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The effects of cloud moisture on Restios, Ericas and Proteas in the Cape Floristic Region.

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Abstract

Recent studies on the interception and utilization of occult precipitation (fog, cloud-borne mist and dew) have revealed that the direct wetting of foliage provides a water subsidy to plants of various ecosystem types. In this study, we investigate the presence of foliar uptake, and the effects of misting on the plant water potential of species representing diverse functional types, namely ericoids, proteoids and restioids in Fynbos species occurring within the Cape Fold mist belt. In this study, foliar uptake after 180-min submergence in distilled water was demonstrated by five of the seven species investigated. These species included all the restioids and ericoids investigated in this study. By contrast, the proteoids *L. conocarpodendron* and *L. laureolum* were found to show no significant amount of foliar uptake or increased leaf water content (%). There was an increase in the average, normalized leaf water content in individuals subjected to misting treatments in both proteoids, *L. laureolum* and *L. conocarpodendron.* Similarly, there was also an overall increase in plant water status, as shown by the increased water potential in individuals that were subjected to the misting treatment. It was found that control individuals showed a decrease in plant water potential (i.e. lost water) during the day, as can be expected when soil water is not replenished. All species showed significant stomatal conductance, during both night and day. Results indicate that misting events have a significant effect on the overall plant water status in all functional types and the presence of foliar uptake in both ericoids and restioids; thus indicating that cloud events may have an important effect on the vulnerability of these species to drought, under the precepts of global climate change.

Introduction

The distribution and survival of vegetation types, such as Mediterranean-Type Ecosystems (MTEs), are strongly controlled by climate (Cowling *et al.*, 1997). Climate predictions for the Cape Floristic region (CFR) indicate a potential increase in the frequency and intensity of drought in these regions, which is of particular concern as the absence of prolonged drought in the CFR may contribute to the presence and maintenance of plant diversity in this region (Hewitson *et al.*, 2005; Midgley *et al.*, 2005; Hewitson and Crane, 2006; IPCC, 2007). Major developments in climatic models afford researchers some certainty when attempting to predict future rainfall availability in these areas. However, the amount of water available to plants within a system is probably not limited to precipitation alone (Breshears *et al.*, 2008).

In the past, rain and snow were considered to be the only types of precipitation contributing to the terrestrial ecosystem water balance and productivity (Mather and Yoshioka, 1968; Limm *et al.*, 2009; Stephenson, 1990). Recent studies on the interception and utilization of fog and/or mist (Simonin *et al.*, 2009; Gouvra and Grammatikopoulos, 2003; Limm *et al.*, 2009; Breazeale *et al.*, 1950; Burgess and Dawson, 2004) have revealed that misting is a significant contributor to climate in a number of terrestrial ecosystems (Weathers, 1999), including coastal mountain regions such as those found in the Cape Peninsula (Burgess and Dawson, 2004).

The primary reason why cloud itself is not currently captured in climate descriptions or climate change models is that cloud droplets are not prone to precipitating out of air, because of their small size and relative mass (Prada and da Silva, 2001). Cloud droplets do not settle on horizontal surfaces, unless intercepted by terrestrial surfaces, as their vertical settling rate is too slow (Burgess and Dawson, 2004). In the event of misting in certain regions of the Cape Peninsula, however, mist is often accompanied by a south-easterly wind. These moisture droplets are blown against the vegetation occurring in the area, where they may coalesce to form larger drops and subsequently fall to the ground, infiltrating the soil (Cavelier and Goldstein, 1989.)

Stem flow?

The wetting of leaves and plant crowns with fog, cloud-borne mist and dew, aptly known as occult precipitation, may provide a water subsidy to ecosystems and can contribute to the overall plant water budget of several species with varying phylogeny, even where soil moisture may not noticeably increase (Boucher *et al.*, 1995; Burgess and Dawson, 2004; Breshears *et al.*, 2008; Ewing *et al.*, 2009). In some cases species may be perfectly adapted to utilizing mist events as a moisture input into the soil, especially where plant morphology enhances the "drip" effect. In addition, misting that occurs either during periods of increased night-time or day-time stomatal conductance appears to benefit all species, due to a suppression of transpiration by the thin water layer that envelops the leaves (Benzing *et al.*, 1978; Barradas and Glez-Medellin, 1999). During night-misting, this facilitates efficient foliar rehydration with xylem water originating from the root zone (Limm *et al.,* 2009).

Misting has several potential effects on plant foliage since it eliminates a large proportion of the atmospheric vapour pressure deficit (VPD) in the air. VPD drives evaporation and transpiration from plant surfaces and these reductions would limit plant transpiration (Burgess and Dawson, 2004). In addition, as the south-easterly wind drives the mist droplets through the vegetation, fog water builds up and wets leaf surfaces (Burgess and Dawson, 2004), and moisture may accrue on plant surfaces beyond the leaf's storage capacity, which would result in dripping from leaves onto soil or the moisture being funnelled to soil via stem flow (Hutley *et al.*, 1997; Burgess and Dawson, 2004). In essence, the presence of misting during summertime drought has been shown to reduce transpiration, increasing soil water content through fog drip from canopy to soil (Azevedo and Morgan, 1974; Dawson, 1998; Ewing *et al.,* 2009). It can also contribute to overall plant water status through direct uptake of fog water deposited on leaf surfaces (Burgess and Dawson, 2004).

These changes in water potential within a plant may greatly affect metabolic function and plant growth by increasing solute transport and photosynthetic rates, even when the change in water potential is small (Burgess and Dawson, 2004; Simonin *et al.,* 2009).
Although cloud interception only provides small quantities of water to the overall water budget of the plant (Monteith, 1963; Limm *et al.*, 2009), this could still provide an essential subsidy to plants, especially if direct foliar uptake occurs into plant leaves. This uptake of water directly into the plant from the atmosphere immediately increases the leaf water content and plant water potential (Grammatikopoulos and Manetas, 1994; Boucher *et al.*, 1995; Yates and Hutley, 1995, Limm *et al.*, 2009).

Foliar uptake has been shown to enhance gaseous exchange in leaves (once dry) (Grammatikopoulos and Manetas, 1994; Martin and von Willert, 2000; Simonin *et al.*, 2009), and increase the rates of plant survival in otherwise arid systems (Stone *et al.*, 1956). In light of the positive influence of foliar uptake on plant water acquisition, this strategy allows plants to occur in seasonally-dry ecosystems, such as the Mediterranean ecosystem investigated in this study. In addition, foliar uptake is not limited to any specific phylogenetic lineage, or ecosystem type (Grammatikopoulos and Manetas, 1994; Yates and Hutley, 1995; Boucher *et al.*, 1995; Martin and von Willert, 2000; Burgess and Dawson, 2004; Oliveira *et al.*, 2005; Breshears *et al.*, 2008; Simonin *et al.*, 2009).

