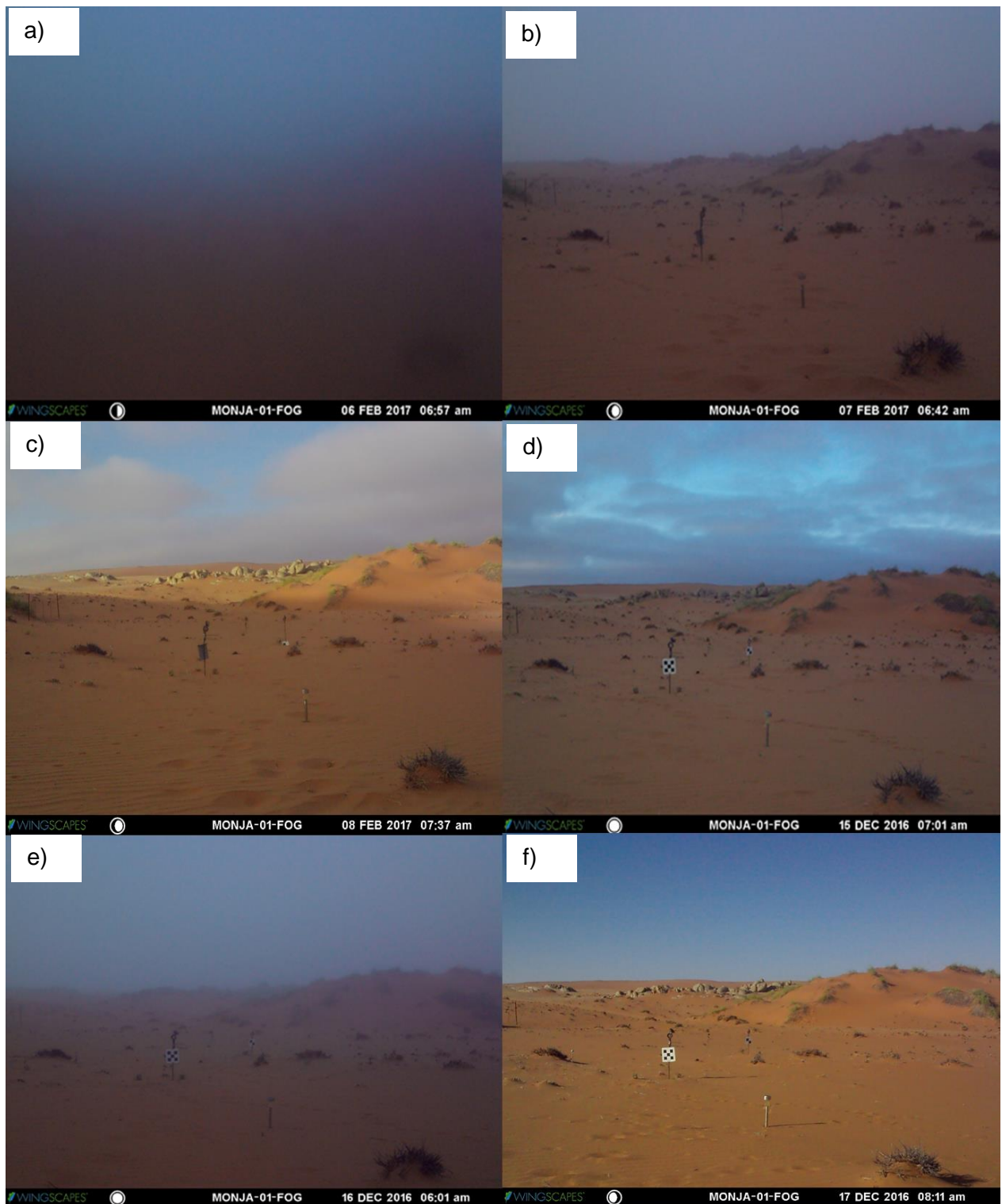


Figure 35: Fog monitoring with a Wingscape time-lapse camera on a) 6 February 2017 (fog = 4), b) 7 February 2017 (fog = 2), c) 8 February 2017 (fog = 0), d) 15 December 2016 (fog = 0), e) 16 December 2016 (fog = 2) and f) 17 December 2016 (fog = 0)

4.8.3 Chlorophyll a fluorescence measurements

The shape of the O-(K)-J-I-P transient is sensitive to stress caused by changes in various environmental conditions. The JIP test, therefore, provides information in terms of the behaviour and vitality of the photosynthetic apparatus of *A. horridus* in response to NRWIs and other abiotic variables. Clear differences in ChIF patterns were observed (Fig. 36) between measurements on nights immediately after fog events (6 February 2017: fog scale = 4; 7 February 2017: fog scale = 2) and days without fog events (8 February 2017: fog scale = 0). The data was normalised to obtain the relative variable fluorescent graphs (Fig. 37).

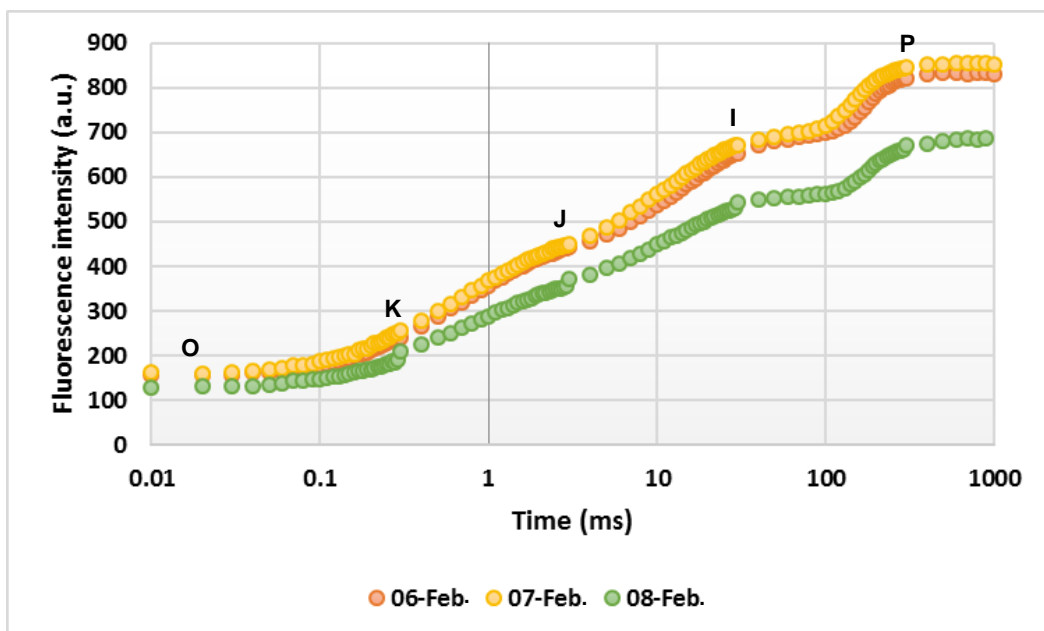


Figure 36: Chlorophyll a fluorescence O(K)JIP transients exhibited by dark-adapted *A. horridus* stems presented on a logarithmic time scale from 10 μ s to 1 s. These transients show the typical O-J-I-P steps at selected time points. This graph compares the OJIP transients of Hummock C, north-western transect, on 6 February 2017 (fog scale = 4), 7 February 2017 (fog scale = 2) and 8 February 2017 (fog scale = 0).

