


Variation in cloud immersion, not precipitation, drives leaf trait plasticity and water relations in vascular epiphytes during an extreme drought

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Abstract

Premise: Epiphytes are abundant in ecosystems such as tropical montane cloud forests where low-lying clouds are often in contact with vegetation. Climate projections for these regions include more variability in rainfall and an increase in cloud base heights, which would lead to drier conditions in the soil and atmosphere. While recent studies have examined the effects of drought on epiphytic water relations, the influence that atmospheric moisture has, either alone or in combination with drought, on the health and performance of epiphyte communities remains unclear.

Methods: We conducted a 10-week drought experiment on seven vascular epiphyte species in two shadehouses, one with warmer and drier conditions and another that was cooler and more humid. We measured water relations across control and drought-treatment groups and assessed functional traits of leaves produced during drought conditions to evaluate trait plasticity.

Results: Epiphytes exposed to drought and drier atmospheric conditions had a significant reduction in stomatal conductance and leaf water potential and an increase in leaf dry matter. Nonsucculent epiphytes from the drier shadehouse had the greatest shifts in functional traits, whereas succulent epiphytes released stored leaf water to maintain water status.

Conclusions: Individuals in the drier shadehouse had a substantial reduction in performance, whereas drought-treated individuals that experienced cloud immersion displayed minimal changes in water status. Our results indicate that projected increases in the cloud base height will reduce growth and performance of epiphytic communities and that nonsucculent epiphytes may be particularly vulnerable.

KEYWORDS

atmospheric moisture, climate change, leaf dry matter content, lifting cloud base, Monteverde, Costa Rica, succulence, tropical montane cloud forest, water potential

Drought can influence plant health and development in a variety of ways including reductions in photosynthetic activity, plant growth, and seed yield (Miyashita et al., 2005; Hussain et al., 2008). Plants have evolved a wide range of adaptations to withstand or avoid drought conditions, including osmotic regulation, stomatal control, and leaf size variability (Blum, 1996; Patakas et al., 2002; Chen and Jiang, 2010; Martin-StPaul et al., 2017). Although such adaptations may increase resistance to drought, extreme water limitation can cause mortality (McDowell, 2011;

Anderegg et al., 2015). Species vary in their vulnerability to drought, so drought-induced mortality can lead to shifts in species abundance and community composition, which have consequences for ecosystem functions and, at large spatial scales, land-atmosphere interactions (Clark, 2004; McDowell et al., 2008). Assessing the impacts of drought on plant communities will help us to predict how particular ecosystems will be affected by shifts in drought regimes.

Vascular epiphytes may be particularly susceptible to drought because they lack access to terrestrial resources and

are largely dependent on water provided by low-lying clouds (Nadkarni, 1985; Benzing, 1998; Gotsch et al., 2015, 2018). Vascular epiphytes in tropical montane cloud forests (TMCFs, defined here as mid- to high-elevation ecosystems in tropical latitudes containing a continuous forest layer that experiences frequent cloud immersion and a high degree of epiphytism) are immersed in low-lying clouds for extended periods of time, which provide them with ample sources of atmospheric water. However, diminishing cloud cover could result in increased drought stress for epiphytes in TMCFs. Projected changes in climate in TMCFs include increases in the number of days without rain and increases in cloud-base heights (Benzing, 1998; Pounds et al., 1999; Still et al., 1999). One model predicted that 100% of the TMCFs in Mesoamerica and the Caribbean will likely experience declines in cloud immersion by 2060, although the degree of the decline varies greatly across the regions studied (Helmer et al., 2019). These changes may lead to additional shifts in microclimatic variables including increases in direct radiation, drought periods and air temperature, and decreases in diffuse radiation and relative humidity (Pounds et al., 1999; Still et al., 1999; Lawton et al., 2001).

Although shifts in many microclimatic variables can affect the health of an epiphyte, increases in the average cloud-base height or the average elevation of cloud formation may be particularly important due to the connection between cloud immersion and vapor pressure deficit (VPD; Gotsch et al., 2017). Increases in canopy VPD, a function of temperature and relative humidity, intensify evaporative demand, which influences the abundance, community composition, and water relations of epiphytes (Gotsch et al., 2017, 2018; Amici et al., 2020). For example, across an elevation and precipitation gradient in a Costa Rican TMCF, epiphyte abundance and diversity were much more strongly correlated with VPD than elevation or precipitation (Gotsch et al., 2017). In another study in the region, Gotsch et al. (2018) found that sap flow in epiphytes was driven more by variation in VPD than precipitation. In cloud forest canopies, the maximum VPD is low (0.2–0.5 kPa), and there are small changes in VPD across sites that have large differences in epiphytic biomass, diversity, and water relations (Amici et al., 2020; Gotsch et al., 2018). These data together suggest that increases in VPD may be a selective agent of epiphyte mortality that causes shifts in the composition and abundance of epiphyte communities.

A decrease in epiphyte abundance is of concern given the ecosystem services that epiphyte communities provide. Epiphyte communities, and the organic soil mats that they create and in which some root, play a significant role in the water cycle via cloud water interception, water storage, and evapotranspiration (Foster, 2001; Köhler et al., 2007; Gotsch et al., 2016; Ah-Peng et al., 2017). Therefore, understanding the responses of epiphytes to drought is a crucial step to anticipate the impacts of their loss to the ecosystem.