There are several different functional morphologies occurring within this region, all of which are likely to have variable responses to drought. These functional groups are represented by large, taxon-specific groups including the tall and deep-rooted proteioid shrubs, the fine-leaved and shallow-rooted ericoid shrubs and finally, the graminoid restioids (Stock *et al.*, 1992). Recent work shows the different response of Proteas, Ericas and Restios to drought; this hints at the importance of cloud moisture in these areas. In attempts to unify the theory surrounding drought induced plant responses, several studies have argued for a "hydraulic strategy" as a key variable in determining the vulnerability of the plant to mortality under drought conditions (West *et al.*, 2012; McDowell *et al.*, 2008; McDowell, 2011). In the context of Fynbos, the variety in plant functional types may result in variable drought responses, and should thus take into account not only growth form, but also the water potential regulation strategy, as investigated by West *et al.* (2012). The water potential regulation strategies that were investigated in the latter study are those associated with plant functional types represented by large, taxon-specific groups; while the shallow-rooted, anisohydric ericoids, the shallow-rooted, isohydric restioids and the tall, deep-rooted isohydric proteoids (Stock *et al.*,1992) were indicated as being likely to show variable responses to drought.

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The study yielded somewhat surprising results, in that some species (namely the ericoids and Restioids) which were predicted to be particularly vulnerable to drought, especially due to their shallow rooting depth, showed resistance. It was found that the shallow-rooted, isohydric restioids, such as *Staberoha cernua* showed no significant die-off response due to the drought. These results, in conjunction with observed increases in soil moisture content after a cloud (misting) event (Figure 1, cloudtips), were hypothesized to indicate that summer misting events may alleviate the water stress associated with extended periods of time when there is limited rainfall. In addition, the soil moisture was retained for longer and at higher concentrations in soil below *S. cernua*, than within bare ground. These results indicate that occult precipitation (fog, dew and cloud water inputs), although not traditionally quantified in physiological experiments, may well serve as an important source of water (Dawson, 2006).

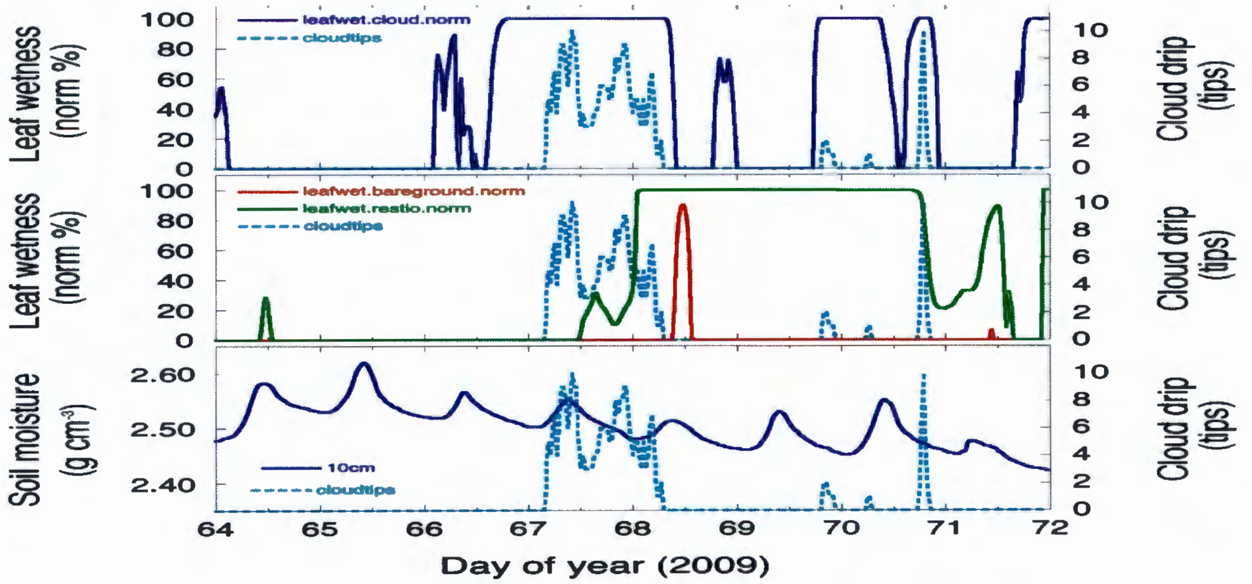

Figure 1: Data obtained from leaf wetness sensor inserted below Staberoha cernua and in bare ground in a drought study in Silvermine. Cloud drip, soil moisture and "leaf wetness" (another measurement of soil moisture) indicated above.

Limm *et al.* (2009) indicated that many studies of foliar uptake could not be considered comparative, as they did not investigate whether foliar uptake was a water acquisition strategy common to a range of diverse and co-occurring species within a single ecosystem. Given the findings of the latter study, and the potential utilization of analytical techniques applied therein, the current investigation considers the importance of cloud moisture inputs into the Fynbos system, especially at the level of individual species and across the distinctly different growth forms of ericoid, proteoid and restioid,.

Our investigation is an attempt to determine whether ericoid, proteoid and restioid species occurring in the Cape Floristic Region are able to 1) take up cloud moisture directly through their leaves, i.e. exhibit foliar uptake, and 2) increase shoot-water potential during episodes when the whole plant is exposed to misting. These increases in shoot-water potential indicate improvements in overall water status of the plant and can occur even in species without the capacity for foliar uptake, because leaf wetness can suppress both day-time and night-time stomatal conductance (transpiration suppression). We hypothesize that misting events can have a significant, positive effect on the plant water status of most functional types occurring within Fynbos and that each functional group will be affected in different ways. In order to test this hypothesis, the foliar uptake capacity, plant water potential and day-time and night-time stomatal conductance of the three species were investigated in this study.

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Materials and methods

The seven Fynbos species investigated in this experiment were collected from the Kirstenbosch nursery. All species were grown under similar conditions and were selected based on their natural prevalence at altitudes and locations frequently exposed to south-easter misting events in summer. All plants were initially grown outdoors and upon collection from the nursery, were transported directly to the Phytotron at the Botany department of the University of Cape Town. The species investigated in this study were intended to demonstrate the effects of misting on each of the functional types (restios, ericas and proteas) occurring within Fynbos, with two or three species representing each functional group.