A significantly higher fluorescence was observed at the last inflexion point of the induction curve (P-step) on days with a fog event compared to those on non-foggy days in early February 2017

(Fig. 37). The significant increase in the I-P phase of the curve after fog events corresponded to the reduction of electron transporters. The days with fog (6 and 7 February) had an overall higher fluorescent intensity (Fig. 37) when compared to a day with no-fog (8 February). This increased fluorescence intensity indicates decreased carbon metabolism on foggy days. Furthermore, this increased fluorescent intensity on foggy days indicates that less absorbed light energy was used for primary photochemistry and results in more energy that is lost through heat or fluorescence to prevent photooxidation. There was no significant difference between the values from successive foggy days on 6 and 7 February 2017 (Fig. 37)

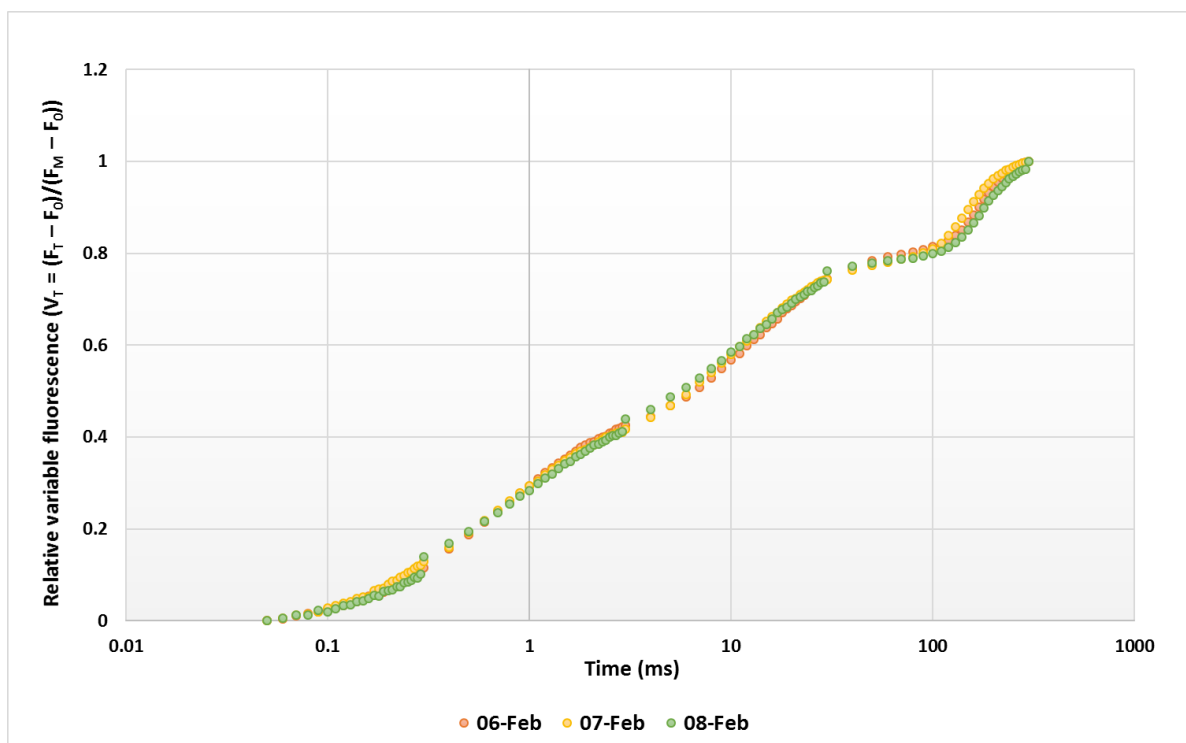


Figure 37: Relative variable fluorescence ($\Delta V = F_M - F_0$) exhibited by dark-adapted *A. horridus* stems presented on a logarithmic time scale from 10 μ s to 1 s. This graph compares the OJIP transients of Hummock C, north-western transect, on 6 February 2017 (fog scale = 4), 7 February 2017 (fog scale = 2) and 8 February 2017 (fog scale = 0)

The parameter F_V/F_M is an indicator of photochemical capacity and is a measure of the PSII quantum efficiency (Fig. 38). Environmental stressors that influence the PS are often associated with a decline in the F_V/F_M and is influenced by nutrient stress. The F_V/F_M value (Fig. 38) was around 0.82 and there were no significant differences between the F_V/F_M values of 6 February

2017 ($p = 0.884$, fog = 4) and 7 February ($p = 0.921$, fog = 2) when compared to 8 February 2017 (fog = 0).

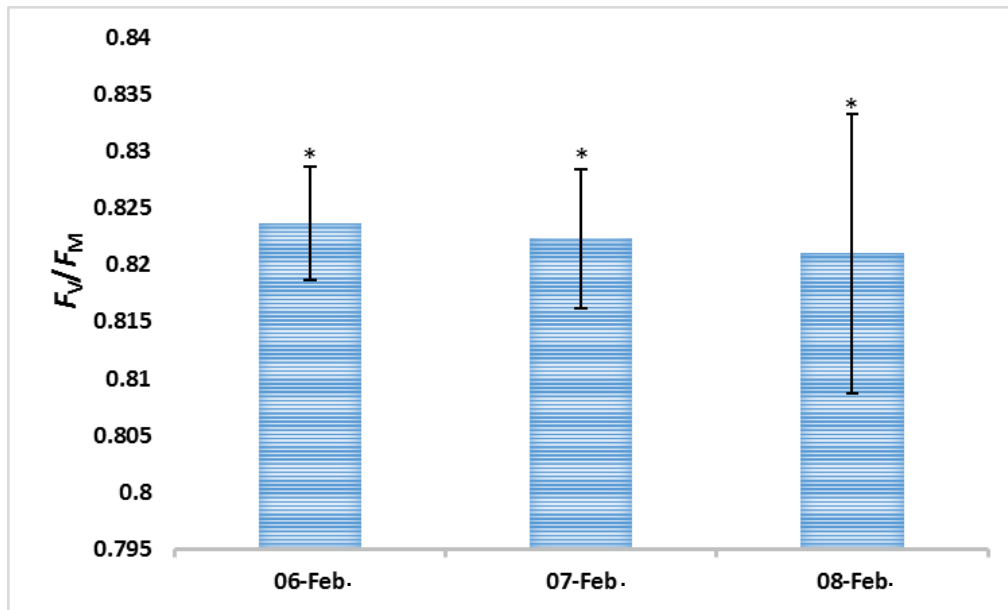


Figure 38: Comparison of the photosystem II quantum efficiency parameter F_v/F_m of 6 February (fog = 4), 7 February (fog = 2) and 8 February 2017 (fog = 0). No significant differences (indicated by *) were found between the F_v/F_m values

F_v/F_m is based on the F_0 and F_m fluorescence values and is often used as a stress indicator, but it is not always sensitive enough to detect subtle differences. The total PI (PI_{total}) parameter is more sensitive and responsive to changes (Fig. 39) and is a better indicator of the overall vitality of a photosynthetic organism. PI_{total} is the product of four partial potentials, namely chlorophyll RC density, electron trapping efficiency, electron transport efficiency and the efficiency of the electron transport flux to PSI electron acceptors. Thus, PI_{total} reflects stress on any of these components. Even though this index is more sensitive, there were no significant differences in the PI_{total} values of *A. horridus* between days with fog events (6 February: $p = 0.707$; 7 February: $p = 0.5$) compared to those on non-foggy days (Fig. 39).