Epiphytes, both herbaceous and shrub functional groups, reduce sap flow and stomatal conductance during drought, presumably to reduce the probability of hydraulic

failure (Gotsch et al., 2015, 2017; Darby et al., 2016). Reductions greater than -1.0 MPa in osmotic potential have been documented in epiphytes from the wet to the dry season as causing a reduction of the turgor loss point, demonstrating an additional strategy that at least some epiphytes have to withstand drought (Gotsch et al., 2017). However, functional groups have different degrees of stomatal regulation and drought vulnerability (Darby et al., 2016; Gotsch et al., 2017). In a shadehouse experiment, rates of photosynthesis and stomatal conductance rebounded following a month-long dry period indicating that epiphytes were generally resilient to short episodic droughts (Williams et al., 2020). Although these drought responses can reduce the effects of seasonal drought, they can be insufficient during a severe drought during which the shutdown of sap flow can precede widespread mortality (Darby et al., 2016).

The ability of epiphytes to recover from short periods of drought is likely aided by a continuum of traits related to leaf water storage (Gotsch et al., 2015). Epiphytes with high water-storage capacity, such as succulents, resist drought by releasing stored water to photosynthetically active cells, a process that buffers them from turgor loss (Schmidt and Kaiser 1987; Monneveux and Belhassen, 1996; Herrera et al., 2000; Zhang et al., 2016; Males and Griffiths, 2017). These studies suggest that drought-tolerance strategies might be particularly important in mitigating the effects of short-term drought, but the impact of prolonged periods of drought on epiphytes in this system is not well known.

Plants can also shift allocation patterns from one leaf cohort to the next to better withstand water limitation. Trees in a variety of biomes exhibit shifts in structural traits during drought, which correlated with lower mortality rates (Greenwood et al., 2017). A reduction in stomatal density can also confer greater drought tolerance by reducing the surface area over which water vapor exits stomata (Hepworth et al., 2015; Hughes et al., 2017; Caine et al., 2019). Whether vascular epiphytes can respond to water limitation via shifts in leaf phenotypes is unknown.

Understanding how variation in atmospheric moisture influences epiphyte responses during an extended drought could inform community responses to future microclimate in TMCFs and improve our ability to determine how shifts in epiphyte abundance will impact ecosystem function. Our study addressed the following questions: (1) Can cloud immersion buffer the effects of a prolonged drought on epiphytes? (2) Do epiphytes exhibit plasticity in the expression of leaf functional traits in response to drought? (3) Do drought responses vary among functional groups of vascular epiphytes? In a shadehouse experiment, we subjected common species of vascular epiphytes from a TMCF to a 10-week drought. We documented responses to drought by measuring water relations and shifts in leaf trait phenotypes to determine whether epiphytes have the ability to adjust allocation patterns in response to microclimatic change.

MATERIALS AND METHODS

Study site conditions

This experiment took place in two shadehouses over 10 weeks in 2018. The study coincided with the dry season and the beginning of the wet season (March through mid-May; Nadkarni et al., 1995). One shadehouse was located in a clearing in the premontane wet forest zone just below the current cloud base (hereafter, lower shadehouse [LSH]) and the other in a clearing in a TMCF (upper shadehouse [USH]). By conducting the experiment in the driest part of the year, we subjected the study plants to as extreme a drought as possible (i.e., highest atmospheric VPDs coupled with no added water). We ended the experiment in May, after 10 weeks, due to the return of the rainy season, which brought low-lying clouds and substantial atmospheric moisture in the form of increased cloud cover and precipitation.

The location of the USH within the current cloud layer led to more persistent atmospheric moisture even during the driest time of year (Table 1). The average relative humidity in the USH was 95%, while in the LSH the average was 86%. Temperature ranged from 11.6° to 23.1°C in the USH and 12.8° to 28.4°C in the LSH. During the study period, rainfall was limited. The region received ca. 134 mm of rainfall in March, 114 mm in April, and 294 mm in May (S. G. Gotsch, unpublished data).

Epiphyte species

The epiphytes used in this study were initially collected for use in a 4-week pilot drought experiment that was conducted in the dry season of 2016. Although the drought-treated plants exhibited decreases in stomatal conductance and photosynthesis, nearly all individuals remained healthy and recovered function within 2 weeks after water was returned (Williams et al., 2020). After the initial experiment, epiphytes remained in the shadehouses in well-watered conditions for almost 2 years before undergoing the second experiment. Given the slow growth of epiphytes, the treatment plants did not experience any crowding or rooting limitation (S. G. Gotsch, personal observations).