The restio species included *Restio multiflorus* (Spreng.), *Restio bifurcus* (Nees ex Mast) and *Staberoha cernua* ((L.f.) T.Durand and Schinz). *R. multiflorus* is typically distributed on the mountains of the south-western Cape, from Piketberg to Bredasdorp, while *R. bifurcus* occurs from Malmesbury to the Cape Peninsula and Shaw's Mountain (Haaksma and Linder, 2000). *Staberoha cernua* was selected as it was also studied by West et al. (2012) but it typically occurs from Ceres and Piketberg to the Cape Peninsula, the Swartberg and Kouga mountains. Erica species included *Erica imbricata* ((L.) D.Don) and *Erica baccans* (L.), both of which are relatively widespread throughout the Cape Peninsula, occurring on mountain slopes in the area (Manning, 2007). *Leucadendron laureolum* ((Lam.) Fourc.) and *Leucospermum conocarpodendron*((L.) H. Buek subsp. *conocarpodendron*) were chosen as representatives of proteoid families as these species were commonly found on location West *et al* (2012).

After purchasing, plants were carefully transplanted into similar sized pots ~~of soil~~ and allowed to acclimate to phytotron conditions of a 14-hour summertime photo-period. The temperatures for day-time and night-time were 20-24 °C and 13-15 °C, respectively. All plants were placed in the same phytotron for the duration of the experiment, in order to minimize variation in environmental conditions. The plants were allowed to acclimate to these conditions for a minimum of three days before any experiments were initiated and soil water availability was standardized by providing 500ml of water to each plant per day for the first five days, after which all plants were allowed to dry-down for two days prior to the commencement of each experiment.

Three primary measurements were taken for this study:

1) Foliar uptake capacity experiment, conducted on six individuals of each species.
2) Day-time and night-time stomatal conductance measurements, both of which were conducted on five individuals of each species.
3) Fog-exposure experiment, conducted on six individuals of each species, three of which were subjected to the treatment (fogging) and three of which were excluded from the treatment (control).

Foliar uptake capacity

The capacity for foliar uptake of each species in this study was measured by calculating the amounts (mg. cm^{-2}) and percentage (%) of water that was absorbed directly into the leaves, shoots and/or photosynthetic culms excised from each plant (Limm, 2009). South-easter misting events can last for several days (Figure 1). In order to simulate the typical conditions for fog exposure and anticipate the greatest potential effects of misting , the foliar water uptake experiment was conducted after two hours of post-sunrise light.

Photosynthetic material from each species was excised according to the functional type they represented. In species where it was possible (*L. conocarpodendron* and *L. laureolum*), a single mature leaf was excised from each plant. In the case of restios a single mature photosynthetic culm was excised from six individuals of each species (*R. bifurcus, R. multiflorus* and *S. cernua*) and where Ericas were investigated (*E. imbricata* and *E.baccans)* a terminal shoot, including the stem and mature leaves, was sampled. Only the youngest, fully mature leaves of each species were excised as this was intended to control for the process of cuticle degradation, brought about over time by chemical and physical weathering, i.e. environmental exposure (Mechaber *et al.* ,1996; Limm *et al.*, 2009).

Once the starting mass of the leaf was measured ($Mass_1$), the whole leaf, culm, or shoot was submerged in distilled water. This was done to account for large amounts of variation in leaf/ shoot morphology and to standardize water availability among all species. Each section of foliage was submerged in separate 200ml glass beakers, with the sealed end of the petiole, stem or culm clipped to the side of the beaker, above the waterline. All leaves were submerged in the water for a total of 180 min. under normal light conditions. This amount of time was indicated by Limm et al. (2009) to be sufficient to allow for absorption of water through foliar uptake, which would be indicated by an increase in leaf water content (i.e. greater leaf mass post-wetting). Subsequent to submergence, foliage was removed from the water and patted dry thoroughly with paper towels. Each section of foliage was then reweighed, and the mass was recorded (Mass $_2$). This was done in order to determine the amount of water that the foliage absorbed. Foliage was then air-dried briefly, in order to allow for the evaporation of any remaining surface water. This surface dry foliage was then weighed again (Mass $_3$), briefly resubmerged in the water, towel dried again and immediately re-weighed (Mass $_4$). This was done in order to account for any measurement error associated with the original towel-drying method. Any increases in mass associated with the rewetting of the foliage was taken to account for the residual water on the leaf surface, as such a brief rewetting period did not allow sufficient opportunity for any additional water absorption. Nice -

The amount of foliar uptake ($mg.cm^{-2}$) was calculated for each leaf by investigating the change in leaf mass after submergence, whilst also taking precautionary steps to account for the residual water remaining on the leaf. This was done as in Limm et al. (2009) using the equation:

$$Foliar\ uptake\ (mg) = \ (Mass_2\ - Mass_1) - (Mass\ _4 - Mass_3\ )$$

In order to standardize the foliar uptake per unit leaf surface area, it was necessary to take the projected leaf/ culm/ shoot area into account. This was done using the software program ImageJ (US National Institutes of Health, Bethesda, Md), taking care to include only sections of the foliage that were submerged. This allowed a comparison of leaf water uptake in mg of water taken up per cm^2 of leaf area. A t-test (α=0.05) was used in order to determine whether the foliage from each species showed foliar absorption that was significantly higher than 0mg water/ cm^2. If significantly different, the leaf was considered to exhibit at least a limited capacity for foliar uptake (STATISTICA7, StatSoft, Inc. 2007).

In order to determine how leaf water content is influenced by foliar uptake, the increase in leaf water content (%LWC) was calculated for each submerged leaf/ culm/ shoot, using the following equation:

$$\% LWC = \left[\frac{(Mass_2 - (Mass_4 - Mass_3) - Mass_{Dry})}{(Mass_1 - Mass_{Dry})} - 1 \right] * 100$$

In this equation, MassDry accounts for the dry mass of the foliage after drying at 65 degrees C for 72h; all other variables are as described above. A single-sample t-test was again used in order to determine whether the increase in leaf water content was significantly greater than 0% after submergence.