When comparing the four partial potentials (Fig. 39), the results showed that there was a significant difference ($p = 0.003$) at the first step (RC/ABS) on 7 February (fog=2) when compared to 8 February (fog=0). This indicates that *A. horridus* expressed a higher absorbance of light

energy, by the reaction centres, on the day when no fog occurred (Fig. 39). The absorbance of light energy was also significantly higher on 6 February ($p = 0.026$) when compared to 7 February.

When comparing days with fog to the day when no fog occurred (Fig. 39), the results showed that there was no significant difference between the trapping of excitation energy ($\phi_o/(1-\phi_o)$), the conversion of excitation energy to the electron transport ($\psi_o/(1-\psi_o)$) or in the reduction of the end electron acceptors ($\delta_o/(1-\delta_o)$).

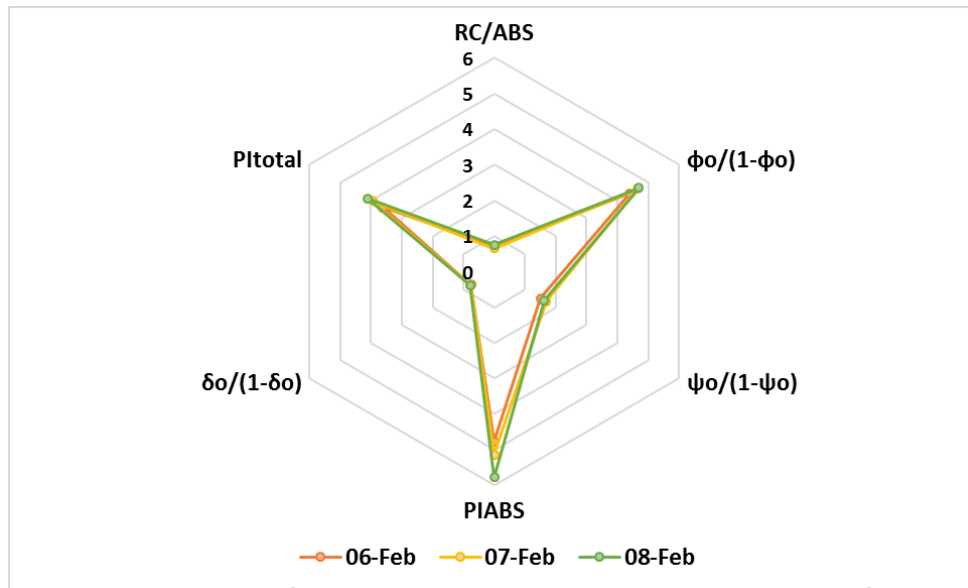


Figure 39: Comparison of the overall vitality parameters, total performance index (PI_{total}), between 6 February (fog = 4), 7 February (fog = 2) and 8 February 2017 (fog = 0). No significant differences (indicated by *) were found between PI_{total} on foggy days and that on the non-foggy day

4.8.4 Chlorophyll a fluorescence on 15–17 December 2016

When comparing the relative variable fluorescence (Fig. 39) of 16 December (fog=2) to the days without fog, the graph illustrated that 16 December had a lower fluorescence intensity in the O-J phase and J-I phase. The difference in the O-J phase of the induction curve was related to the closure of PSII RC in response to the reduction of the Q_A and was determined by the ratio between the trapping rate of light energy by Q_A and the reoxidation rate by Q_B . The lower fluorescent intensity in the J-I phase of the induction curve after fog events was parallel to the reduction of

the Q_B^- , including PQ, Cyt *b₆f* and PC. This lower fluorescence intensity indicates that more absorbed energy was used for the reduction of the Q_B^- on the day when fog occurred compared to the days without fog. However, 16 December had a higher fluorescence intensity in the I-P phase of the curve this corresponds to the reduction of electron transporters including ferredoxin, intermediary electron acceptors and NADP on the PSI side. This increased fluorescence intensity on the day with fog indicates that less energy was used for the reduction of the end electron acceptors than when compared to days with no fog (Fig. 39)

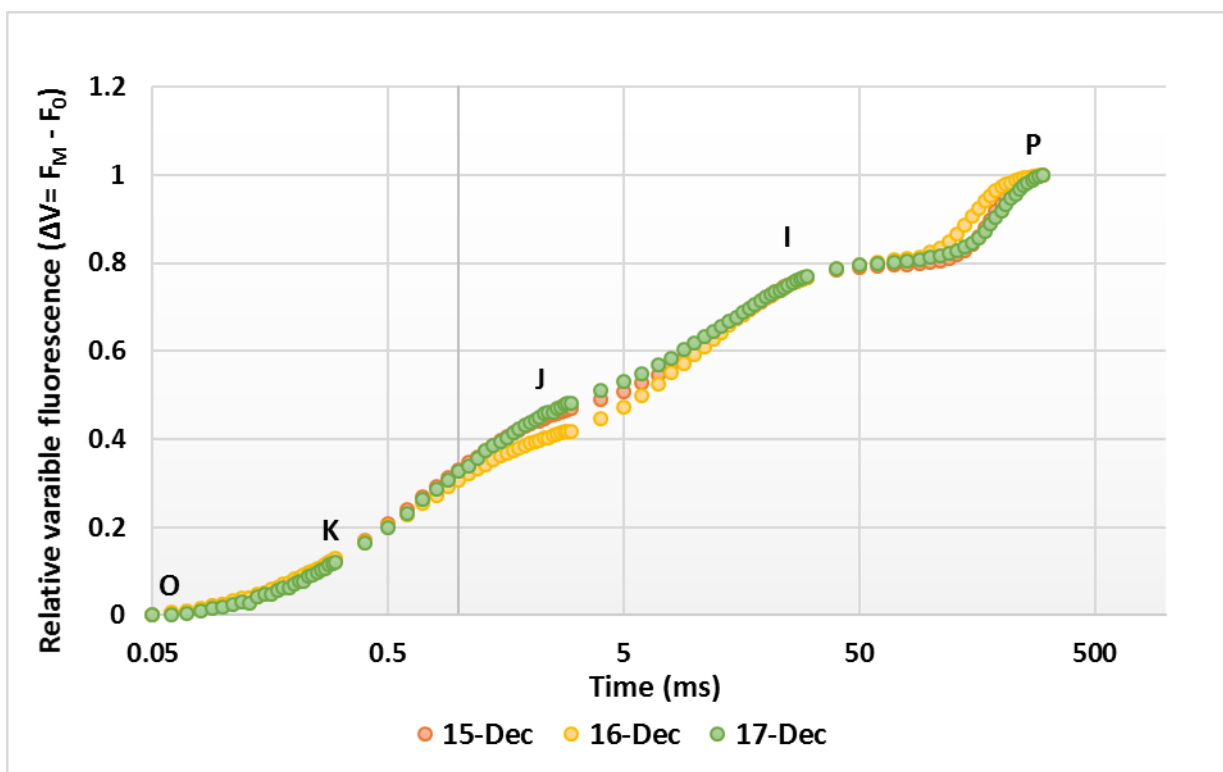


Figure 40: Relative variable fluorescence at 0.05 ms (O-step), 0.3 ms (K-step) and 2 ms (J-step), 30 ms (I-step) and 300 ms (P-step) on 15 December (fog = 0), 16 December (fog = 2) and 17 December 2016 (fog = 0). The mean value across all three days is indicated by the dotted line

The difference in relative variable fluorescence (Fig. 41) was obtained by comparing the values on 16 and 17 December 2016 with those on 15 December 2016. Results revealed a more

pronounced ΔK -band (0.3 ms) on 16 December (fog = 2) after a fog event relative to that on 15 December (fog = 0) (Fig. 41). The ΔK -band represents the balance between electron donation from the oxygen evolving complex (OEC) to $P680^+$. Moreover, the positive ΔK -band indicated lower water-splitting efficiency due to the disruption of the OEC. There was also a more pronounced ΔI -band on 17 December (Fig. 41) relative to that on 15 December, suggesting that there was an accumulation of Q_B^- and a disruption in the flow of electrons beyond Q_A^- . Lastly, there was a pronounced unknown positive Δ -band at 180 ms on 16 December and a negative Δ -band on 17 December (Fig. 41). This may be related to the inhibition of the Rieske iron-sulphur (FeS) protein Cyt *b₆f*.

