Occult moisture inputs in the fynbos: foliar moisture uptake in Ericaceae, Restionaceae and Proteaceae

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Abstract

'Occult' precipitation may be an important moisture source that can impact specific plant responses to water stress in certain ecosystems. Species in many ecosystems have been shown to use this moisture source via foliar moisture uptake. The Cape Peninsula and the surrounding southern coast experience high altitude mountain cloud cover in the summer dry period. Previous work on species that exist exclusively in the cloud belt found that species from the Erica and Restionaceae family were able to absorb water through their leaf surfaces, while members of the Proteaceae were unable to do so (Gibson, 2012). Adding to the body of research, this study explores whether foliar uptake occurs in species that do not exist in the cloud belt of the Cape Peninsula and the relative importance of this method of uptake in positively impacting a plants water balance during a cloud event. Direct foliar uptake was assessed at the leaf level using two methods; submersion and mist exposure. The importance of foliar uptake was measured at the whole plant level by exposing whole plants to cloud in a mist chamber. By covering the soil in a subset of plants, we were able to isolate the importance of foliar uptake relative to uptake via drip. Results found that the restios and ericas showed a stronger ability to take up moisture via their leaves than protea species, which showed little ability to do so. In contrast to this, the mist exposure method showed no significant uptake in any species except *E. quadrangularis.* This disparity is possibly due to specific leaf morphology. All species showed significant hydration in response to a whole plant cloud event in both the covered and uncovered treatments. The significant hydration in response to an occult event suggests that certain low land species are capable of utilizing small occult inputs despite the lack of regular summer cloud events as seen in the cloud belt species. As family patterns of foliar moisture uptake seen in cloud belt species (Gibson 2012) are conserved in the surveyed low land species, it can be concluded that moisture uptake is not a trait specific to cloud belt species, and represents a family level pattern of ability.

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Introduction

‘Occult’ precipitation may be an important moisture source that can impact specific plant responses to water stress in certain ecosystems (Weathers 1999; Burgess and Dawson, 2004; Limm et al, 2009; Johnstone and Dawson, 2010; Matimati et al, 2013). ’Occult’ is the term given to moisture sources such a fog, mist and cloud – inputs not traditionally considered to significantly impact the moisture content of a plant. However, evidence suggests that there is a large amount of moisture available in occult precipitation events (Marloth 1904, 1907; Olivier, 2004) and that this moisture source is utilized by a large number of species across diverse habitats (Limm et al, 2009; Limm and Dawson, 2010; Goldsmith et al, 2013; Matimati et al, 2013).

Cases of reliance on fog and cloud moisture can be found in many forests worldwide. This includes the tropical montane forests in Costa Rica (Goldsmith, 2013), cloud forests in Venezuela (Cavelier et al., 1996), semi-arid forests in Chile (Del-Val et al., 2006), and subtropical forests in Australia (Huntley et al, 1997). Reliance on occult precipitation also extends into arid and hyper-arid areas such as the Namib Desert, the Succulent Karoo (Matimati et al, 2013) and the chaparral in California (Vasey et al, 2012).Perhaps the most rigorously researched example of occult precipitation use is the marine coastal forests on the west coast of North America. *Sequoia sempervirens* D. Don (Californian Redwood) relies heavily on dry season marine fog and canopy drip of condensed fog is thought to be a crucial moisture subsidy (Burgess and Dawson, 2004; Limm et al, 2009). Inputs from drip alone can account for up to 30% of the total annual water input (Burgess and Dawson, 2004).

Occult events can impact the water potential of a plant via suppression or uptake processes (Dawson, 1998; Burgess and Dawson, 2004; Limm et al, 2009). A suppression effect occurs when vapour saturates the air and results in a transpiration depression due to a reduction in atmospheric vapour pressure deficit (VPD). The lowered VPD decreases the diffusive gradient between the inner leaf surfaces and the outside air, slowing the net diffusion out of the leaf and reducing transpiration rates. The decrease in transpiration rate enables plants to more efficiently conserve water resources, especially during periods of drought (Johnstone and Dawson, 2010) as well as allowing the plant stem and leaf potential to equilibrate more effectively (Huntley et al, 1997). Most plants that transpire during the day will experience a suppression event in response to a day time occult event, making it a general ecosystem level effect.

Uptake processes involve direct absorption of moisture provided by occult events. Occult moisture finds an ideal settling medium in the large surface area provided by the foliage canopy as the relative permeability of the foliage medium to air allows moisture deposition onto leaf surfaces (Burgess and Dawson, 2004). Particularly in the presence of wind, occult events can result in moisture pool build up on plant surfaces. Should this moisture accrue to an extent that the foliage can no longer support its weight, moisture droplets fall to the ground. This process (termed 'drip') results in a moisture pool in the soil directly below the canopy that can be exploited by the roots (Hutley et al, 1997). The alternate uptake process involves the direct uptake of moisture through the leaf surface. There is a growing body of evidence to suggest that foliar moisture uptake occurs in many ecosystems worldwide (Boucher et al, 1995; Yates & Hutley 1995; Hutley et al, 1997; Martin & von Willert 2000; Burgess and Dawson, 2004; Limm et al, 2009; Goldsmith et al, 2013; Matimati et al, 2013).

Foliar moisture uptake occurs via two main pathways: through specialised pores or directly through the cuticle. Studies involving *crassula* species found specialised epidermal hydathodes that can play a role in water uptake as well as water exudation (Yates and Hutley, 1995; Martin and von Willert, 2000). In certain arid species, epidermal hydathodes may represent an adaptation to arid conditions where regular occult precipitation events provide water films on leaves (von Willert et al., 1992). Species that lack clear hydathodes are assumed to take up moisture directly through the cuticle (Hutley et al, 1997). The effectiveness of this pathway is influenced somewhat by leaf age (Martin and von Willert, 2000). Older and more weathered leaves will have more damage to their cuticle, rendering it more permeable to water (Limm et al, 2009). Regardless of the pathway, foliar moisture uptake is an effective method of reducing foliar water stress especially during peak stress times in the dry season (Hutley et al, 1997; Burgess and Dawson, 2004; Limm et al, 2009).

Studies into the ability of species to take up moisture through their leaves are particularly important in areas that experience regular occult events. One such area is the Cape Peninsula of South Africa. The Cape Peninsula and the surrounding southern coast experience high altitude mountain cloud cover in the summer, formed by south easterly winds that cause clouds to cover high elevation areas by convective overturning and subsequent radiation loss (Olivier, 2004). Instead, the Evidence suggests that these cloud events hold large amounts of moisture. Marloth (1902; 1905) found that including deposited cloud moisture in the water balance of Table Mountain resulted in a 16-fold increase in amount of water available compared to normal rainfall amounts. Nagel (1956) estimated the mean intensity of fog precipitation on Table Mountain at 3.75 mm/hr compared to the 1.84 mm/hr of rainfall.