Daytime and night-time stomatal conductance:

The average rates of both day- time and night-time nocturnal conductance ($_{sn}$) were measured on five plants of each species, using an Infra-Red Gas Analyzer (Li-Cor 6400 Portable Photosynthesis System, Li-Cor Europe), with a fluorescent head. This was done in order to determine two things; whether any or all of the species lost water via night-time transpiration, and what the rates of daytime stomatal conductance were in each species. Night-time stomatal conductance measurements were made after three hours of post-sunset darkness in the phytotron (23h00); whereas daytime conductance measurements were made between 10h00 and 15h00, as this was expected to provide valuable information regarding an interpretation of the results for the water potential experiment. In the restioids, *S. cernua, R. bifurcus* and *R. multiflorus*, multiple culms were measured in parallel, in an attempt to fully cover the IRGA aperture for the duration of the readings. In the restioids, *E. baccans* and *E. imbricata,* a single shoot bearing a multitude of leaves was measured. The total projected leaf area included within the aperture for each measurement was calculated using ImageJ. All "corrected" data was then investigated by calculating the mean individual plant conductance (as 10 measurements were taken for each reading), and the species' mean was subsequently calculated using these individual averages. A single-sample t-test (α = 0.05) was used in order to identify any significant amount of stomatal conductance (above 0mmol.cm^{-2}.s^{-1}). In addition, a one-way ANOVA was used in order to determine whether species differed significantly from one another in terms of their potential water loss (i.e. conductance) (STATISTICA7, StatSoft, Inc. 2007).

Fog exposure

In order to measure how day-time misting affected the overall water status of the plants, a comparison was made between plants exposed to misting (treatment) and other individuals of the same species that were not (control). All plants were otherwise subjected to similar conditions, having all been subjected to the same phytotron conditions before the treatment began. Treatment (+ fog) and control (-fog) conditions were imposed on the (potted) plants for 16 hours (between 06h00 and 22h00), in order to both maximise misting effects and provide a realistic representation of a natural misting event (as found by West et al. 2012). Plants in the "treatment" group were placed in a 2m x 1.5m x 1m chamber, created from PVC pipes, with the joints and frame of the chamber covered in clear PVC sheeting (Figure 2).

Figure 2: Misting chamber created for the purposes of this study.

The chamber contained two computer fans mounted on retort stands, in order to circulate the air and mist within the chamber. All sides of the chamber could be closed by means of a flap, and all piping and cables etc. were carefully insulated details to preserve the integrity of the experiment. In addition to each computer fan, a data logger was mounted on each retort stand in order to verify that the surroundings within the treatment chamber remained fully water saturated. Three of the plants were randomly assigned to the "with-fog" treatment, while three were excluded from the fogging treatment. The misting chamber contained the allocated plants, as well as a 5-disk ultrasonic mist-generating device (Chaoneng Electronics, ex Nanhai, Guangdong, China), capable of producing 400ml/ hr. The mist generator was furthermore placed in a flotation device, in order to ensure that it remained submerged in sufficient water to allow it to function (approximately 7cm required). This device was submerged in a 121 bucket of water, which was fitted with a hosepipe and topped up every four hours.

Plant water potential measurements were made on three leaves/ culm and/ or shoots of each individual plant (excl. *L. conocarpodendron,* which had only a single usable shoot, and *L. laureolum,* which often allowed for only two measurements prior to and two measurements post the misting event). All water potential measurements (Ψ_{wp}) were made using a Scholander pressure chamber (PMS instruments, Corvallis, OR, USA). As such, only a single water potential measurement was taken from each *L. conocarpodendron* individual, and comparisons were made between the water potential of treatment and control plants, after the misting events.

Foliage sampling and plant measurements were conducted within an eight hour interval (between 22h00 and 06h00 hours), and were completed just before the start of the day-time treatments (with and without fog). These measurements were taken in two hours post-sunset darkness in order to minimize water potential variability associated with midday transpiration and photosynthesis. After finishing these measurements (for the pre-mist data), the side of the treatment chamber was closed and sealed, the fans connected to a 6V battery and the ultra-sonic mist generator was switched on. This misting "event" was simulated for 14 hours.

After the fogging event, the side of the misting chamber was opened briefly, in order to remove individual plants for sampling. After removing a plant, the mist generator was switched on for approximately two minutes, in order to ensure that relative humidity remained the same within the chamber. Care was taken to ensure that each section of the foliage to be excised was patted dry with tissue paper in order to minimize the effects of pressure-induced water uptake by the leaf/ culm/ shoot. All individuals were re-sampled at the same time as the previous night, in order to assess the plant water status (and leaf water content in the case of the proteoids).

The effect of misting on plant water status was assessed by investigating the raw plant water potential for both the treatment and control individuals, pre- and post- misting. These raw results were then analyzed utilizing multiple independent t-tests, in order to determine whether there was a significant difference between pre- and post- misting individuals, both for the treatment and the controls. In addition, the change in plant water potential from pre-mist to post-mist was determined according to the following equation:

$$\psi_{diff} = \psi_{post} - \psi_{pre}$$

where pre- is the water potential of all individuals before the misting treatment (excluding *L. conocarpodendron*) and post- is the water potential of all individuals after the misting treatment (including *L. conocarpodendron*). The magnitude of this change between treatment (+mist) and control (- mist) was assessed for individuals of each species using multiple, independent t-tests.

Synthesis

In order to determine the effects of foliar uptake, day-time and night-time stomatal conductance on the plant water status, multiple regression analyses were performed (STATISTICA7, StatSoft, Inc. 2007). These analyses were intended to indicate the relative importance of foliar uptake and suppression of transpiration in each species, for individuals subjected both to the control and to treatment effects.

Results

Foliar uptake capacity experiment

Foliar uptake was established to occur in five of the seven species investigated in this study, subsequent to the submergence of their foliage in distilled water for a period of 180-min. These species (*E. imbricata, E. baccans, R. bifurcus, R. multiflorus and S. cernua)* showed significant ($p<0.05$) levels of water absorption into the leaves, indicated by changes in both the percentage of leaf water content (Fig. 3a) and direct foliar uptake in mg per unit leaf area (mg. cm^{-2}) (Figure 3b). Interestingly, these species encompassed all the ericoids and restioids investigated in this study. In contrast, the proteoids *L. conocarpodendron* and *L. laureolum* were found to show no significant increases in the foliar water content and no differences were found, whether the foliage was pubescent (h) or smooth (nh).