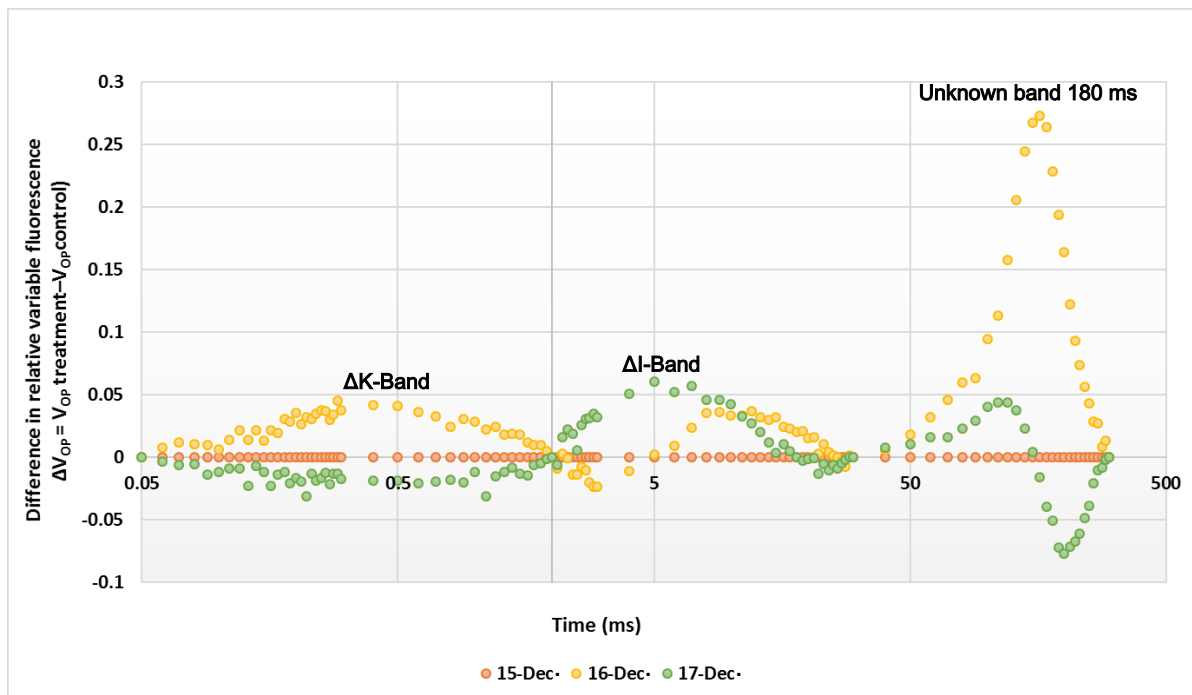


Figure 41: Differences in relative variable fluorescence, double normalised to reveal different bands at ΔV_{OJ} (0.05 and 2 ms), ΔV_{JI} (2 and 30 ms) and ΔV_{IP} (30 and 300 ms). $\Delta V = (V_{\text{treatment}} - V_{\text{control}})$ for 15 December (fog = 0), 16 December (fog = 2), and 17 December 2016 (fog = 0); 15 December was used as the reference date

The difference in relative variable fluorescence was obtained by comparing measurements from 16 and 15 December with those from 17 December 2016 (Fig. 42). Results for 16 December

showed a ΔK -band at 0.3 ms of the induction curve (Fig. 42), indicating a lower water-splitting efficiency of the OEC on 16 December (fog = 2) than on 17 December (fog = 0). Results revealed a negative ΔI -band (2 ms) on 15 and 16 December (Fig. 42), suggesting that fog had a stimulating effect on the reduction of Q_B^- and an increase in the electron flow beyond Q_B^- . The positive ΔH -band on 16 December (Fig. 42) depended on the reduced secondary quinone acceptor (Q_B^{2-}). This band suggests that *A. horridus* plants were less able to produce electron flow beyond Q_B^{2-} after a fog event than after days with no fog. Furthermore, the results revealed a hidden, negative ΔG -band at 100 ms on 15 December that reflected the reduction of the protonated secondary quinone acceptor (Q_BH_2) and is related to PSI. Lastly, there was a pronounced band at 180 ms on 16 December (Fig. 42), the provenance of which is unknown as it has not yet been described in the literature.

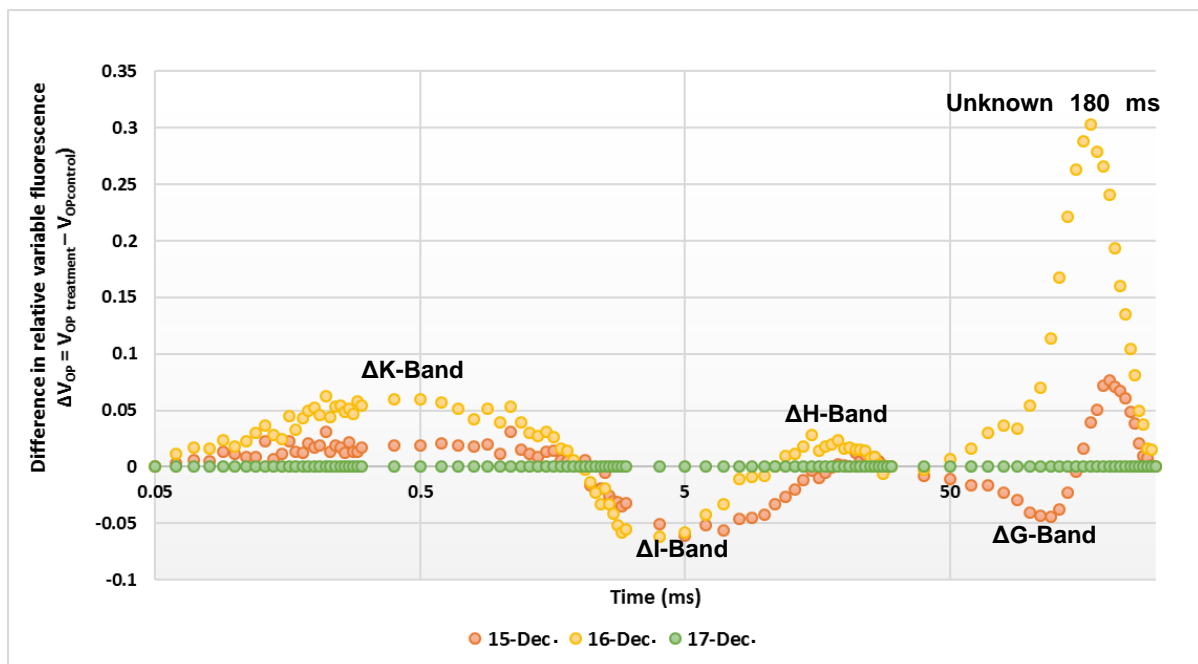


Figure 42: Differences in relative variable fluorescence, double normalised to reveal different bands at ΔV_{OJ} (0.05 and 2 ms), ΔV_{JI} (2 and 30 ms) and ΔV_{IP} (30 and 300 ms). $\Delta V = (V_{\text{treatment}} - V_{\text{control}})$ for 15 December (fog = 0), 16 December (fog = 2) and 17 December 2016 (fog = 0); 17 December was used as the reference date

4.8.5 *Effects of temperature and wind*

In the extreme environmental conditions of the Namib Desert, *A. horridus* plants are exposed to various other daily environmental stressors (e.g. temperature and wind) that may also influence photosynthetic potential. It was therefore important to take into consideration other abiotic influences when comparing the ChlF data after days with fog to those after days with no fog.