In preparation for the 2016 experiment, epiphytes were collected with their associated mats of arboreal soil from four host trees in a tropical montane landscape in central Costa Rica. Two of the host trees were located in the Monteverde Cloud Forest Reserve (10°18'19"N, 84°47'39"

W, 1550 m a.s.l.), which had a mean annual temperature, relative humidity, VPD and total rainfall of 17.0°C, 98.2%, 0.038 kPa and 3148 mm, respectively, at the time of the collection (Williams et al., 2020). The other two host trees were located in the Curi Cancha Reserve (10°18'23"N, 84°48'16"W, 1480 m a.s.l.) where mean annual temperature, relative humidity, VPD and total rainfall were 17.9°C, 94.0%, 0.131 kPa, and 2993 mm, respectively, at the time of the collection (Williams et al., 2020).

All host trees were species of *Ficus* and among the largest trees in the area, with continuous coverage of epiphyte mats across branches. We selected sections of main branches for sampling based on qualitative assessments of abundance in the study site to ensure that we had adequate sample sizes of the most common species. Selected epiphyte mats were cut with a hand saw to a length of approximately 60 cm, peeled from the entire branch surface, and lowered to the ground in plastic bags. The epiphyte mats were then transported to the two shadehouses (Williams et al., 2020).

The seven most frequently collected species were *Clusia* sp., *Disterigma humboldtii*, *Elaphoglossum* sp., *Nephrolepis pendula*, *Peperomia* sp., *Pleurothallis dolichopus*, and *Stenospermation sessile* and were the focus of the majority of our measurements. We characterized each species as succulent or nonsucculent, depending on the presence of a distinct layer of water storage cells (i.e., hydrenchyma, Table 2). *Clusia* sp. is a mixture of two species of the same genus, *C. palmana* and *C. flavasepala*, which are difficult to distinguish in the field. *Elaphoglossum* sp. is also a mixture of two species, *E. glabellum* and *E. latifolium*. *Peperomia* sp. is one member of the genus *Peperomia*, which has not yet been positively identified to species. *Clusia* sp. and *Pleurothallis dolichopus* were not present in the upper shadehouse. *Peperomia* sp. was not present in the lower shadehouse. A minimum of three individuals per species per treatment were included in the study; however, as a result of varying growth rates, leaves were not produced each week by all individuals (Table 2).

Experimental design

Two shadehouses were constructed: the LSH in a clearing in the premontane wet forest zone just below the current cloud base and the USH in a clearing in the TMCF (Figure 1). These locations were chosen due to their differences in elevation and atmospheric conditions including temperature, relative humidity, and light availability. The LSH was warmer, drier, and

TABLE 1 Average, minimum and maximum of microclimate variables in the upper and lower shadehouses

Shadehouse	Light ($\mu\text{mol m}^{-2} \text{s}^{-1}$)			Air temp. (°C)			RH (%)			VPD (kPa)		
	Avg	Min	Max	Avg	Min	Max	Avg	Min	Max	Avg	Min	Max
USH	8.5	0	175	16.8	11.6	23.1	95	58	100	0.12	0	1.13
LSH	50	0	315	18.5	12.8	28.4	85.7	41	100	0.39	0	2.06

Notes: Light, solar radiation measured with a porometer; Air temp., air temperature; RH relative humidity; VPD, vapor pressure deficit.

TABLE 2 Focal taxa, functional groups, and sample sizes of epiphytic vascular plants used to measure functional traits during a shadehouse drought experiment in the montane tropics

Species	Family	Functional group	Shadehouse	<i>n</i>
<i>Clusia</i> sp. ^a	Clusiaceae	Succulent	Lower	6
<i>Disterigma humboldtii</i>	Ericaceae	Non-succulent	Upper	13
			Lower	29
<i>Elaphoglossum</i> sp. ^b	Dryopteridaceae	Non-succulent	Upper	17
			Lower	18
<i>Nephrolepis pendula</i>	Nephrolepidaceae	Non-succulent	Upper	26
			Lower	17
<i>Peperomia</i> sp. ^c	Piperaceae	Succulent	Upper	8
<i>Pleurothallis dolichopus</i>	Orchidaceae	Succulent	Lower	15
<i>Stenospermaton sessile</i>	Araceae	Non-succulent	Upper	7
			Lower	12

^a*Clusia* sp. is a mixture of two species of the same genus, *C. palmana* and *C. flavasepala*.

^b*Elaphoglossum* sp. is also a mixture of two species, *E. glabellum* and *E. latifolium*.

^c*Peperomia* sp. is one member of the genus *Peperomia* that has not yet been positively identified to species.



FIGURE 1 (Left) View of the exterior of the upper shadehouse as workers repair the roof. (Right) Student helper waters the experimental plants in the lower shadehouse

brighter than the USH, which in conjunction with the experimental drought, created a higher stress environment that simulated the warmer and drier conditions projected in the region due to climate change (Pounds et al., 1999; Still et al., 1999; Lawton et al., 2001).