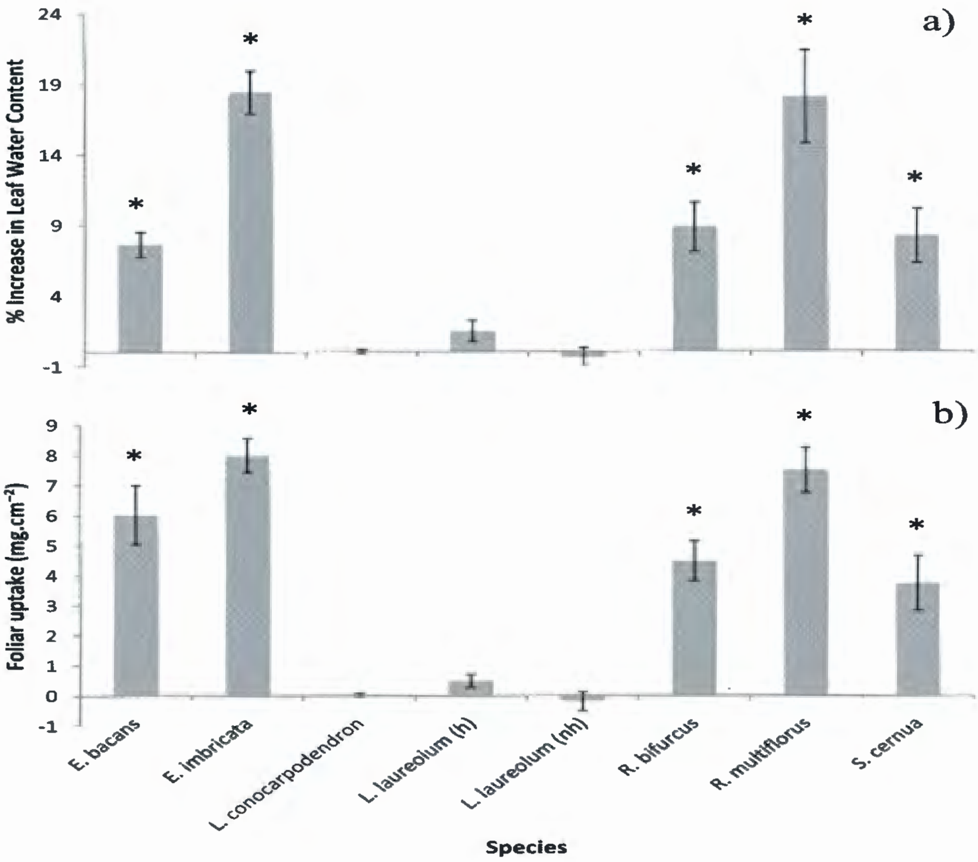

Figure 3 a, b Direct absorption of water by leaves, including hairless (*L. laureolum-* nh and *L. conocarpodendron*) and pubescent (*L. laureolum-* h) proteoid leaves, photosynthetic shoots (ericoids) or culms (restioids) of each species after being submerged in distilled water for 180min (n = 6). a) Average (±SE) % increase in leaf water content. b) Average (±SE) foliar uptake (mg) of water per unit of leaf area (cm^2). Asterisks indicate water absorption significantly different from the 0mg/cm^2 or 0% reference constants, as determined in multiple one-tailed t-tests (α=0.05, df = 5).

Daytime and night-time stomatal conductance

There was considerable variation in stomatal conductance, both during the day (Figure 4a) and during the night (Figure 4b). All species showed a stomatal conductance greater than 0 mmol.cm^{-2}.s^{-1}. The restios *R. multiflorus* and *R. bifurcus* displayed surprisingly high night-time stomatal conductance. Overall, *R. multiflorus* was found to have the highest levels of stomatal conductance, which were significantly different to all other species (Daytime $p<0.05$, df= 294, MS=6558.6 ; Night-time $p<0.05$, df=326, MS=2426.9). *R. bifurcus* also showed relatively high stomatal conductance in both daytime and nighttime measurements.

E. baccans was found to show the greatest variability in stomatal conductance, despite having a significantly different daytime conductance to all other species ($p<0.05$, df=294, MS=6558.6).

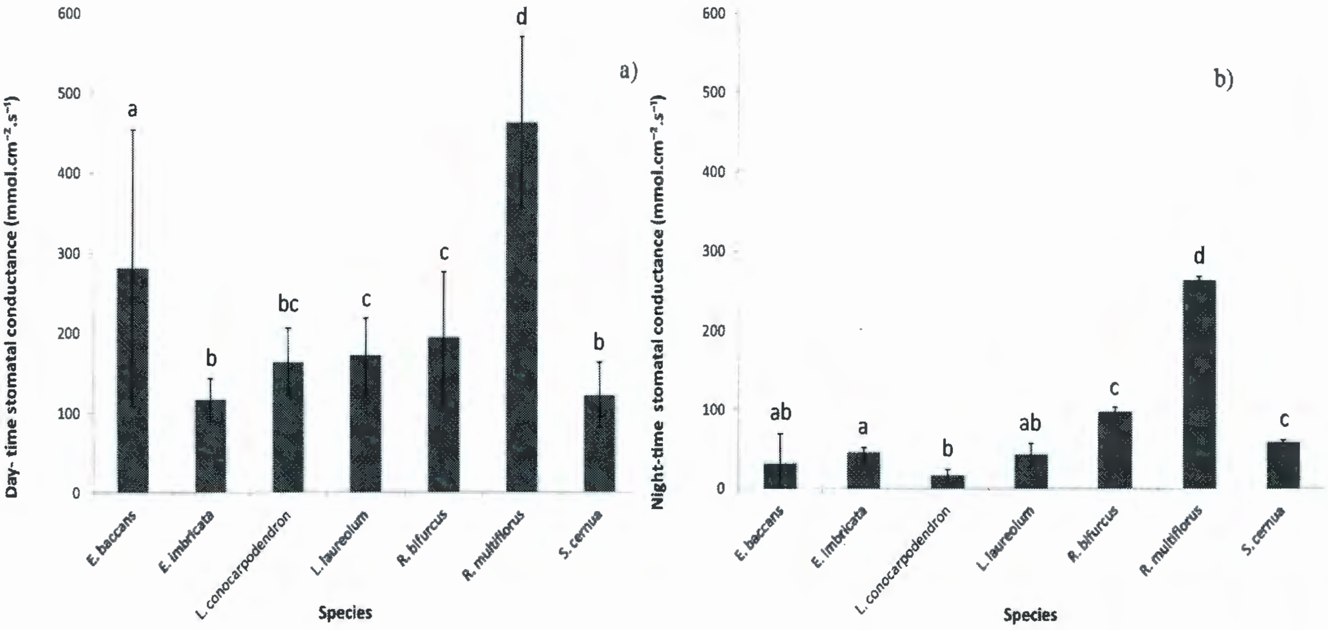

Figure 4 a, b The mean a) day-time and b) night-time stomatal conductance for all species. Each species showed conductance rate which was greater than the 0mmol $H_2O.m^{-2}.s^{-1}$ as determined by a t-test. ANOVA results indicated a significant difference between species, with Tukey-HSD Post-Hoc grouping (α=0.05) indicated by the letters (a, b, c, d).