Though some work has been done on the process of fog moisture utilization and foilar moisture uptake in South Africa, research on the Cape Peninsula is sparse. The most recent work on cloud moisture utilization by high altitude mountain fynbos in the Cape Floristic Region found that while select species from the families Ericaceae and Restionaceae show the ability to take up moisture directly through their leaves, surveyed species from the Proteaceae family showed no ability to do so (Gibson, 2012).

This study explores whether foliar uptake capacity occurs in species that do not exist in the cloud belt of the Cape Peninsula. We can test the hypothesis that foliar moisture acquisition is a common strategy to particular families by comparing the results of this study to the family uptake pattern observed by Gibson (2012). If foliar uptake occurs in species in the low lands as well as those in the cloud belt, it suggests that such ability is a common family occurrence rather than a trait that could possibly be an adaption to regular cloud events at high altitudes. This study will also test how important foliar moisture uptake is in positively impacting the overall moisture balance of a plant during a cloud event. In order to test these two questions, the study measures foliar uptake capacity for six low land species from the families Restionaceae, Ericaceae and Proteaceae, by administering a cloud treatment with covered and uncovered soil in order to separate the foliar uptake from the drip process.

Methods:

Study species:

Two species from each of the Ericaceae, Restionaceae and Proteaceae family were selected for study. The species used in this experiment were selected for their occupation of low land habitats (low altitudes <500m) within the Cape Peninsula and the greater Cape area. The Erica family was represented by *Erica quadrangularis* and *Erica verticillata,* the Protea family by *Leucadendron argentuem* and *Leucadendron levisanus* and the Restionaceae family by *Elegia tectorum* and *Thamnocotus insignis*.

In this study, 12 individuals of each species were grown in an outdoor environment at Kirstenbosch garden centre. Due to limited stock in the nursery, only eight individuals of *L. levisanus* and *E. quadrangularis* were sourced. The plants were all housed in the same phytotron with conditions set to a 14-hour photoperiod, with sunrise at 06h00 and sunset at 20h00. The daytime and night-time temperatures were set to 23°C and 15°C respectively. The plants received 250 ml of water at sunrise each morning. The plants were given a week for acclimatization, after which the dry-down period began and water was withheld for three days. Foliar uptake capacity experiments began on the morning of the third dry day.

Foliar uptake capacity experiment:

The foliar uptake capacity of each species was assessed in two methods: the submergence method and the mist method. The submergence method tested the ability of each species to absorb water via their leaves, taking care to avoid pressure forcing from deep submergence under the water surface. The misting method tested how applicable uptake rates gained from submergence methods are in predicting foliar moisture uptake from a cloud event.

Both methods were conducted two hours after sunrise (08h00). Leaf material was harvested from every individual of every species. Care was taken to ensure the youngest fully mature leaf material was used in order to reduce the impact of cuticle degradation on leaf permeability (Mechaber et al. 1996; Martin and Von Willart, 2000; Limm et al, 2009). For *L. argenteum* and *L. levisanus*, a single fully mature leaf was harvested. For *E. tectorum* and *T. insignis*, a fully mature photosynthetic culm was harvested. From *E. verticillata* and *E.*

quadrangularis, a single terminal shoot consisting of a stem with multiple mature leaves was harvested. After excising photosynthetic material, the exposed petiole was promptly sealed using waterproof, non-porous glue. After sealing, the leaves were photographed using a Canon IXUS 220 digital camera and the submerged area of the photosynthetic material later determined using ImageJ (US National Institutes of Health, Bethesda, Md).

After photographing each specimen, all leaves were weighed to obtain the initial mass (mass 1). In the submergence method, leaves were then placed into individually labelled plastic sample bags. Each bag was filled with 20 ml of deionised water and then sealed. All bags were then floated on the surface of a large shallow tank (1.0m X 2.5m X 0.2m) filled 5 cm deep with deionised water. The material remained submerged for 180 minutes (Limm et al, 2009) in order to allow significant foliar moisture uptake to occur. After the submergence period, the material was removed and lightly dabbed with tissue paper to remove excess surface water before being reweighed to obtain mass 2. To account for potential error in weight due to the surface wiping, the leaf material was then briefly allowed to air dry to remove surface moisture via evaporation. It was reweighed to obtain mass 3 before being re-submerged in the tank for 10 seconds. Excess moisture was removed from the leaf surface and the leaf was then weighed a final time to give mass 4. The total foliar moisture uptake (mg) for each leaf was calculated according to equation 1.

Equation 1:

$$foliar\ moisture\ uptake\ (mg) = (mass_2 - mass_1) - (mass_4 - mass_3)$$

Using equation 2, the foliar moisture uptake per unit surface area was calculated (mg.cm^{-2}) for every individual of each species.

Equation 2:

$$foliar\ moisture\ uptake\ per\ leaf\ area(mg.cm^{-2}) = \frac{total\ foliar\ moisture\ uptake\ (mg)}{total\ surface\ area\ (cm^2)}$$

By floating the leaves on the surface of the water with a thin layer of water bathing the entire leaf surface, pressure forcing that may occur with deep submergence under the water surface was avoided.

The misting method was done in the same way as the submergence method, with material harvested, sealed, photographed and weighed. The material was then placed on a raised wire mesh in the fully saturated cloud chamber for 180 minutes before being removed. The material was tissue dried and then weighed to obtain mass 2. After a brief air dry period, the material was weighed again and placed back into the fog chamber for one minute. Once removed, the material was dried and weighed for a final time. The total foliar moisture uptake (mg) for each leaf was calculated according to equation 1 and the foliar moisture uptake per leaf area ($mg.cm^{-2}$) was calculated using equation 2.

Average foliar moisture uptake per species ($mg.cm^{-2}$) was calculated for the submergence and the mist method. Uptake per species ($mg.cm^{-2}$) for both the submergence and the mist method was tested using a single sample t test (α=0.05) establish whether levels of uptake were significantly higher than 0 $mg.cm^{-2}$. A two sample t test (α=0.05) was used to test whether the submergence and mist uptake averages differed significantly from each other in each species.

Cloud exposure experiments

The cloud exposure experiments assessed the impact of a day time cloud event on the water potential of water stressed plants. The cloud chamber was built from a PVC piping frame fitted with clear PVC sheeting. The chamber measured 2m x 1.5m x 1m with an access flap to allow plants to be placed inside. Two small fans on mounted retort stands inside the chamber, one at each end of the chamber. The fans ensured that the water saturated air was circulated. The chamber was filled with vapour using a 5-disk ultrasonic fog device (Chaoneng Electronics, ex Nanhai, Guangdong, China) submerged in a 12 l bucket of water with a floatation device to ensure enough water covered the machine. The 12l bucket was topped up via the attached hose pipe at four hour intervals during the experiment.