In preparation for the study, the canopy light was measured along the harvested branches in each of the host trees using the photodiode on an AP4 Porometer (Delta-T Devices, Cambridge, UK). Hourly from 8:00 to 12:00 hours, 10 measurements were made along each of the canopy branches on three non-rainy mornings, regardless of whether they were sunny or cloudy (S. G. Gotsch, unpublished data). These measurements were then used to select a covering for the shadehouses that would simulate the canopy light environment. A locally sourced, knitted,

green, 70% horticultural shade cloth, under the roof, provided conditions that most closely mimicked the in situ conditions. A mixture of clear and opaque roofing panels was used to exclude rainfall for the duration of the study and to moderate temperature spikes on sunny days. In addition, we found that this type of roof provided the structural integrity needed to withstand the high winds at the study site and to help match the light environment with the canopy. Within both shadehouses, we installed wooden benchtops to simulate the in situ substrate underlying the epiphyte mats. We designated separate benches for the control (manually watered to saturation daily) and drought treatment (unwatered for the duration of the experiment, hereafter, “drought-treated”). Before transplanting, epiphyte mats were sorted by target species and were separated to

evenly distribute the most frequently collected species between treatments in the shadehouses. However, whole mats were transplanted onto the benches rather than sectioning out individual epiphytes to minimize disturbance to the plants. As a result, species distributions were unbalanced across the benches and between shadehouses. In the USH, there were 60 target individuals for the control treatment and 101 drought-treated individuals. In the LSH, there were 84 target individuals for the control treatment and 156 drought-treated individuals.

Because the goal was to assess phenotypic shifts between leaf cohorts, we measured leaves that were produced and matured after the onset of the experimental drought. Leaves were defined as mature if they had achieved the color, size, and texture of the rest of the leaves on the plant. Young leaves were noticeably paler in color, smaller, and softer to the touch. Leaf maturity was tracked by weekly visual inspection of the plants. Small immature leaves were tagged as new growth, visually monitored, and harvested for measurements when the leaf fully expanded. Leaves were produced at different intervals over the 10-week period, depending on the growth rate of the individual. Plants in the USH produced a total of 39 leaves in the control and 54 leaves in the drought treatment, while plants in the LSH produced a total of 50 leaves in the control and 81 leaves in the drought treatment. Epiphytes are generally slow growing, so most study epiphytes produced only one new leaf during the experiment although some did produce as many as three leaves. Additionally, because growth rates varied, leaves were not produced each week by all individuals; therefore, it was not possible to acquire a weekly sample size large enough to include time as a factor in the analyses. The study species also varied in leaf morphology, so not every response variable could be measured on each species.

With the exception of leaf thickness, traits were measured weekly if newly produced mature leaves were available. Traits included water potential, stomatal conductance, specific leaf area (SLA), leaf dry matter content (LDMC), stomatal density, and stomatal length. Most of these variables do not change throughout the lifespan of the leaf. Water potential is not fixed, but its measurement is destructive. To minimize the impact on the study plants, we measured water potential on leaves that were harvested to measure other traits. Stomatal conductance is also not fixed, but it is highly dependent on fixed stomatal properties, so we measured it on newly produced mature leaves as well. Unlike many of the other traits, leaf thickness can vary greatly throughout the lifespan of the leaf due to the shrinking and swelling of hydrenchymal cells (Syvertsen and Levy, 1982, Gotsch et al., 2017, Williams et al., 2020). Therefore, leaf thickness was measured at the conclusion of the experiment and used to compare water loss between the nonsucculent and succulent functional groups after 10 weeks of drought. Since this measurement is nondestructive and quick, leaf thickness was measured on all available individuals in the control and drought beds in each shadehouse.

Shadehouse microclimate

Temperature and relative humidity were recorded every 15 min throughout the experiment using HOBO Pro v2 Data Loggers (Onset Computer Corp., Bourne, MA, USA) inside both shadehouses. Hourly averages for VPD were calculated for each shadehouse (Gotsch et al., 2017). Soil moisture of the epiphyte mats was quantified weekly as volumetric water content (VWC) using a Hydrosense II CS616 Water Content Reflectometer (Campbell Scientific, Logan, UT, USA) outfitted with a 30-cm-long probe. Ten soil moisture measurements were taken in each plant bed, and the probe was inserted at a 45-degree angle to ensure complete submersion. Weekly measurements were scaled to a percentage of the maximum VWC in each shadehouse during the study period to ensure comparable values across the two shadehouses. We averaged the percentages of maximum values to obtain a monthly value for each shadehouse. Weekly data showed consistently higher values in the LSH in comparison to the USH.

Physiological and morphological variables

We measured water potential and stomatal conductance to determine differences in the water relations of control and drought-treated individuals. Water potential was measured with a Scholander-style pressure chamber (Model 1505D Pressure Chamber Instrument, PMS Instrument Co., Albany, OR, USA) at the leaf level (except for *D. humboldtii* for which we measured shoots because the leaves were too small for the pressure chamber). *Nephrolepis pendula* did not generate enough leaves to allow water potential measurements. Stomatal conductance of each newly produced leaf was measured using a dynamic diffusion porometer (AP4, Delta-T Devices, Cambridge, UK). We did not obtain stomatal conductance for *D. humboldtii* because its tiny leaves were incompatible with the porometer. Stomatal conductance was measured at midday before harvesting the leaf from the plant. Once harvested, leaves were placed in plastic bags to prevent water loss, and the water potential was measured immediately upon returning to the lab.