Fog-exposure experiment

Plants subjected to the misting treatment showed an immediate increase in plant water potential from their initial state, taken before (pre-mist) and after (post-mist) the event (Figure 5 a, b). All species were found to display significantly different ($p<0.05$) pre- and post- mist plant water potential (ψ) for treatment plants, whereas no significant difference ($p>0.05$) was found to occur between the pre- and post- mist measurements in the control plants. These data show that misting has a significant effect on plants, and plants subjected to misting tend toward a water potential value which is closer to that of a fully hydrated plant (ψ

= 0). This trend indicates that misting has an effect on plant water status, even when plants are not under high levels of plant water stress. The absence of significant changes in water potential within control species and the inconsistencies within the trend (some species were found to slightly increase plant water status during the course of the experiment, whereas some were found to lose plant water.

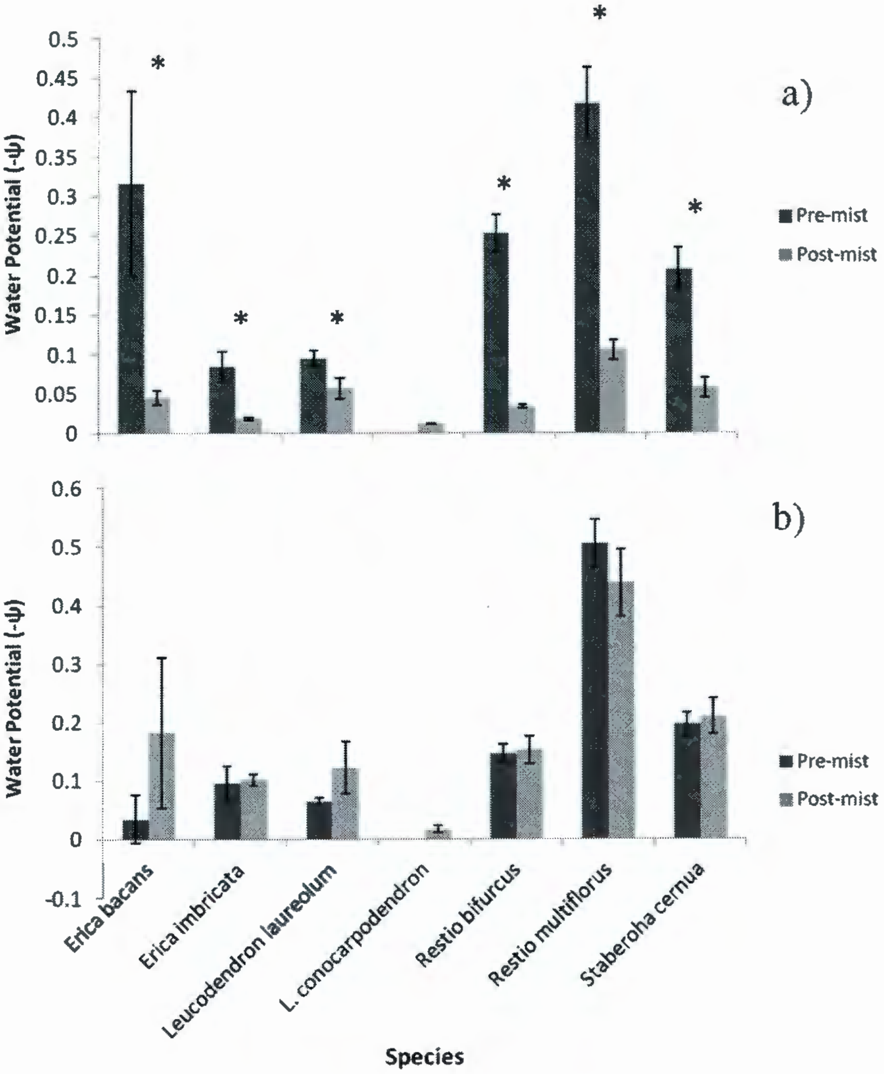

Figure 5 a, b Raw data indicating average pre- and post- mist Water Potential (-ψ) for individuals of each species subjected to a) the treatment effect and b) the control effect.

As indicated above, there was an overall increase in plants water status, as shown by the increase in water potential in individuals which were subjected to the misting treatment. Furthermore, it was found that control individuals showed a decrease in plant water potential (i.e. lost water) during the day, as expected when soil water is not replenished (Figure 6). These increases in water potential indicate that plants placed in the treatment chambers were

Synthesis

Regression analyses indicated variable linear relationships between changes in plant water potential status of plants subjected to misting, and their respective foliar uptake (mg. cm^{-2}), day-time and night-time stomatal conductance (Figure 7). In each case, inclusion of all plant species results in insignificant trends. However, each time the outlier is removed, there is an associated switch to significance. These outliers were *E. imbricata* (Figure 7a) , *R. multiflorus* (Figure 7b) and *E. baccans,* respectively.

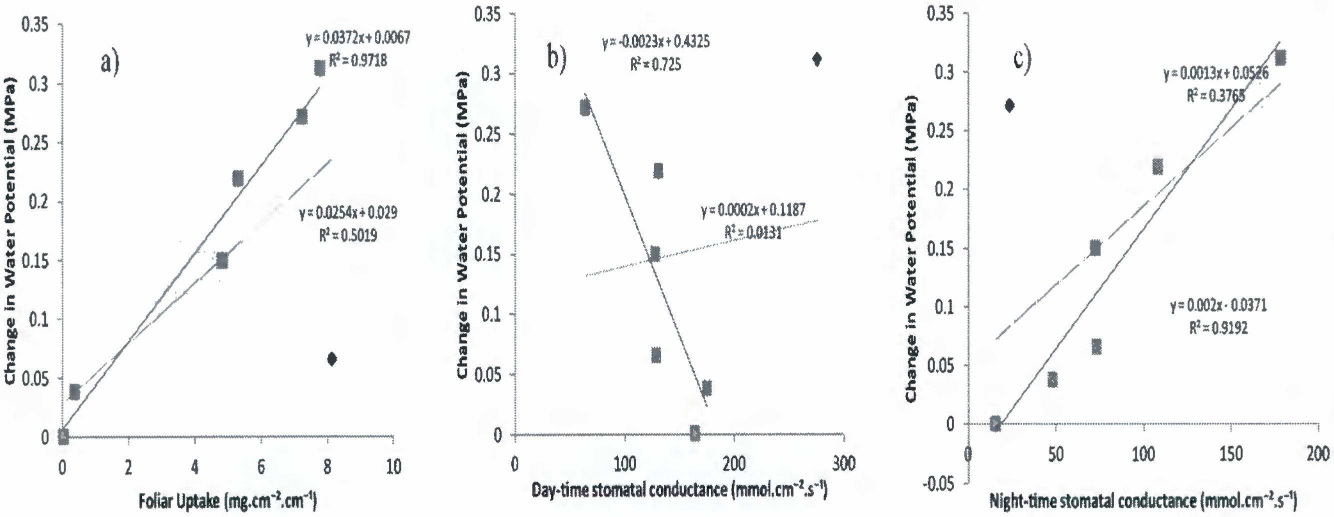

Figure 7 a, b, c The effects of Foliar Uptake, Day-time and Night-time stomatal conductance on changes in plant water potential of those individuals subjected to the treatment effect. Multiple linear regression analyses serve to indicate that there are significant relationships between the treatment plant water potential, and components which may contribute significantly to the overall water status of the plant. Each dark point indicates the presence of a single outlier species. The dashed trendline indicates the regression equation before outlier removal, whereas the solid line indicates the linear trend between the variables, once the outliers have been removed.