The maximum RH was 96.5% around 03:50 on 16 December 2016 (fog = 2) (Fig. 43), while the maximum temperature was 40.5°C on 17 December 2016 (fog = 0). Statistical analysis (Tukey's test; $p = 0.012$) showed that 17 December had a significantly higher daily temperature than that of 15 December. In contrast, there was no significant difference between the temperature of 16 and 17 December ($p = 0.53$) or between that of 15 and 16 December ($p = 0.175$). Furthermore, there was no significant difference (Holm-Šídák test) between the RH on foggy days (16 December) and that on non-foggy days (15 December: $p = 0.96$; 17 December: $p = 0.87$). This indicates that the increase in ChlF after a fog event was not the result of differences in temperature or RH. However, temperature may influence photosynthetic potential and could possibly be the reason for the pronounced ΔI -band (Fig. 42) when comparing 15 and 17 December, days on which no fog was present but with significantly higher temperatures on 17 December (fog = 0) than on 15 December (fog = 0) ($p = 0.012$).

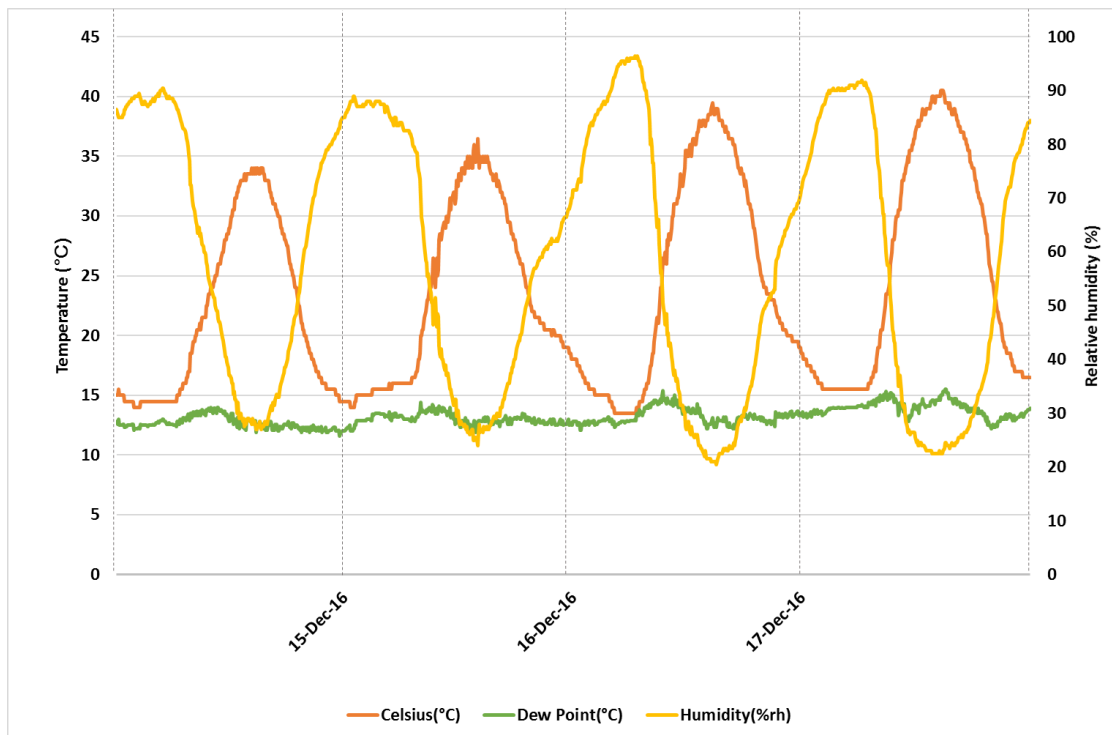


Figure 43: Daily fluctuations in air temperature (°C), relative humidity (%) and dew point temperature (°C) for 15–17 December 2016 at 5 min intervals recorded with a Lascar temperature-humidity logger

Variations in the overall plant vitality in response to temperature were also evaluated (Fig. 44). Average daily temperature (24 h period) ranged from 15 to 35°C from August 2016 to 7 February 2017 on days when fog occurred. The PI_{total} and average daily temperature of all three *A. horridus* hummocks on days with fog (18 July 2016, 01 November 2016, 11 November 2016, 29 November 2016, 16 December 2016, 06 February 2107 and 07 February 2017) were compared to the PI_{total} and average daily temperatures of the same plants during one of hottest weeks (29 March–04 April 2017), when there was no fog incidence. The results show a negative correlation between the average temperature and the overall vitality of *A. horridus* plants (Fig. 44). All three *A. horridus* hummocks had a significantly higher PI_{total} ($p < 0.001$) after cooler days when fog occurred compared to that on hotter days when no fog occurred.

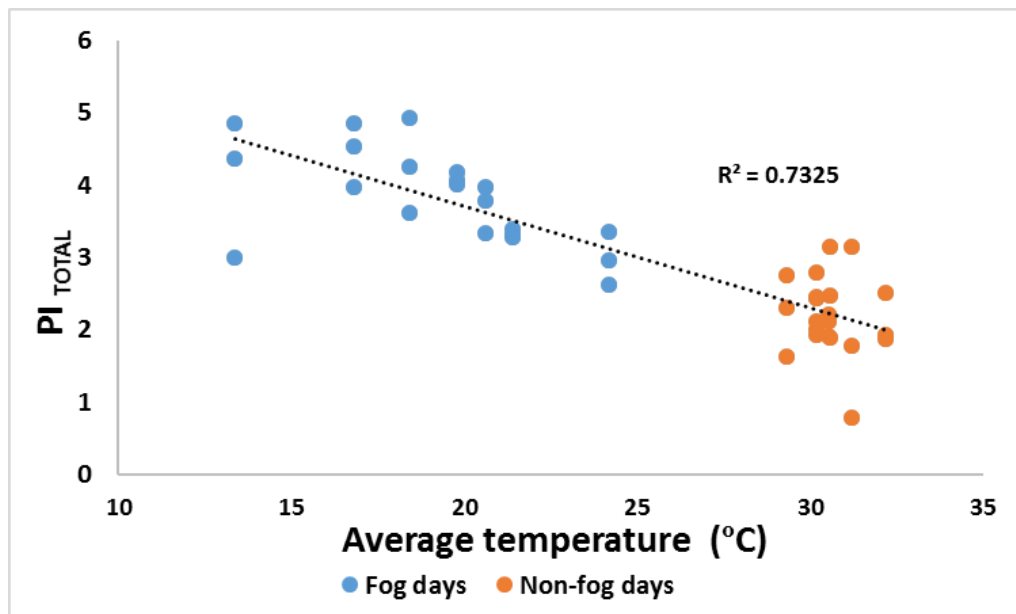


Figure 44. Correlation between average daily temperature and overall vitality of *A. horridus* plants over a period of 9 months.