The SLA and LDMC of new leaves were calculated to assess the ability of epiphytes to respond to drought by altering resource allocation (Poorter and Remkes, 1990; Poorter and de Jong, 1999; Wilson et al., 1999). The SLA was calculated as the fresh leaf area divided by leaf dry mass. The LDMC was calculated as the dry mass of the leaf divided by its fresh mass. Each leaf was weighed immediately following water potential measurements. We measured fresh leaf area with a portable leaf area meter (CI-202, CID Bio-Science, Camas, WA, USA). Each leaf was dried at 60°C until mass was constant, then weighed.

Stomatal density and size, traits that determine maximum theoretical stomatal conductance and relate to drought tolerance (Franks and Beerling, 2009; Hughes

et al., 2017), were quantified by first applying a thin layer of clear nail varnish on the bottom surface of the leaf. Once dried, the varnish was peeled from the leaf surface, and the impressions were viewed and digitally photographed at 40–400× magnification with a compound light microscope (Motic BA210E; Richmond, BC, Canada). Three impressions were made per leaf; the location of the impression was approximately equidistant between the midrib and the outer edge of the leaf. Impressions were made on 1–3 leaves per individual depending on the availability of leaves. We measured the density and length of the stomata using ImageJ (Rasband, 1997), and the values of each impression were averaged to obtain a value for each leaf.

We also measured the thickness of the leaf lamina at approximately the midpoint between the midrib and the outer edge of the leaf. This trait is highly correlated with leaf water potential and has been suggested as a tool to monitor water stress (Syvertsen and Levy, 1982). We measured the thickness of four leaves per individual and averaged those values using a thickness gauge (Mitutoyo USA Model 547-500S; Aurora, IL, USA). Between 91 and 252 individuals were measured per treatment in each shadehouse for leaf thickness.

We also visually assessed the overall health of all plants in the upper ($n = 103$) and lower shadehouses ($n = 158$) throughout the experiment. Before the initiation of the study, the green and the yellowing or desiccating leaves were counted on each plant to calculate the percentage of leaves that were green and healthy. In this way, leaves undergoing normal senescence at the beginning of the experiment were not associated later with leaf shedding or desiccation due to drought.

Statistical analyses

All response variables, with the exception of plant health, were analyzed separately for each shadehouse due to unequal species distributions and sample sizes. Leaf water potential, stomatal conductance, and leaf thickness were log-normally distributed. These traits were evaluated using mixed-model ANOVAs, to analyze the effects of treatment (fixed) and species (random). In addition, differences between the control and drought groups were evaluated for each species using ANOVAs. The SLA, LDMC, stomatal density, and stomatal length were not normally distributed. Differences between the control and treatment groups were evaluated using Wilcoxon tests on species averages in each shadehouse.

We also examined differences in the effect of drought on succulent versus nonsucculent species. For leaf water potential, leaf thickness, and stomatal conductance, we performed mixed-model ANOVAs for each shadehouse to analyze the effects of treatment and succulence and their interaction. In addition to these fixed factors, species was included as a random factor in the analyses. For the rest of the traits, which were not normally distributed, trait

comparisons for the well-watered versus drought-treated plants were examined in succulent and nonsucculent groups using Wilcoxon tests on species averages.

Data for plant health were analyzed only for drought-treated individuals since all control plants remained 100% healthy throughout the experiment. Plant health data were not normally distributed; therefore, a Wilcoxon test was conducted for each week of the experiment to test for significant differences in plant health of drought-treated individuals between the two shadehouses. Means \pm the standard error of the mean are presented. Analyses were conducted using JMP v. 10 (SAS Institute, Cary, NC, USA).

RESULTS

Shadehouse microclimate

Mean VPD in the LSH (0.35 ± 0.04 kPa) was approximately three times higher than in the USH (0.11 ± 0.02 kPa) throughout the study and highest in both shadehouses in April (Figure 2A). The lowest VPD in both shadehouses was in May, coinciding with the beginning of the wet season (LSH 0.28 ± 0.04 kPa; USH 0.08 ± 0.02 kPa). The difference in the soil volumetric water content between the control and drought groups increased with the progression of the dry