Half of the individuals of each species were randomly assigned to either the uncovered soil treatment or the covered soil treatment. Covered soil treatments attempted to prevent drip from occurring and, by comparison to the uncovered soil treatment, ascertain the relative importance of foliar moisture uptake versus drip in impacting the water potential of stressed plants during a cloud event. The six individuals in each treatment were further split into control and experiment groups, each consisting of three individuals. Two of the plant species did not have sufficient numbers to use both covered and uncovered soil treatments and so *L. levisanus* and *E. quadrangularis* were not included in the covered soil experiment and four individuals were in each of the control and experiment group.

The cloud exposure experiments occurred for a 16 hour period, beginning at sunrise (06h00) and ending at 22h00 after two hours of post sunset darkness. The experiments occurred in a neighbouring phytotron set to the same conditions. At 22h00 of the night before, all individuals of a particular species were relocated to the neighbouring phytotron and the covered group had their soil isolated using parafilm over the soil surface and the drainage holes of the pots. Measurements of plant water potential (Ψ) were conducted over the next eight hours using fine scale Scholander pressure chamber (PMS instruments, Corvallis, OR, USA), with measurements taken on a single culm/terminal shoot per individual.

Experiment group plants of both covered and uncovered treatments were placed in the fully saturated cloud chamber at 06h00 (sunrise). After placement of plants in the cloud chamber, the chamber was sealed and the fans connected to a 6 V battery. The control plants were placed on the bench opposite the chamber. At 22h00, after 16 hours of exposure, the chamber was briefly opened to remove individual plants for post water potential measurement. To allow for the loss of moisture due to the flap opening, the fog machine remained on until all plants were removed. Measurement of post water potential was done in the same order as the pre-water potential measurements.

The average species difference in pre and post water potential was calculated for the control and experiment series in each of the covered and uncovered soil treatments. A two sample t test ($\alpha=0.05$) was used to assess whether control and experiment groups differed significantly in water potential difference in both soil treatments. In each of the covered and uncovered experiment groups, an ANOVA was performed with a post-hoc tukey test to determine whether the water potential difference for experiment plants differed significantly between species. Additionally, the experiment group water potential difference averages for the uncovered and covered soil treatments were tested for significant difference within species using a two sample t test ($\alpha=0.05$).

Regression analysis

In order to explore the variance in the cloud exposure data, regression analysis was performed on all six species. For each species, individual plant pre-water potentials (MPa) were plotted against the change in water potential (MPa) for both experimental and control series. Significant slopes were used calculate the treatment effect of cloud, the difference between the experimental series change in water potential and the control series change in water potential at a particular pre-water potential. This analysis was repeated on Gibson's 2012 data set of six high altitude fynbos species.

Stomatal conductance measurements:

In order to assess the relative gain of each species from day time transpiration suppression in a cloud event, day time stomatal conductance ($mmol.cm^{-2}.s^{-1}$) of each individual was measured using the Infrared Gas analyser (Li-Cor 6400 Portable Photosynthesis System, Li-Cor Europe).

All measurements occurred between 10h00 and 15h00 to catch the peak photosynthetic periods. Conductance measurements were taken 10 times on a single photosynthetic shoot of every individual of each species. For both restio species, multiple culms were used in parallel to fully cover the IRGA aperture. For both erica species and *L. levisanus,* a mature terminal stem with multiple small leaves was used. For *L. argentum*, a single mature leaf was used. The area of the photosynthetic material placed in the IRGA chamber was photographed and later determined using ImageJ (US National Institutes of Health, Bethesda, Md).

Average conductance per species ($mmol.cm^{-2}.s^{-1}$) was calculated and an ANOVA was performed followed by a Tukey multiple comparison test to establish whether daily conductance ($mmol.cm^{-2}.s^{-1}$) differed significantly between species.

Results:

All species were able to take up moisture (α=0.05) in the submergence method (Figure 1). These patterns varied by family, with the restio species showing the greatest ability while the proteas showed the least. Foliar uptake rates were much lower in the mist method compared to the submergence method and most species showed no significant foliar uptake (α=0.05). *Erica quadrangularis* was the exception to this, with similar rates of foliar uptake under both methods (Figure 1).

The experiment groups of both the covered and uncovered soil treatment showed positive hydration in response to misting (Figure 2 A and B). The control groups for both soil treatments showed a negative water potential difference, getting more stressed over time. The difference between control and experiment means was significant for all species in the uncovered soil treatment, and all species except *E. tectorum* in the covered treatment

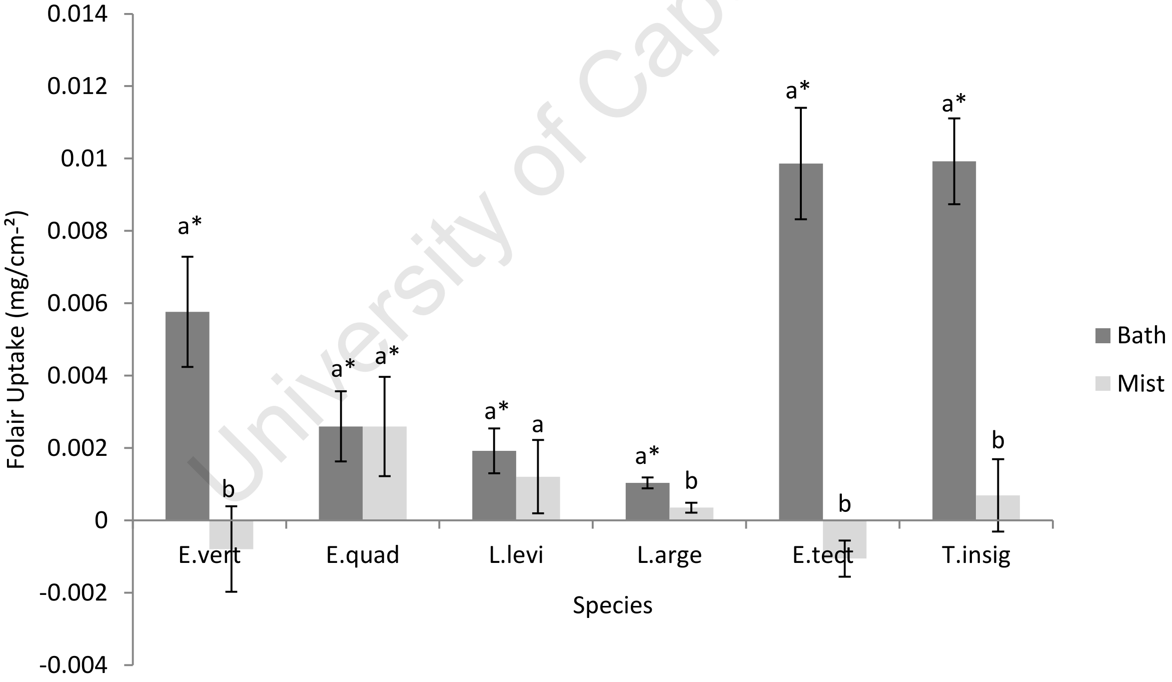

Figure 1: Plot showing the foliar uptake (mg/cm-²) for both the submergence and the mist method for all species. Asterisks (*) indicate mean foliar uptake (mg/cm-²) is significantly different ($p<0.05$) from 0 mg/cm-² using a one sample t-test. Letters show the result of pairwise comparison within each species between the mist and the submergence foliar uptake (mg/cm-²) using a two sample t test assuming equal variance. Differing letters (a and b) within the same species indicate a significant difference ($p<0.05$) in foliar uptake (mg/cm-²) between the two methods. Vertical lines show standard error.