Discussion

Cloud moisture events may counteract the effects of limited water availability and plant stress experienced by Fynbos species, during summer drought periods. As indicated, misting has several effects on the water flux experienced by a plant. These effects can be separated according to "uptake" effects, where the plant directly absorbs the water into its foliage or via the root system, or "suppression" effects, where a thin film of water that has settled on the plant acts as a barrier to stomatal conductance (Benzing et al., 1978; Barradas and Glez-Medellin, 1999), allowing the plant to re-equilibrate its own moisture with that of the soil.

Leaf wetness may increase plant hydration in two ways; by direct absorption of the water into the leaf and also by the suppression of water loss from the leaf during the misting event, which assists in enhancing the rehydration process (where the plant water potential re-equilibrates with that of the soil). This would be especially effective for plants if exposed to a misting event during the night-time (Limm et al., 2009), although a significant amount of water is lost during the day, as shown in the plant water potential of individuals that were not exposed to misting events.

Foliar water uptake has been shown to occur in various different ecosystems across terrestrial biomes and henceforth the Fynbos biome can be included among these. Direct absorption of water into the foliage occurred in two diverse functional groups included in this study. After experiments in which plant foliage was immersed in distilled water, it was clear that the ericoids and restioids exhibited leaf surface permeability to water and therefore had the capacity to absorb water directly (Figure 3) into the photosynthetic tissue (Limm, 2009), whether culms, shoots or leaves. This indicates that this water uptake strategy is not limited to a single species or lineage in the system, and occurs over a great diversity of leaf morphology and growth habit, from the fine-leaved ericoids to the spindly restio culm.

The prevalence of this foliar uptake in restioids is not surprising, as these have been shown in several studies (e.g. see Figure 1) to entrap and condense cloud, allowing a significant amount of cloud water to infiltrate the soil as drip (Marloth, 1903; 1905). The addition of this moisture to the soil and the ability of the restios to practice foliar uptake may be what allows them to overcome their vulnerability as shallow-rooted individuals and also allows them to react quickly to any moisture inputs (such as both regular and occult precipitation).It seems as if restios show a perfect combination of "uptake" and "suppression" affects, and exhibit a plant morphology that enhances the drip effect. The architecture of the restios and their ability to absorb water directly into their foliage indicates that cloud is likely to be a significant contributing factor to their survival. Restioids, in general, have a shallow rooting system and should thus respond rapidly to any increases in soil moisture. In addition, restios were determined by West *et al.* (2012) to be isohydric. This means that they should also have a fair amount of stomatal control, although the results for *R. multiflorus* seem to contradict this. However, *R. multiflorus* had a unique morphology, in that photosynthetic material occured in large tufts dispersed along the central axis. This species is likely to be affected quite substantially by misting events, not only because of its architecture, but also due to "suppression" and "uptake" effects induced by the cloud moisture.

Overall, the restioids and ericoids were found to be more tightly coupled to environmental variation than the proteoids. *E. imbricata* was found to show the highest overall foliar uptake (Figure 3), but were surprisingly unresponsive when it came to the effects of misting on overall plant water status. This may have been a side effect of sampling where different experiments occurred at different times, thus allowing a window of time for the individuals of this species to die. In addition, plant water potential was quite close to zero prior to the misting treatment, and this may have resulted in the anomalous data point in Figure 7a; the water potential of all plants that were subjected to the misting treatments were found to trend toward zero, post-mist. This also occured for *E. imbricata,* although this species started at a plant water potential that was already quite close to zero. This means that any changes in the plant water potential due to the treatment effect would not have been as great as species that initially have a lower water potential.

The apparent absence of foliar uptake capacity in the proteoids could serve as an indicator that these species do not require this type of water input, especially as these species tend to be deep-rooted in the field and may thus be buffered from the worst of the drought impact (West et al., 2012). In addition, the limited amount of water uptake into these leaves may indicate the presence of a somewhat waxy cuticle and the evidence of pubescence may influence the ability of the leaves to take up the water.

This indicates that foliar uptake capacity could rise with increases in plant drought stress. It should be noted, however, that the impact of cloud is proportionate to how stressed the plant is; if a plant is well hydrated, then misting will have marginal effects. Nevertheless these results indicated that uptake can occur even across a small water potential gradient between the inside and outside of the plant (Slatyer, 1960; Rundel, 1982; Limm et al., 2009). In addition to the potential effects of pre-mist plant water status on plant response, it is necessary to bear in mind that different species of plant will have different reactions when they experience drought stress. This is because plant functional morphology likely plays an important role in plant response to misting events; Ericoids and restioids are more coupled to environmental variation, whereas proteoids are more buffered (deeper roots, strong stomatal control). Species such as *R. multiflorus* further support this- where a species which was morphologically very different (with tufts of photosynthetic material that were highly susceptible to Foliar uptake and illustrated a large amount of day-time and night-time transpiration. This species displayed the greatest potential response to water fluxes in the environment, as it showed high levels of foliar uptake, as well as high night-time and day-time stomatal conductance, and a large change in water potential.