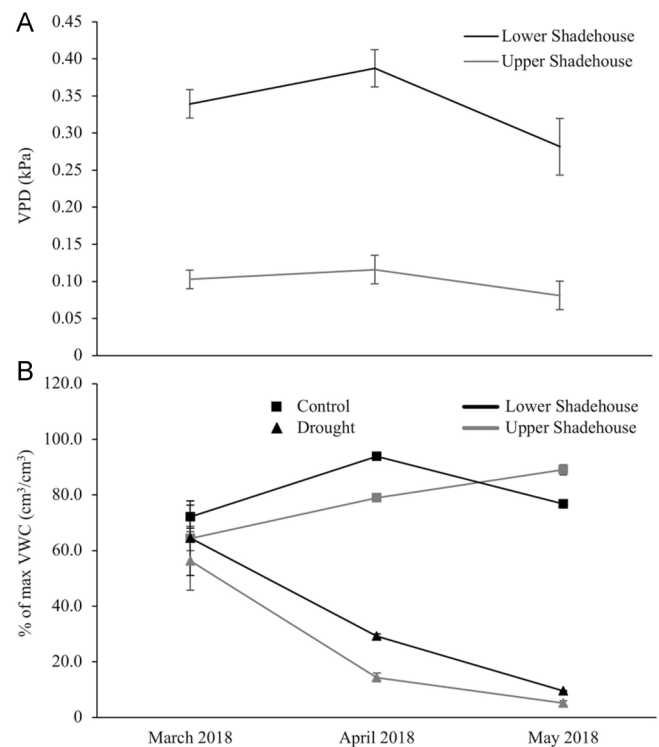


FIGURE 2 (A) Mean vapor pressure deficit (± 1 SE; A) in the two shadehouses, one located in the premontane wet forest (lower shadehouse) and one located in the tropical montane cloud forest (upper shadehouse), during the 10-week drought experiment. (B) Average percentage of maximum volumetric soil water content (VWC; ± 1 SE) of the control and drought-treatment beds in the lower and upper shadehouses

season (Figure 2B), with the lowest values in May toward the end of the experiment (USH: control $76\% \pm 1$, drought $9\% \pm 0.2$; LSH: control $89\% \pm 2$, drought $5\% \pm 0.8$).

Leaf traits and plant health

Leaf water potential (Ψ_l) and stomatal conductance (g_s) were significantly lower in the drought treatment in both shadehouses (USH, Ψ_l : $F_{1,45.84} = 21.98$, $P \leq 0.0001$ and g_s : $F_{1,71.12} = 13.66$, $P = 0.0004$; LSH Ψ_l : $F_{1,97.74} = 90.31$, $P \leq 0.0001$ and g_s : $F_{1,64.38} = 120.09$, $P \leq 0.0001$). Mean leaf water potential of the drought-treated individuals in both shadehouses was significantly lower than for the control groups, and water potentials were lower for the drought-treated plants in the LSH (-3.29 ± 0.27 MPa) than in the USH (-1.41 ± 0.18 MPa) (Figure 3A). Stomatal conductance was low for drought-treated individuals in both shadehouses (LSH 9.75 ± 2.62 mmol m⁻² s⁻¹; USH 11.41 ± 1.60 mmol m⁻² s⁻¹), and stomatal conductance of the control individuals in the USH (30.50 ± 6.90 mmol m⁻² s⁻¹) was less than half that in the LSH (83.74 ± 11.40 mmol m⁻² s⁻¹) (Figure 3B).

Leaf dry matter content was significantly higher for drought-treated individuals in both shadehouses (Figure 4;

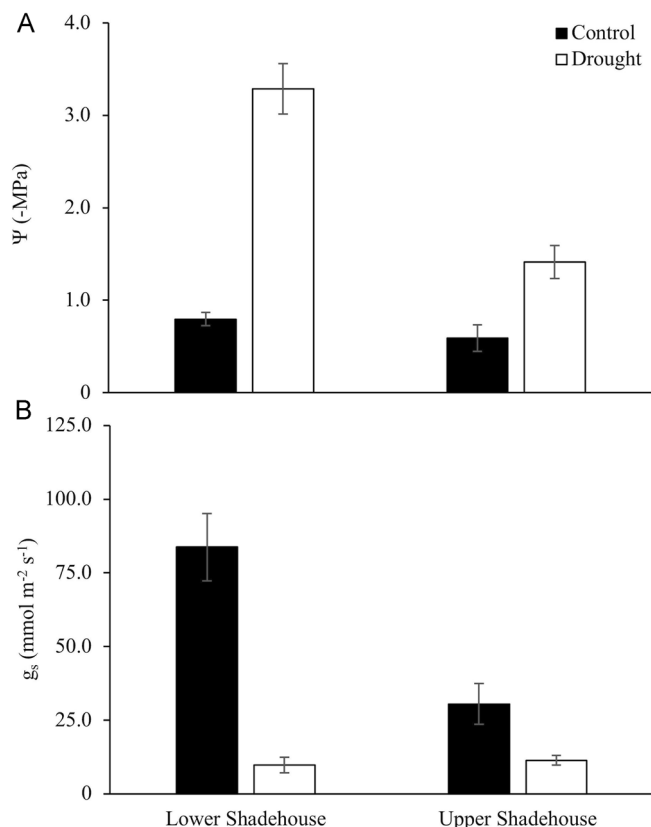


FIGURE 3 Mean water potential (± 1 SE; A) and stomatal conductance (± 1 SE; B) for epiphytic vascular plants in the 10-week drought treatment. Measurements were taken on newly mature leaves produced after the initiation of the drought treatment and were averaged across species over the 10 weeks