(Figure 2). When comparing amount of water potential change in response to misting between species, it was found there were two homogenous groups. One group contained only *T. insignis* (df=5, MS=1.082, F=5.870 p>0.005) and the other group contained the rest of the species (Figure 2A). More interspecific difference was seen in the covered treatment soil; water potential change (MPa) formed a gradient between species; *T. insignis* had the highest difference value, significantly different to that found in *E. verticillata* and *E. tectorum* and intermediate of that found in *L. argenteum* (df=3, MS= 0.844, F=5.580, p=0.028).

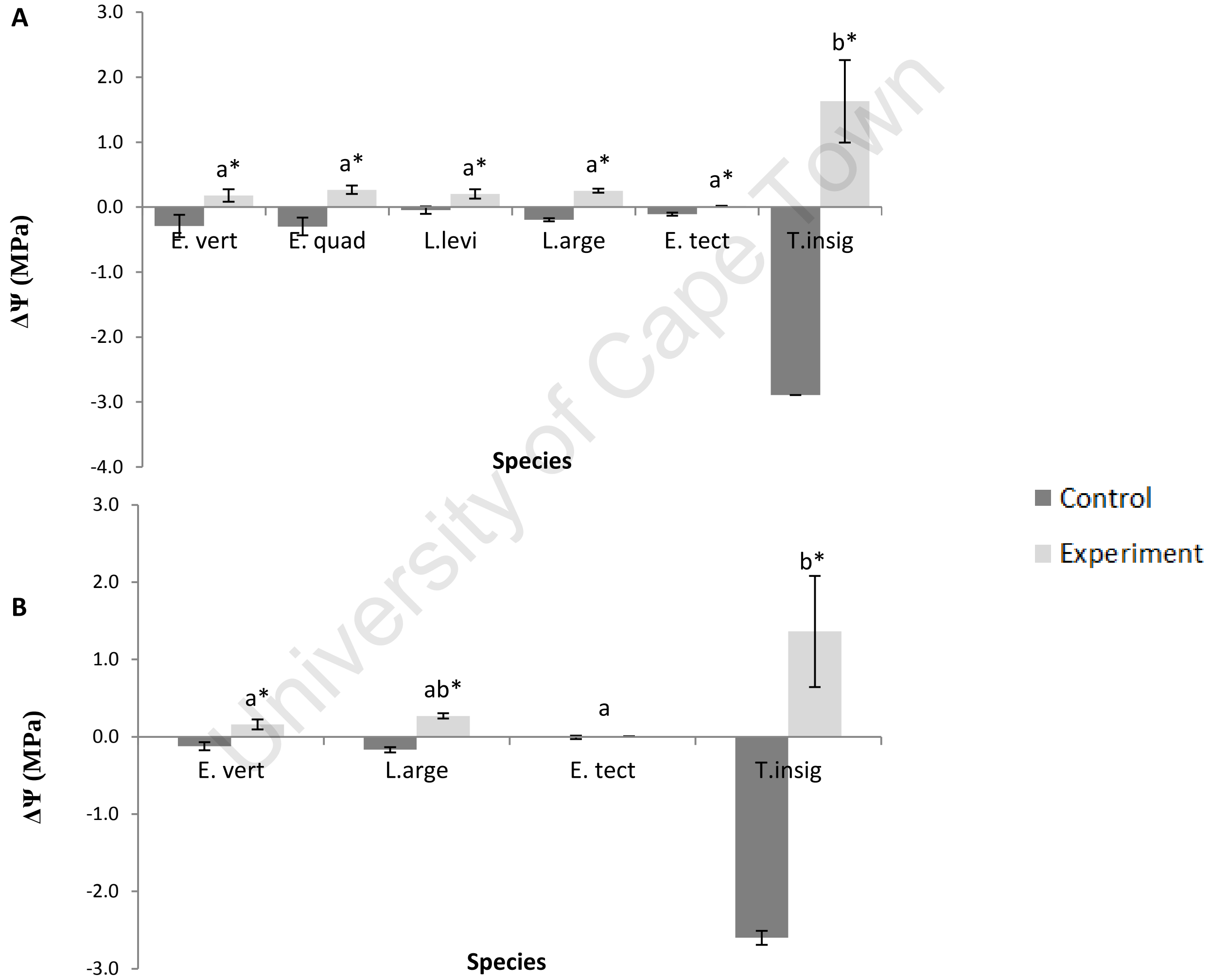

Figure 2: Plot showing the difference in water potential (Ψ) between pre and post treatment water potential measurements for **(A)** uncovered soil and **(B)** covered soil treatments. Both control (no cloud exposure) and experiment (cloud exposure) treatments are shown in each group across all species. Vertical bars indicate standard error. Asterisks (*) indicate a significant difference (p<0.05) in t –test between control and experiment treatments within each species. Letters indicate homogenous groups (p<0.05) of experiment group difference in water potential according to separate post-hoc Tukey tests on each covered and uncovered data sets.