Wind may exacerbate the already severely desiccating conditions of the Namib Desert. The average wind speed (Fig. 45) on 15 December (fog = 0) and 16 December (fog = 2) showed no significant difference. However, 17 December had a significantly higher wind speed ($p = 0.001$) than 16 December did as well as a significantly higher ($p < 0.001$) wind speed than 15 December did (Fig. 45). There was also a slight difference ($p = 0.045$) in the average wind direction on 15 December (west-southwest) and 16 December (west-northwest).

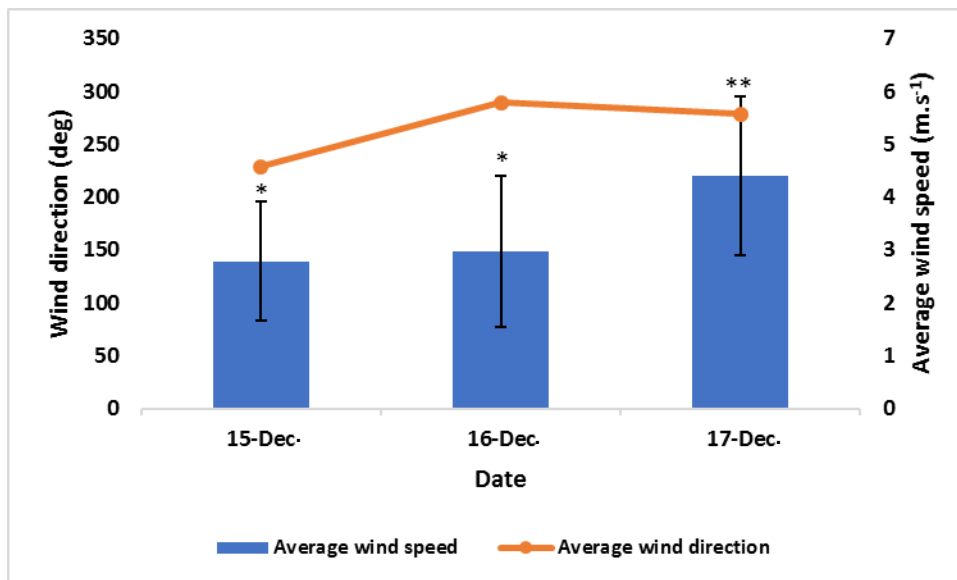


Figure 45: Average wind direction and speed on 15 December (fog = 0), 16 December (fog = 2) and 17 December 2016 (fog = 0); * indicates no significant difference in the average wind speed and ** indicates a significant difference in the average wind speed

4.9 Discussion

In the hyper-arid Namib Desert, plants are exposed to various environmental stressors that constantly change. Water availability is the most severe stressor, particularly as it is an important limiting factor in photosynthesis, plant growth and productivity (Van Heerden *et al.*, 2007). Therefore, rainfall pulses are essential (Eckardt *et al.*, 2013) and the ability of endemic Namib organisms to survive between rare pulse events can be linked to other sources of moisture, e.g. fog input (Soderberg *et al.*, 2014). It is therefore important to understand how an endemic plant such as *A. horridus* has adapted to tolerate and exploit rapidly changing environmental conditions in the Namib Desert.

PSII has a higher resistance to water stress than PSI does and a negative impact will only occur under persistent drought conditions (Kalaji *et al.*, 2016). Plants are known to adjust the energy distribution between PSII and PSI and to activate alternative sinks to protect the photosystems against desiccation. Results of this study indicate that on days with fog occurrence *A. horridus* plants had an increase in the F_0 (0.05 ms) value when compared to non-foggy days. This parameter represents the number of open PSII RCs or the number of Q_A in its oxidised state

(Martinazzo *et al.*, 2012). The increased F_0 value may indicate an excess excitation energy due to uncontrollable heat dissipation that ultimately results in irreversible damage to PSII (Martinazzo *et al.*, 2012). The fluorescence intensity at the I-step (30 ms) and P-step (F_M) exhibited significantly greater values on days with fog occurrence and parallels the partial and total reductions of the Q_B^- and Q_B^{2-} , respectively, and the accumulation of Q_A^- . This indicated a rapid increase in polyphasic ChlF, which reflects accumulation of the Q_A and PQ pools on foggy days (Van Heerden *et al.*, 2007; Martinazzo *et al.*, 2012). This accumulation on foggy days could indicate decreased electron transport of PSII. A decrease in F_M could be related to the denaturation of chlorophyll proteins (Kalaji *et al.*, 2016).

The OJIP transients for *A. horridus* plants revealed a K-step (0.3 ms). This change of the OJIP transient to a O(K)JIP transient is related to impaired oxygen evolution in PSII. The K-step represents the rapid closure of the PSII RC due to slower electron donation at the donor side of PSII (Kyseláková *et al.*, 2011; Campos *et al.*, 2014 Krüger *et al.*, 2014). The K-step is exhibited in several higher plants (e.g. *Cycas revolute*, *Permelia* sp. and *Juniperus* sp.) that are native to dry and hot climates. The K-step is sometimes associated with heat-induced partial inhibition of the OEC or changes in the structure of the antenna of PSII, resulting in altered energy distribution between the pigment protein complexes and the photosynthetic system (Srivastava *et al.*, 1997; Oukarroum *et al.*, 2012). The results obtained for *A. horridus* indicate that heat dissociated the OEC and decreased the number of electrons supplied to the PSII RC. The reoxidation of Q_A^- continued by transferring electrons to Q_B , but due to a decrease in electrons on the donor side the fluorescence intensity decreased again and formed the K-step (Srivastava *et al.*, 1997). This K-band visible in the OJ(K)IP transient of *A. horridus* could possibly indicate that the plant was exposed to continuous heat stress, resulting in changes in the structure of the PSII antenna complexes and could be a form of physiological adaptation. This alteration in the energy distribution might possibly protect the *A. horridus* plant against photodamage.

The photosynthetic process is the most sensitive to high temperatures, which result in changes in the reduction-oxidation properties of PSII electron acceptors by reducing the efficiency of

photosynthetic electron transport in both PSII and PSI (Kalaji *et al.*, 2016). Heat may destroy the donor side of PSII RCs, resulting in a reduction in the electron donation capacity of the RCs and a slower JI rise of the OJIP transient and the ΔK -band, as observed in *A. horridus*. Meteorological results indicate that there was no significant difference in average daily temperatures when comparing days with fog to days with no fog. However, maximum temperatures of above 35°C were recorded on all three days in December 2016, which exceeded the optimal temperature (23–25°C) for C₃ plants (Hopkins & Hüner, 2008). Ehleringer and Mooney, (1978) found that some desert C₃ shrubs can achieve high photosynthetic yields at temperatures greater than 40°C. It is possible that *A. horridus* has a slightly higher temperature optimum than C₃ plants in general and that it has special morphological adaptations to deal with increased heat stress (Chapter 4), but the plant is suspected to have physiological adaptations to survive in this hot and dry environment.