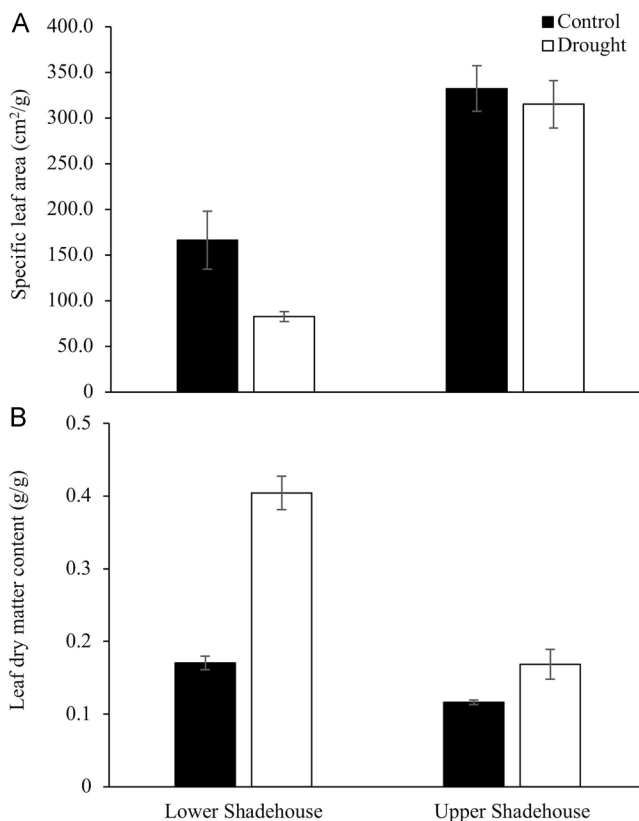


FIGURE 4 Mean specific leaf area (± 1 SE; A) and leaf dry matter content (± 1 SE; B) for individuals during a 10-week drought experiment. Measurements were taken on newly mature leaves produced after the initiation of the drought treatment and were averaged across species and measurement periods

LSH: $\chi^2 = 5.02$, $df = 1$, $P = 0.025$; USH: $\chi^2 = 3.93$, $df = 1$, $P = 0.047$). Drought-treated individuals in the LSH exhibited a mean LDMC of 0.40 ± 0.02 g/g (in comparison with 0.17 ± 0.01 g/g for control plants), while in the USH the mean was 0.17 ± 0.02 g/g (in comparison with 0.11 ± 0.003 g/g for control plants). This shift amounted to a 68% increase in structural carbon allocation in the LSH and a 42% increase in the USH. In the LSH, drought-treated individuals had an SLA of 82.42 ± 5.58 cm²/g, while the control individuals and an SLA of 166.19 ± 31.67 cm²/g; thus, newly mature leaves of the drought-treated plants were 67% smaller and denser than in the USH, though these differences were not significant when evaluated for the small number of species averages using a Wilcoxon test. In contrast, SLA was similar for both treatment groups in the USH (Figure 4). Stomatal density and length did not vary significantly between the control and treatment in either shadehouse.

Leaves were significantly thinner on drought-treated than control plants in both the lower ($F_{1,1550} = 406.19$, $P \leq 0.0001$; 0.66 ± 0.05 mm vs. 0.84 ± 0.04 mm) and upper shadehouses ($F_{1,949.2} = 103.14$, $P \leq 0.0001$; 0.30 ± 0.15 mm vs. 0.38 ± 0.02 mm). Leaf retention on drought-treated plants did not differ between the two shadehouses in the

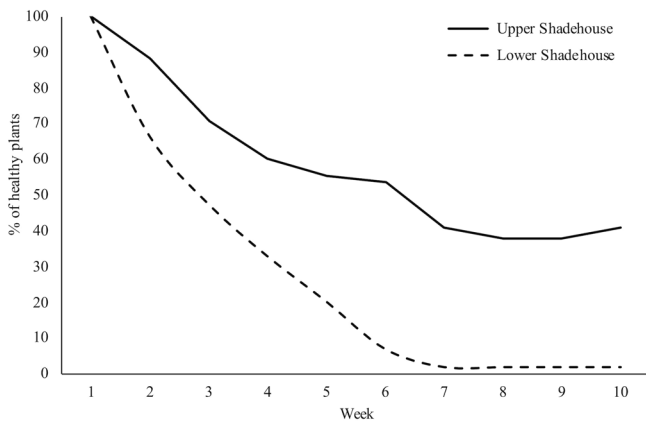


FIGURE 5 Proportion of healthy plants in the drought treatments in the lower ($n = 158$) and upper shadehouses ($n = 103$). The health assessment of the control individuals remained relatively constant at 100% health in both shadehouses (data not shown). Health decline resulted from chlorosis, desiccation, necrosis, and/or senescence of leaves. Since each week is a calculated proportion, no error bars are reported

first week of the experiment (Figure 5). However, the overall health of the drought-treated individuals consistently decreased throughout the experiment in both shadehouses, with the drought-treated individuals of the lower shadehouse exhibiting a more severe response to the drought ($P \leq 0.001$ for weeks 2–10). At the end of the experiment, 43% of the individuals in the upper shadehouse and only 2% of the individuals in the lower shadehouse were similar to their baseline assessment.