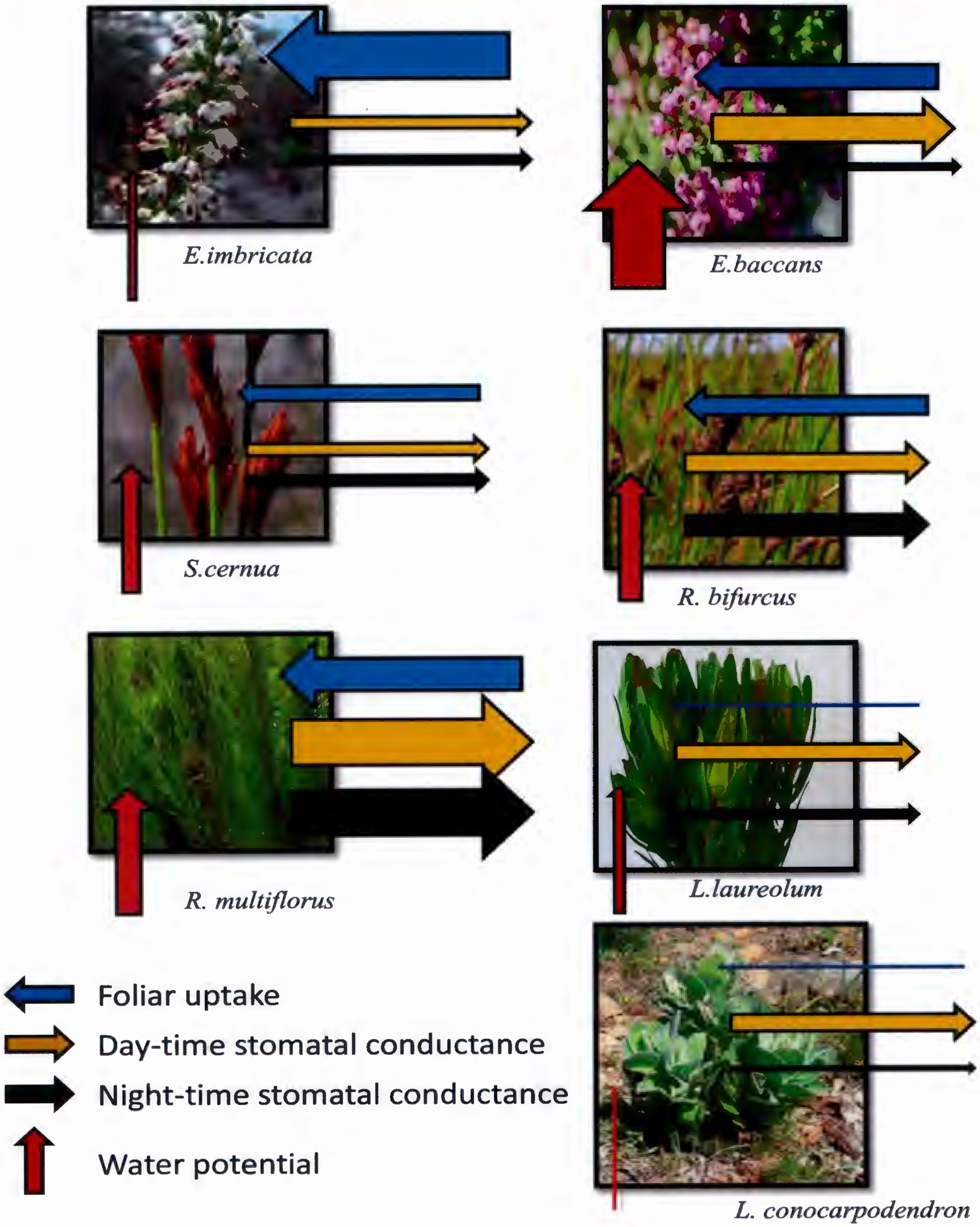

Figure 8: Relative importance and effects of Foliar uptake, Day-time stomatal conductance, Night-time stomatal conductance and Water Potential.

The Foliar uptake arrow illustrates the amount of water that can be absorbed by a species, in response to a misting event. The breadth of the arrow represents the foliar uptake capacity of a species, relative to the maximum overall foliar uptake measured. The Day-time stomatal conductance and Night- time stomatal conductance arrows illustrate the amount of stomatal conductance (i.e. potential water loss from each plant species) . In both cases, arrow breadths represents the conductance rate of the species, relative to the maximum rate measured for the different types of stomatal conductance. The Water Potential arrow indicates the effect of the misting treatment on a species, on the level of entire plant (the breadth of the arrow represents the treatment effect of a species, relative to the maximum overall treatment effect). Species are ranked in order of the ratio of foliar uptake to day-time stomatal conductance. This indicates how influential each potential water loss and/ or gain is, at the level of the plant. E. imbricata came first in ranking, as it displayed the highest foliar uptake capacity : in terms of the day-time and night-time stomatal conductance ratio. L. laureolum and L. conocarpodendron, on the other hand, were ranked last as they did not show any foliar uptake capacity, whereas both had substantial amounts of day-time and night-time stomatal conductance.

The synthesis figure indicates the variability in the "uptake" and "suppression" variables within a functional group. Although individual species could be interpreted as showing substantially different reactions to misting events, it should be noted that when ranked according to the Foliar uptake: Day-time stomatal conductance ratio, ericoids showed the greatest ratio of foliar uptake. This indicates that restioids can be categorized as a morphological type that typically tends toward "uptake" of cloud moisture, rather than a simple suppression of stomatal conductance. On the other hand, the proteoids were found to show limited "uptake" of cloud moisture, indicating that these plants do not display any gain.

In this study, it was also found that nocturnal conductance occurs in all the species (Figure 4 a,b) investigated, further confirming the occurrence of nocturnal conductance across a variety of different ecosystems (Donovan et al., 1999; Snyder et al., 2003; Dawson et al., 2007, Limm et al., 2009). Although rainfall constitutes the greatest input of water into the leaves of these plants, it is clear that misting events, such as those recorded by West et al. (2012) have a significant effect on the plant water status of the plant species occurring in Fynbos. These misting effects would be greatest during summer drought periods, as plant water status is likely to be dominated by root uptake during the rainy season.

This rapid response is also influenced by the fact that restios tend to be isohydric (i.e. they retain a high water plant water potential even during periods of drought; an effective strategy if an intense drought occurs for a short period of time, as elongated drought periods may result in the controversial "carbon starvation". In addition, the presence of isohydry in these plants may result in their ability to absorb the water through their culms; as the stomata responds to changes in the soil and plant water potential quite rapidly. Although the pathway of direct foliar uptake is not yet understood, these species could also have pores for direct water uptake.

When considering this data in light of what was discovered by West et al. (2012), we argue that although species tend to have distinct 'response pathways' to droughting, the effects of misting may play a considerable role in the maintenance and future survival of these species.

Conclusion

Misting was shown to make significant contributions to the overall plant water status. These contributions occur not only in the form of drip and subsequent soil uptake, but also through "suppression" and "uptake". These results indicate that cloud moisture plays an important role in the hydrological cycles occurring in the Fynbos ecosystem. Cloud events are a distinct synoptic phenomenon, the occurrence of which can be predicted with relative certainty. As such, we argue that occult moisture should also be a component of global change models.

Further studies should seek to improve sample size, and potentially expand the experiments to include species which are not presently affected by misting events.

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