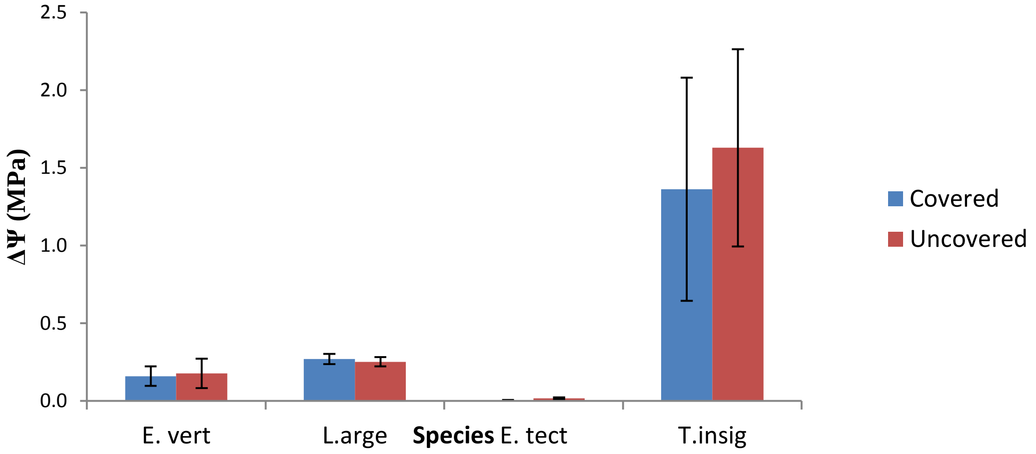

Figure 3: Plot showing the change in water potential (Ψ) for covered soil experimental treatment (blue bars) and uncovered soil experimental treatments (red bars). No significant difference was found between treatments within species (α=0.05). Vertical bars indicate standard error.

Comparison of the experiment group water potential differences (MPa) for both covered and uncovered soil treatment (Figure 3) revealed that there was no significant difference between treatments within each species (α=0.05).

Regression analysis (Figure 4) revealed that pre-water potential (MPa) significantly predicted the difference in water potential (MPa) for the pooled experimental data for *E. verticillata* (β=- 0.972, t=-8.5351, p<0.001), *E. quadrangularis* (β=-0.933, t=-3.1561, p<0.05), *L. levisanus* (β=-1.070,t=-13.5341,p<0.005) and *T. insignis* (β=-0.520, t=-3.0689, p=0.05). *Leucadendron argenteum* was very close to significant with a p value of 0.055 (β=-0.232342188, t=-2.672280388). For the pooled control data set, pre water potential (MPa) was not significantly able to predict difference in water potential (MPa) for any species.

Stressed plants gained more water during a cloud treatment in all species except for *E. tectorum* (Figure 4). In a regression analysis of Gibsons (2012) data on montane species in the Cape Peninsula (Figure 5), the same pattern was observed. The species data showed the pre water potential (MPa) significantly predicted difference in water potential (MPa) for *E. baccans* (β=-0.974, t=-25.98, p<0.025), *E. imbricata* (β=-0.869, t=-51.1504, p<0.02), *R. bifurcus* (β=-0.933, t=-88.65, p<0.01), *R. multiflorus* (β=-1.368, t=-12.85, p<0.05) and *S. cenura* (β=-1.197, t=-42.85, p<0.015). *Leucadendron lauroleum* showed no significant relationship.

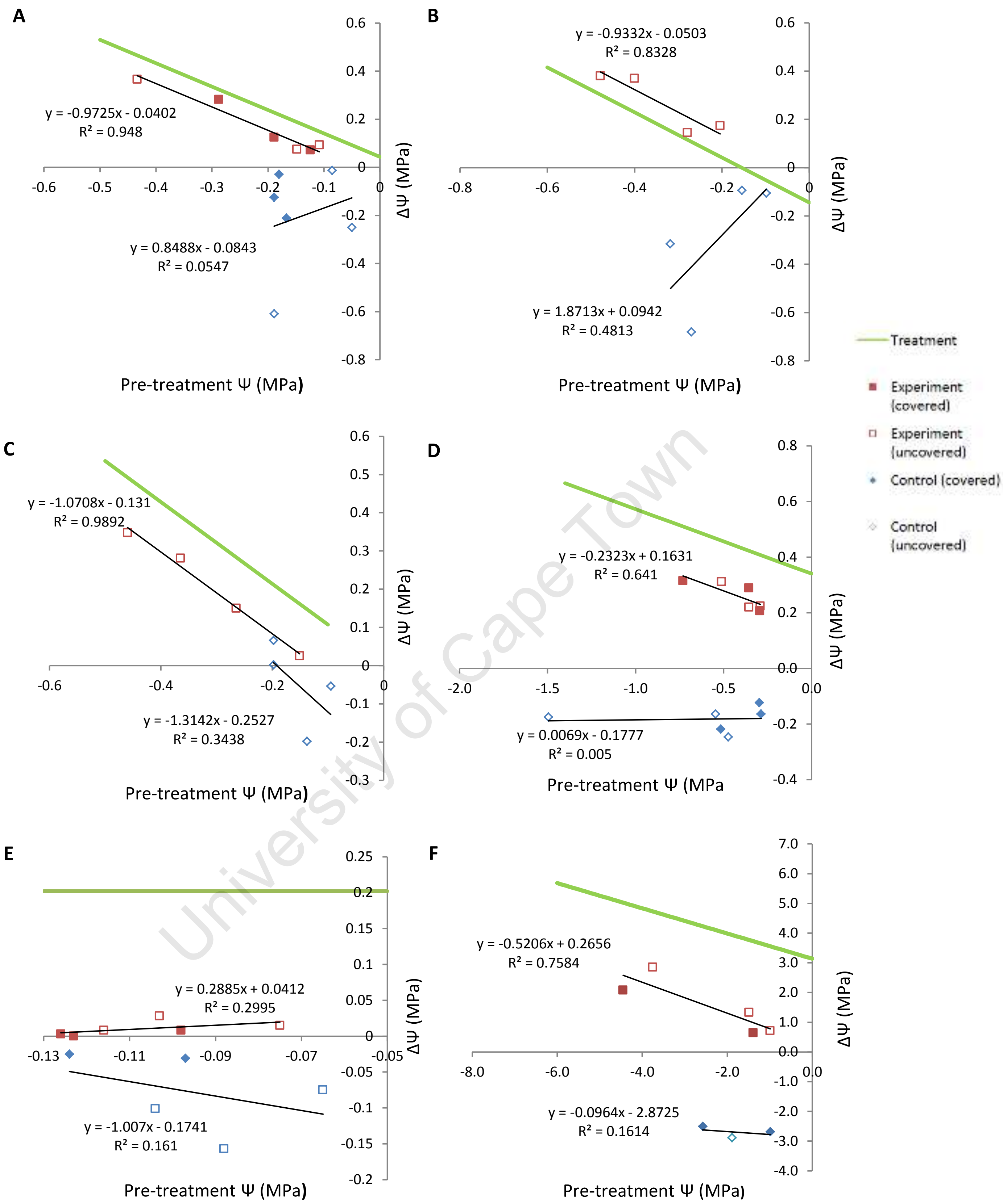

Figure 4: Regression analysis of pre-treatment water potential (Ψ) against change in water potential between pre and post measurements (Ψ) for the control (blue series) and experiment (red series) groups for (A) *Erica verticillata* (B) *Erica quadrangularis* (C) *Leucadendron levisanus* (D) *Leucadendron argentum* (where dashed line indicates near significant experiment slope form treatment effect) (E) *Elegia tectorum* and (F) *Thamnochortus insignis.* Treatment effect of cloud (defined as the water potential difference between a control and an experiment plant beginning at the same pre water potential after 16 hours of treatment) is shown by the solid green line.