Thermal stress also causes significant changes in the OJIP transient intensity and is affected by the activity of the electron acceptor side of PSII and by the functional state of the donor side (OEC) of PSII (Strasser *et al.*, 2004). This results in an increased F₀ value and a decreased F_M value, with an additional ΔK -band at 0.3 ms (Oukarroum *et al.*, 2012). *Acanthosicyos horridus* did have increased F₀ and decreased F_M values on 15 December compared to those on 16 December, but a more distinctive ΔK -band was visible on the foggy day. This indicated that the *A. horridus* plant is exposed to thermal stress and that it might be able to adjust its energy balance by affecting the functional state of PSII. Drought is also known to influence the amplitude of the I-P phase of the O(K)IP curve and has been recorded as the slowest phase of the induction curve. The IP phase parallels the electron transport through PSI. Furthermore, the amplitude of the IP phase corresponds to the re-reduction of PC and is related to the PSI leaf content (Kalaji *et al.*, 2016). The results indicated that *A. horridus* was not drought stressed but that the presence of fog had some influence on PSI and resulted in an unknown Δ -band at 180 ms.

The difference in relative variable fluorescence revealed a hidden ΔK -band after fog events and on 17 December (fog = 0). This indicated a response to the equilibrium established for electron transport. A positive ΔK -band parallels an imbalance between the electron donor side of PSII and

electron acceptor sides (Strasser, 1997) and reflects the dissociation of the OEC, resulting in non-water electron donation to PSII, accompanied by a gradual decrease in the rate of photochemical processes (Strasser *et al.*, 2004).

Interestingly, the double normalisation of ΔV_{OJ} of the *A. horridus* plants revealed a distinct positive ΔK -band (0.3 ms) after fog events relative to that after non-foggy days. The ΔK -band is a known phenomenon in plants exposed to elevated temperatures with increased stem temperature and is influenced by variations in the energetic relationship between PSII. It is further considered to be due to the separation of the OEC and electron transport between pheophytin and Q_A . Moreover, Van Heerden *et al.* (2007) found that C_3 desert plants *Zygophyllum prismatocarpum* E. Meyer ex Sond. and *Augea capensis* also exhibited a ΔK -band (0.3 ms) during dry periods in a field investigation. In contrast, the same desert plants grown under drought conditions in a laboratory investigation did not exhibit this band. The ΔK -band, therefore, indicates heat stress and is parallel to the dissociation of the OEC (Van Heerden *et al.*, 2007), resulting in an imbalance in PSII between the electrons that leave the RC in the electron acceptor side and the electrons donated by the donor side (Campos *et al.*, 2014; Strasser, 1997). The meteorological data indicate that the *A. horridus* plants had a higher temperature on 16 December, when fog occurred, than on 15 December, a non-foggy day, resulting in a more pronounced ΔK -band on 16 December. This indicated that the presence of fog did not decrease the daily temperatures and that even if fog was present, other environmental factors played important roles in the photosynthetic potential

Furthermore, *A. horridus* plants had a distinct negative ΔK -band on 17 December (fog = 0) when compared to 15 December (fog = 0), even though 17 December had a higher maximum temperature (40.5°C) than 15 December did (35°C). The negative ΔK -band (0.3 ms) indicated that the OEC had an increased water electron donation to PSII and was accompanied by an increase in the rate of photochemical processes (Krüger *et al.*, 2014; Guissé *et al.*, 1995). This indicated an ability in *A. horridus* plants to recover the balance between the electrons at the acceptor and donor sides of PSII (Martins *et al.*, 2015). However, 17 December had a significantly

higher wind speed than 15 and 16 December did and, even though the air temperature was higher on 17 December, this increase in wind speed could possibly have resulted in the convective cooling of the *A. horridus* stems and therefore a lower operative temperature of the stems. This could further be the result of increased evaporative cooling due to increased stomatal conductance in response to the decrease in the boundary layer between the *A. horridus* stems and the atmosphere.

Plants are simultaneously exposed to heat and drought stress and Oukarroum *et al.* (2012) found that plants respond differently to a combination of environmental stressors than they do to a lone stressor. Interaction between heat and drought stress was found to result in enhanced thermostability of PSII (Oukarroum *et al.* 2012), which may be due to increased thermotolerance caused by an accumulation of organic osmolytes (proline and glycine betaine) observed in some plants under drought stress (Ashraf & Foolad, 2007). Such interaction may result in the disappearance of the positive ΔK -band. The formation of a negative ΔK -band in *A. horridus* plants on non-foggy days may therefore indicate increased thermotolerance on days when no moisture input occurred due to an increase in a secondary metabolite. Some members of the Cucurbitaceae family, including *A. horridus*, are known to have bitter compounds, known as cucurbitacins, and other secondary metabolites, which should be considered for future investigation.

The formation of a positive ΔI -band (5 ms) for *A. horridus* on non-foggy days paralleled the inhibition of the reduction of end electron acceptors, such as Fd_{red} and $NADP^+$, and is due to a higher reduced state of the PQ, Cyt b_6/f and PC pool (Yusuf *et al.*, 2010) or a may indicate lower activity of PSI (Krüger *et al.*, 2014). The ΔI -band reveals that the activity of PSII involves PSI-dependant control. Schansker *et al.* (2003) suggested that the positive ΔI -band may indicate the inactivation of ferredoxin- $NADP^+$ oxidoreductase (FNR). *Acanthosicyos horridus* had a hidden negative ΔI -band (5 ms) on 16 December (fog = 2) and this suggests increased activity of PSI in *A. horridus* plants on foggy days. The function of PSII is the most sensitive to temperature stress and changes in the daily temperature. Because this was a field study, it was difficult to focus on

the influence of a single abiotic factor. *Acanthosicyos horridus* was simultaneously exposed to different environmental conditions that might have had similar or opposite interactions with the plant. Only PSII was investigated and it is therefore possible that fog may have a more pronounced effect on the photosynthetic potential of PSI, but this was beyond the scope of this study.

The negative ΔH - and ΔG -bands are related to the function of PSI. These, together with the unknown Δ -band, could possibly be related to Cyt b_6/f complexes, resulting in increased re-oxidation of PQH₂, which leads to a decreased reduced PQ pool (Ilík *et al.*, 2006). The Cyt b_6/f complex controls electron transport from PSII to PSI while performing the Q-cycle (Laisk *et al.*, 2009).

The results for quantum yield indicated no significant difference between the F_v/F_M parameter between all three days in December 2016, with a value around 0.82 for all three days. The average F_v/F_M values for C₃ plant species growing under optimal conditions are approximately 0.83–0.85 (Pfündel, 1998) and stress conditions are known to significantly reduce these values. The results of this study indicate that *A. horridus* did not seem to be under severe stress and that fog did not influence the quantum yield, i.e. create more optimal conditions. However, Van Heerden *et al.* (2007) found that the F_v/F_M parameter decreased to a lesser extent than the PI parameter did in response to drought stress and was more sensitive to these changes than F_v/F_M was. Furthermore, Lawson *et al.* (2013) suggested that the unaffected F_v/F_M value demonstrated protection of the photosystems through photorespiration. It is possible that *A. horridus*, like *Rhazya stricta*, can increase photorespiration in response to an increase in air temperature. Photorespiration acts as mechanism to dissipate excess excitation energy when internal CO₂ concentrations are low by acting as an energy sink for the end products of the photosynthetic ETC.

Overall, measurements of ChlF did not seem to indicate symptoms of drought stress in *A. horridus*, away from the Kuiseb River, and indicated that the *A. horridus* plants were connected to a consistent underground water source. It is possible that the water supply in sandy soils exerts

control on stomatal conductance in C₃ succulents, including *A. horridus*. This can only be confirmed by stomatal conductance measurements, that was not measured during the scope of this study. The strong diurnal fluctuations in the leaf water potential, in soil with abundant moisture, is possibly the result of active daytime transpiration. This relatively high seasonal water potential and might be the result of a deep taproot system that can reach underground water. These types of species exhibited drought-avoiding attributes and water wasting characteristics (Monson and Smith, 1982). Plants with these attributes avoid drought stress through rapid water absorption.