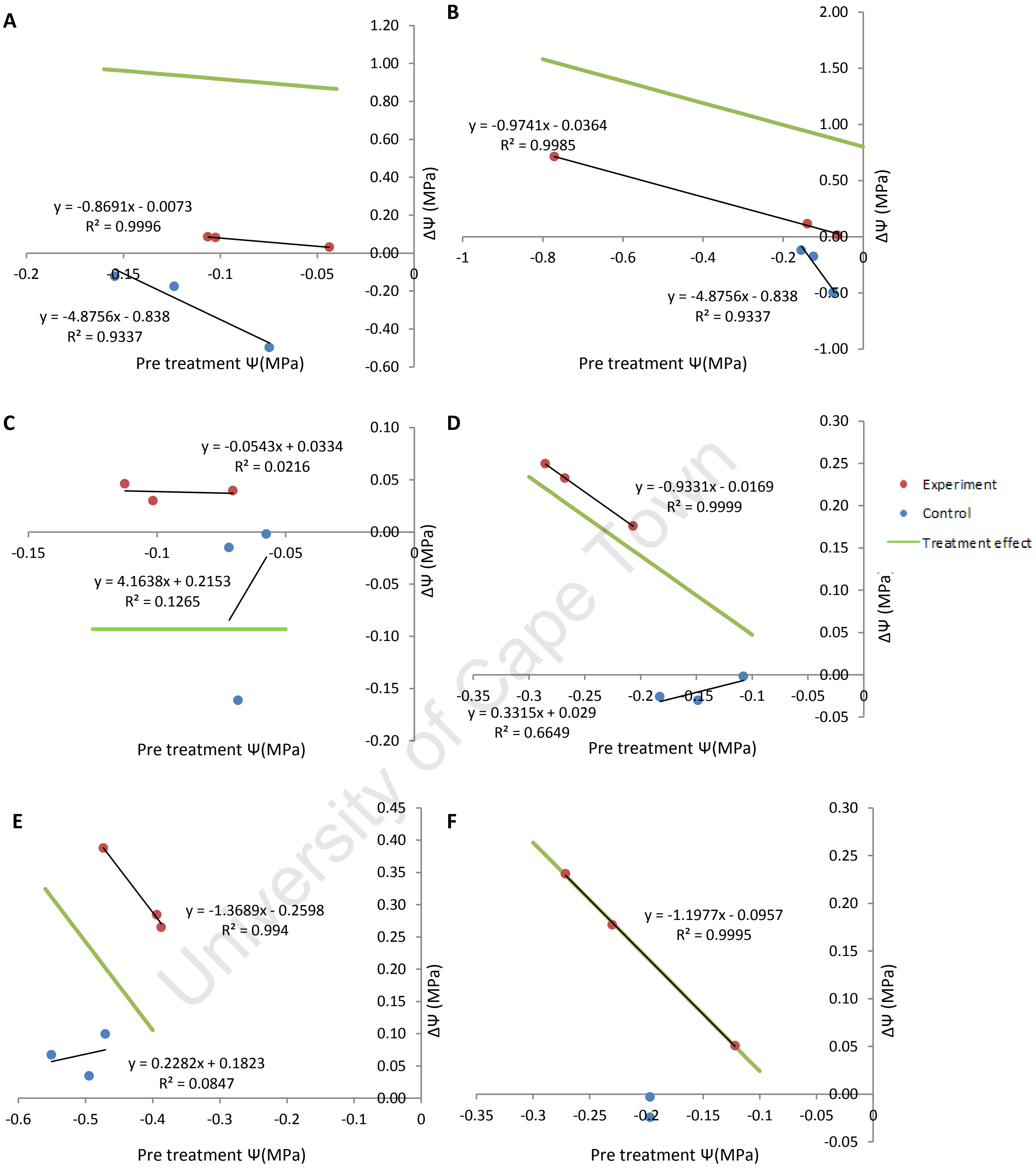

Figure 5: Regression analysis of Gibson (2012) data showing pre-treatment water potential (Ψ) against change in water potential between pre and post measurements (Ψ) for the control (blue series) and experiment (red series) groups for (A) *Erica imbricata* (B) *Erica baccans* (C) *Leucadendron lauroleum* (D) *Restio bifurcus* (E) *Restio multiflorus* and (F) *Staberoha cernua.* Treatment effect of cloud (defined as the water potential difference between a control and an experiment plant beginning at the same pre water potential after 16 hours of treatment) shown by solid green li

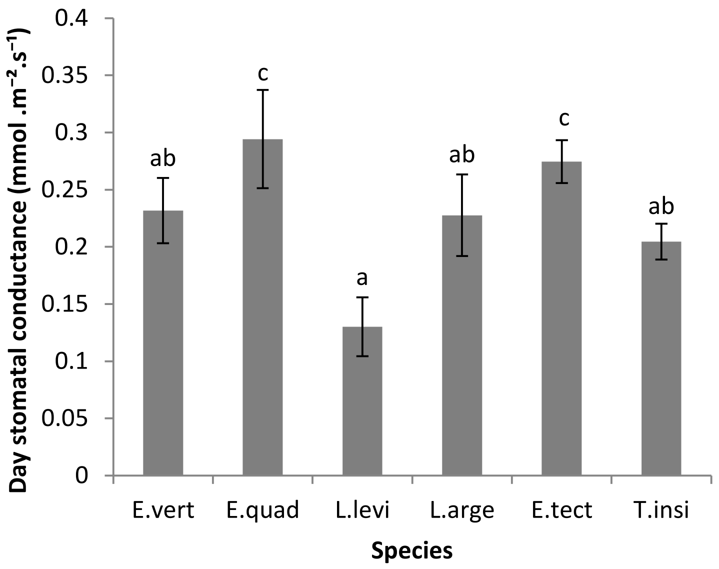

Figure 6: Plot showing the average day time stomatal conductance (mmol $.m^{-2}.s^{-1}$) for each species. All species show a day time stomatal conductance rate that is significantly ($p<0.05$) higher than 0 mmol $.m^{-2}.s^{-1}$ as assessed by an independent one sample t test. ANOVA tests show significant differences in conductance between species. Letters a, b and c indicate homogenous groups ($\alpha=0.05$) according to a posthoc Tukey test. Vertical lines indicate standard error.

All species showed conductance rates significantly higher than 0 mmol $.m^{-2}.s^{-1}$ (Figure 6). No significant structural group pattern existed. *Leucadendron levisanus* had the lowest conductance rate ($\mu=0.13$ mmol $.m^{-2}.s^{-1}$, SD=0.0727 mmol $.m^{-2}.s^{-1}$) and was significantly lower than any other species ($p>0.05$). *Erica verticillata*, *Leucadendron argenteum* and *Thamnochortus insignis* had intermediate levels of conductance relative to the study. *Elegia tectorum* and *Erica quadrangularis* had the highest conductance rates.

Discussion:

Family patterns of foliar moisture uptake seen in cloud belt species (Gibson 2012) are conserved in the surveyed low land species, with restios and ericas showing a stronger ability to take up moisture via their leaves than protea species, which showed little ability to do so. The relative importance of foliar moisture uptake in plant hydration during a cloud event was found to be high, as all species showed significant hydration in response to a whole plant cloud event in both the covered and uncovered treatments. The significant hydration in response to an occult event suggests that these low land plants are capable of utilizing small occult inputs despite the lack of regular summer cloud events as seen in the cloud belt species. Foliar moisture uptake is therefore not a trait specific to cloud belt species, though it may be important in facilitating life in the cloud belt.

Though all species showed the ability to allow moisture uptake via the leaves, vast differences in rate occurred between the two foliar moisture uptake methods. Most species show significant uptake using the submergence method but no discernible uptake when exposed to cloud, suggesting that ability to allow water through the leaf surface may not necessarily translate into foliar moisture uptake of occult precipitation in the field. Previous studies may have exaggerated the rate at which such uptake occurs in the field by using submergence of a leaf to test the species ability to exhibit foliar moisture uptake. This is especially true for methods when the leaf is held under the water surface ('dunking' methods), as the higher pressure under the water surface may result in more water uptake than is typical.

Leaf morphology may be important in the disparity in foliar moisture uptake rates between methods. *Elegia tectorum* and *T. insignis,* the species showing the strongest submergence uptake, have a vertical, rounded culm structure. When misted, vapour coalesces on the smooth culms and forms beads of moisture that run off the rounded surface. This differs vastly from the conditions of submergence when the culm surface is consistently covered by water. *Leucadendron argenteum* has leaves are covered in a smooth but dense layer of air-filled hollow hairs. When submerged, the water may be able to percolate through this dense hair layer. However, in the misting treatment, moisture again simply condenses on the hairs and runs off the leaf surface. The hairy leaf of *L. argentum* suggests that this species would not be able to take up cloud moisture through its leaves in the field. An additional for the difference in uptake between the submergence and mist method is only one side of the leaf surface was exposed to vapour due to the placement of the leaves on fine mesh grid. Comparing the lack of uptake of during single leaf misting with the significant hydration during whole plant misting suggests that the amount of time the leaves were placed in the cloud chamber may not have been sufficient to allow discernible uptake by the plants.

Erica quadrangularis was the only species to show comparable rates of foliar moisture uptake with both methods. This is due to the water capturing capabilities of small leaves – the species had the smallest leaf size out of all surveyed species and the fine mesh created by the small leaves is very efficient at capturing and holding moisture droplets. The same principle is used for fog harvesting screens that utilize small intermesh spaces to effectively condense and capture fog moisture (Gultepe et al, 2007). The result is the micro-leaves are bathed in moisture for the duration of the misting, mimicking conditions created by the submergence method hence resulting in similar levels of uptake. Supporting this, it was noted the *E. quadrangularis* samples were particularly wet when removed from the cloud chamber.

Despite the lack of uptake during misting of single leaves in most species, a large difference in water potential was seen between the more stressed control and more hydrated experiment plants during whole plant exposure. Furthermore, the difference between covered and uncovered whole plant exposure treatments was not significant, suggesting that foliar moisture is a very important method of moisture acquisition during a prolonged occult event. Though an interpretation of these results may also be that drip is not a significant source of moisture for these species, this is probably as the experiment design was not able to mimic field conditions accurately enough. Soil moisture increase in the field is a result of drip from a large canopy surface area of many densely packed plants. Realistically, the experiment probably did not have a large enough leaf surface area or a wide enough soil area in the pots to create and capture sufficient drip as to significantly impact the soil moisture.

Though foliar moisture is clearly an important method via which the plants may hydrate during an occult event, the extent of hydration was largely determined by the pre-water potential. The more water stressed a plant was when the cloud event commenced, the more it gained during a cloud treatment. This is because more water stress means a greater the water potential gradient between the plants tissue and the outside environment. Though every care was made during the experimental set up to ensure all individuals were given the same amount of water before a uniform dry down period, the pre water potential within species was variable. Future attempts to distinguish the relative important of drip versus foliar moisture uptake in moisture balance should either rear individuals from seed with a strictly controlled water input, or allow a much longer acclimatization period to nursery obtained individuals to more adequately equalise water potential between individuals of a species.

The increasing treatment effect with decreasing pre-water potential suggests that the rate of uptake changes according to its starting water potential, with more water stressed plants taking up water faster than less water stressed plants. Given that drip is a passive occurrence and should theoretically occur at the same rate in all conspecific individuals of similar size experiencing the same cloud event, this suggests that the rate at which foliar moisture uptake occurs changes down a gradient of water stress. Cloud events may then differ in importance of water input depending on its temporal occurrence. Late summer cloud events, where plants have witnessed months of severe water depletion, may be crucially important.

The finding that certain low land species can utilize cloud moisture has important implications for drought survival. Though cloud events are not typical for low land areas, the presence of foliar moisture uptake ability in these species means that they can take advantage of small occult moisture inputs into the system, such as early morning dew. Moisture input in the fynbos system is then not only limited to precipitation, and small occult inputs may provide important subsidies for plant survival, particularly during the hot, dry summer.