The positive ΔK -band on days with fog input might indicate that the plants' operative temperature and soil temperature might increase, e.g. Balghouthi *et al.* (2005) found that wet soils had a higher surface temperature during the day and a lower surface temperature at night when compared to dry soils. It is possible that fog and other NRWIs cause heat stress in *A. horridus* plants, i.e. they may deteriorate when receiving more water input and would do best in very dry and hot environments.

Increased temperatures, higher than the optimum, result in a decrease in physiological and metabolic processes of C₃ plants. Furthermore, the response of carbon metabolism to elevated temperatures is believed to be the main limiting factor for photosynthesis (Lawson *et al.*, 2013). Increased temperature will indirectly influence carbon metabolism by decreasing stomatal conductance and reducing carbon assimilation (Lawson *et al.*, 2014). Temperature can also directly influence carbon metabolism by affecting the activity of several enzymes of the Calvin cycle particularly, namely rubisco activase. This enzyme plays an important regulatory role by removing sugar phosphates from the rubisco catalytic site and can also influence photosynthetic performance. Rubisco specificity is sensitive to temperature changes and the specificity for CO₂ decreases and the water solubility of oxygen (O₂) increases, resulting in photorespiration to avoid photodamage (Lawson *et al.*, 2014). It is also known that increased temperature reduces photosynthetic electron transport due to PSII damage. Plants may have certain traits that enable them to tolerate heat stress (Lawson *et al.*, 2013).

This relationship between photosynthetic potential and heat stress was clearly visible in the decrease in the PI_{total} in response to increased temperature, indicating that temperature stress decreases the overall vitality of *A. horridus* plants. However, the results further suggest that *A. horridus* is acclimated to high temperatures and that temperature stress does not result in lasting photoinhibition. The decrease in photosynthetic potential with increased temperatures is, therefore, rapidly reversible.

Other abiotic factors like wind speed can be beneficial for *A. horridus* by decreasing stem temperature through evaporative cooling. The water potential measurements (Chapter 4) were lower for the *A. horridus* shoots in the dominant wind direction and could further indicate increased evaporative cooling due to increased stomatal conductance. This would be beneficial for *A. horridus* by decreasing the stem temperature and reducing heat stress. However, dust storms can also result in excessive dust cover on desert shrubs, which can induce heat stress effects due to dust particles absorbing more near-infrared solar irradiance, together with decrease in the evaporative cooling of the leaf surfaces (Sharifi *et al.*, 1997).

4.10 Conclusion

Acanthosicyos horridus is exposed to extreme environmental conditions that change constantly. *A. horridus* is a C_3 plant that generally seems to have a higher temperature optimum than that of other C_3 plants. ChIF indicated that *A. horridus* plants are tolerant to heat stress and that the negative effect of temperatures above 40°C on the photosynthetic potential is reversible. Fog precipitation may influence PSI and should be further investigated, together with gas exchange.

CHAPTER 6: SYNOPSIS AND FUTURE PROSPECTS

5.1 Introduction

This study investigated the adaptations of an important keystone species, *Acanthosicyos horridus*, to highly variable abiotic factors within its Namib Desert habitat. This study focused on the adaptations and strategies that are important for the survival of desert plants, in particular those that allow them to cope with extreme aridity, rare and unreliable rainfall events and high temperatures.

5.2 Principal findings

The hypothesis that the exposure of *A. horridus* to environmental stress, specifically heat, will result in decreased photosynthetic potential was supported by the negative correlation between air temperature and overall plant vitality. However, *A. horridus* seemed to be heat tolerant, as heat stress did not result in irreversible damage to the photosystems. Furthermore, the plants were able to recover their photosynthetic potential after exposure to air temperatures above 40°C. It is also possible that a decrease in the stem water potential could result in enhanced thermostability of PSII. This is possibly due to the accumulation of organic osmolytes as the stem water content decreases. Wind was found to have a positive effect on photosynthetic potential by decreasing the stem temperature through convective cooling. This increase in wind speed is suspected to result in a decrease in the boundary layer between the stem and atmosphere, increasing stomatal conductance and evaporative cooling of the *A. horridus* stems.

The assumption that the structural shoot features of *A. horridus* might enable retention and absorption of fog precipitation was supported by the time-lapse photography and the aerial absorption test. The grooves on the stem of *A. horridus* enable directional water movement along the stem. Furthermore, the hydrophobic wax layer and trichomes on the stem of *A. horridus* make the surface more wettable, which allows water retention on the stem surface after a fog event. However, the pathway for water absorption is still unknown and should be further investigated.

The assumption that the photosynthetic potential is improved by direct aerial absorption of fog precipitation could not be confirmed during this study due to conflicting results. Results indicated that *A. horridus* showed signs of heat stress and dissociation of the OEC on the day of a fog event. It is possible that the increase in the stem water potential on days with fog events resulted in a decrease in the concentration of stem osmolytes, resulting in a decrease in thermostability. In contrast, it is possible that the increase in the shoot water potential after fog absorption may result in increased stomatal conductance and, therefore, an increase in evaporative cooling. This increased stomatal conductance would also lead to increased carbon assimilation and ultimately increased biomass production. However, this has not been proven and gas exchange measurements should be considered for future research.

5.3 Future studies

A major limitation in this study was the lack of information available on the root system of *A. horridus*. Further investigation is needed to determine whether *A. horridus* has surface root hairs that assist in the absorption of fog precipitation. The indirect influence of fog events and other NRWIs still needs to be investigated through gas exchange measurements, including the VDP of the atmosphere during a fog event.

Another major limitation of this study was the lack of gas exchange measurements. Future investigation on *A. horridus* -fog interaction should combine photosynthetic data with gas exchange measurements as well as further investigation into the effect of NRWIs on PSI. Gas exchange measurements would provide valuable information on the stomatal response to NRWIs

In addition, another limitation in this study was that plants in the natural environment are exposed to several stresses simultaneously, such as the *A. horridus* plant that is exposed to conditions of heat stress and high light intensity. It is, therefore, difficult to identify the effect of a single stress factor using ChIF and different stress factors may yield similar effects on the photosynthetic system. It would be valuable for future investigations to consider exposing *A. horridus* to a single

environmental stressor in a greenhouse setting and to compare this to plant responses in the natural environment.

There are *A. horridus* plants that occur outside the fog belt in Namibia and it would be valuable to repeat this study on different *A. horridus* populations along a fog gradient. Fog is the highest at the coast (west) and decreases towards the interior (east). This could provide information on population dynamics and evolutionary adaptations.

Although the value of this unique plant is long-recognised, some fundamental questions need to be investigated to exploit it as an agronomic field crop. This project is part of a broad, transdisciplinary programme at GTRC. Addressing this issue of understanding the fog-capturing abilities of *A. horridus* to understand its moisture requirements, and how the habit of individual plants may guarantee their survival in one of the most extreme environments on earth will enable cultivation of the *A. horridus* plant. Cultivation will secure a threatened resource, and allow for up-scaling of harvesting and processing of this indigenous natural product that will provide income-generating opportunities for local people. The *!Aonin*, associated with *A. horridus* for centuries, are historically disadvantaged and still disenfranchised, while their livelihood options are strongly curtailed as their traditional land is included within a national park.

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