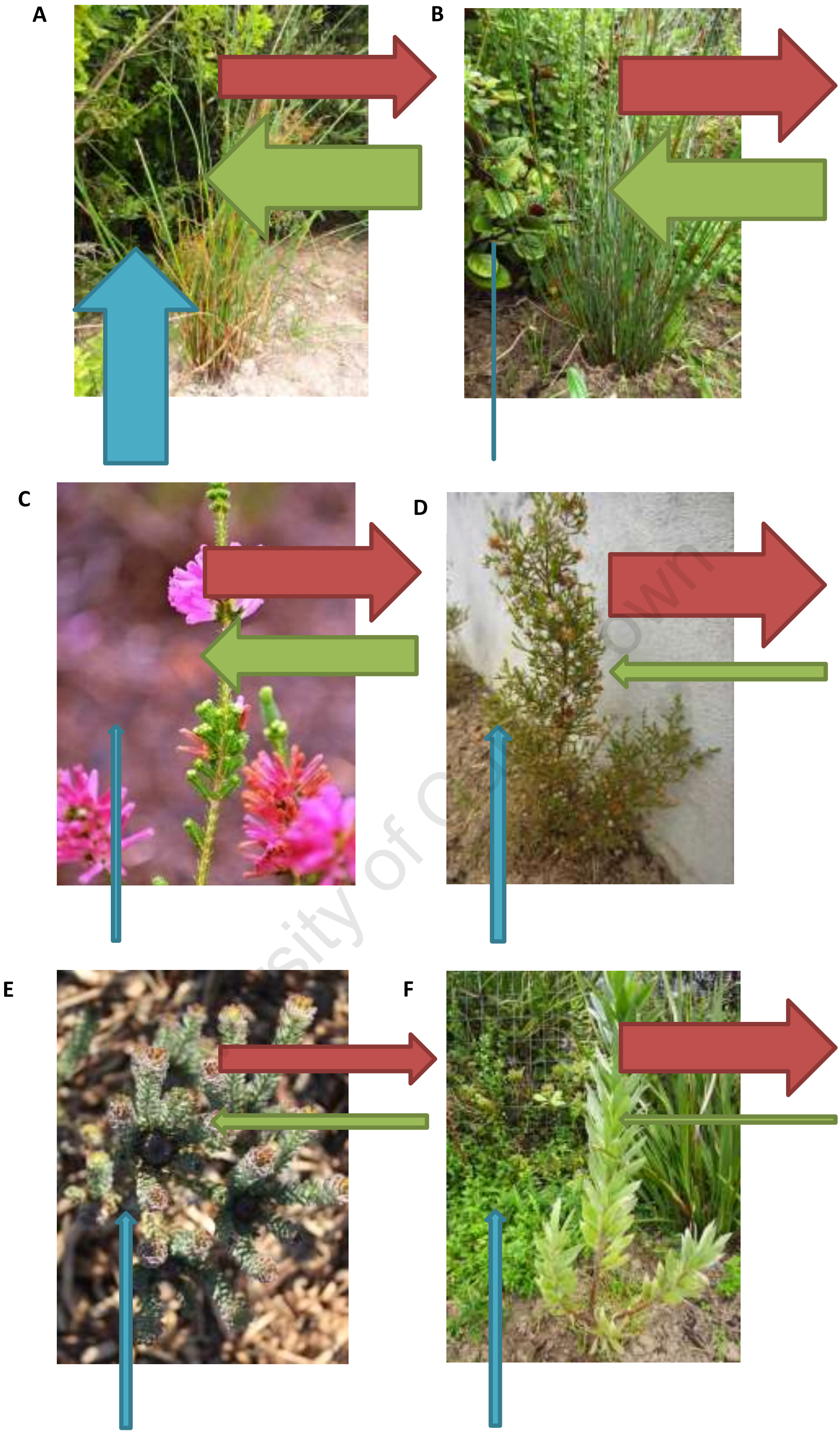

Figure 7: Infographic showing relative rates of day time stomatal conductance (red arrows), foliar uptake (green arrows) and treatment effect of cloud chamber in terms of change in water potential (MPa) (blue arrows) for (A) *Thamnochortus insignis* (B) *Elegia tectorum* (C) *Erica verticillata* (D) *Erica quadrangularis* (E) *Leucadendron levisanus* and (F) *Leucadendron argentum.* The breath of the arrows represents the rate of each process relative to the species with the maximum rate for that process.

This infographic (Figure 7) shows the relative rates of day time stomatal conductance (mmol .m^{-2}.s^{-1}), foliar uptake (mg. cm^{-2}) and change in water potential in cloud chamber (MPa) between the six low land species investigated by this study.

Both restio species show high daily conductance and high foliar uptake when water stressed. The result of this for *T. insignis* is a large loss of water during the dry down period and a consequent large amount of uptake during a cloud event. The large amount of water loss during dry down suggests that this species still transpires during times of stress. Due to this, suppression effects will be particularly important for this species. The importance of the suppression effect is supported by the large amount of water loss experienced by the control group that did not experience the effect (Figure 2 A and B). The particularly fast rate of uptake during a cloud event suggests that *T. insignis* has a cuticle that is particularly permeable to water (unlikely given their occurrence in a summer drought area) or it has specialized hydathodes for uptake.

Given the similarities in rate of uptake and daily conductance rates recorded for both *T. insignis* and *E. tectorum*, one would expect *E. tectorum* to show the same water stress response. However, *E. tectorum* showed the least water potential change in response to cloud. This may be due to the sample as the species was particularly difficult to stress; the normal three day dry period resulted in mass death of individuals. A second attempt used a two day dry period was not sufficient to stress the plants adequately and so pre-water potentials before cloud exposure were very close to zero and little gain was shown.

The restios responses to stress, particularly that of *T. insignis*, are important in describing heterogeneity in the family's response to drought. A recent drought study in the fynbos found that surveyed species of restios - *Hypodiscus aristatus* (Thunb.) Krauss and *Staberoha cernua* (L.f.) T.Durand & Schinz - were isohydric and in drought situations they closed their stomata in order to preserve water (West et al, 2012). This study suggests that this is not a species wide pattern, and there is some degree of differing strategies in different species of the same family.

Leucadendron argenteum and *L. levisanus* show the first and second lowest rates of foliar moisture uptake respectively, suggesting that occult moisture may not be a usable moisture source for these species. Proteas generally have deeper roots than either the erica or restio family (West et al, 2012). These deep roots may decouple them from surface water fluxes and the generally thick leaves work to conserve water loss (West et al, 2012). In insulating them from smaller scale water fluctuation, such morphology may also prevent exploitation of occult moisture via the leaves. High rates of stomatal conductance for *L. argenteum* mean that this species may benefit from suppression effects to a greater extent than *L. levisanus*. The large leaf surface area and the smooth hairs on the surface of the leaves suggest that *L. argenteum* may benefit from drip in the field, though no discernible benefit was gained in this study.

The erica species both show relatively high rates of day time stomatal conductance so stand to gain from transpiration suppression during a cloud event. Both show intermediate foliar uptake rates, placing third and fourth in comparison to the other species and therefore both stand to gain from both moisture uptake during a cloud event. *Erica quadrangularis* may be particularly suited to the utilization of occult moisture with it small leaf morphology working to condense and capture moisture directly against the leaf surfaces.

Conclusion:

Family patterns of foliar moisture uptake are conserved between the high and low land species that we observed. Functional groups tend to benefit from an occult event in different ways: proteas seemed to benefit more from transpiration suppression, whereas the ericas and restios are able to absorb moisture directly though their leaves in an occult event. The strong response shown in this study by both low land and high altitude species to a cloud event suggests that occult preciptation events are an imporant and usable moisture input into the fynbos system. Moisture input in the fynbos system is then not only limited to precipitation and this is important to consider in a host of other studies including drought response studies as well as modelling attempts to discern the future distribution of species in response to climate change.

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