Drought effects on succulent vs non-succulent epiphytes

We found differences in the drought responses between succulent and nonsucculent epiphytes. In the LSH, leaf water potential of both succulent and nonsucculent species was significantly reduced in response to drought (Figure 6C; effect of treatment: $F_{1,96,9} = 4.71$, $P \leq 0.0001$). However, nonsucculent individuals had a much greater average reduction in water

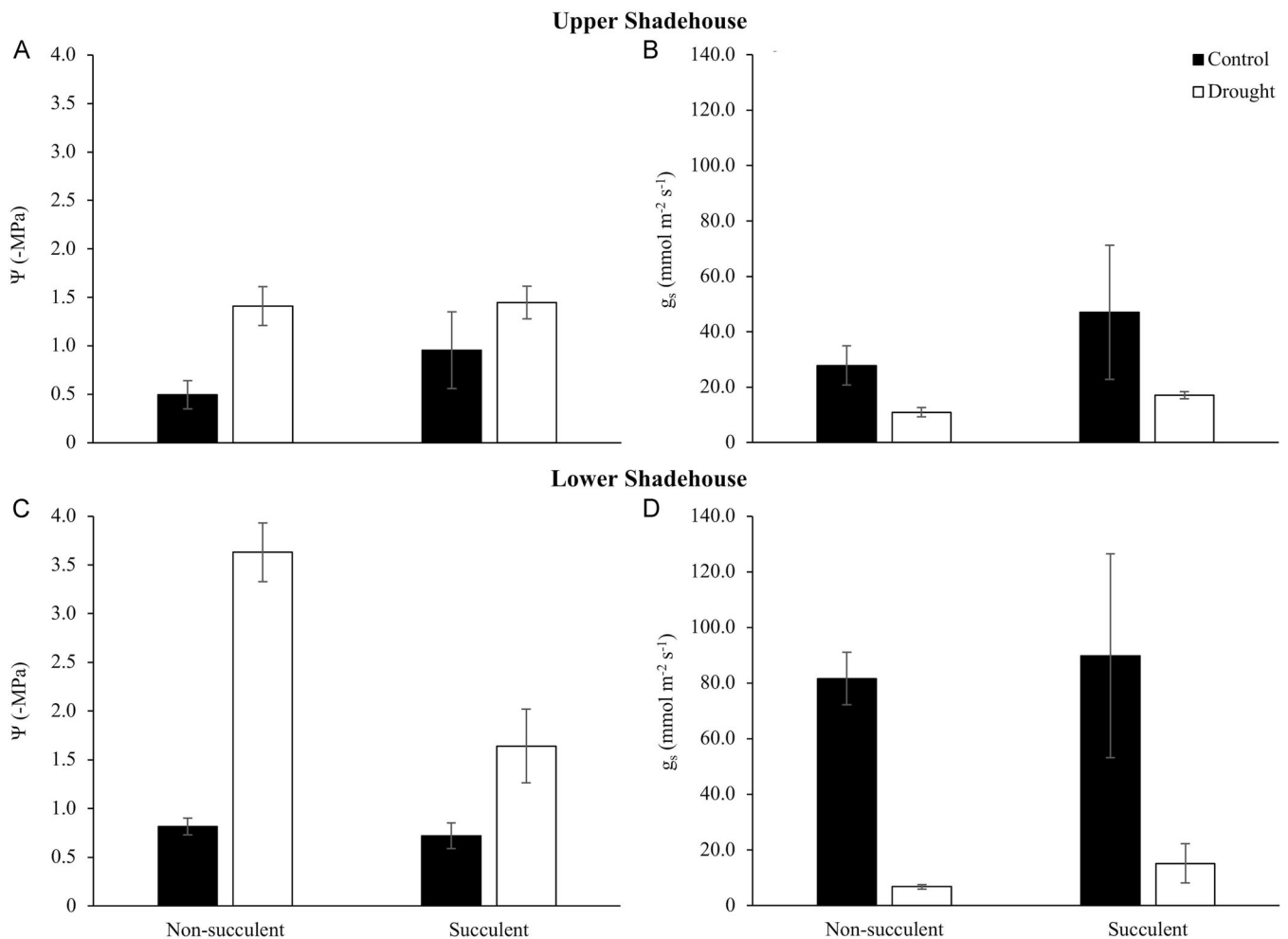


FIGURE 6 Mean water potential (± 1 SE; A, C) and stomatal conductance (± 1 SE; B, D) of nonsucculent and succulent epiphytes in the upper and lower shadehouses during the 10-week drought experiment. Traits of newly mature leaves that were produced after the start of the drought treatment and were averaged across species and measurement periods

potential (-3.63 ± 0.30 MPa) than in succulent plants (-1.64 ± 0.38 MPa). In the USH, although the overall effect of the treatment was significant, the interaction term was also significant, indicating that succulent and nonsucculent species responded differently to the drought (effect: treatment, $F_{1,44.24} = 16.58$, $P = 0.0002$; treatment succulence, $F_{1,96.9} = 4.71$, $P = 0.0323$) (Figure 6A). Nonsucculent individuals that underwent drought in the USH had lower water potentials than in the nonsucculent control plants (control -0.49 ± 0.15 MPa; drought -1.41 ± 0.20 MPa; Figure 6A), while drought had no effect on the water potential of succulents in the USH (Figure 6A). Average reductions in water potential were much greater for the nonsucculent individuals in the LSH compared to the USH (-3.63 MPa vs. -1.41 MPa, respectively). In both shadehouses, drought caused a significant reduction in stomatal conductance, regardless of whether the plants were succulent (effect of treatment: USH $F_{1,69.97} = 4.9$, $P = 0.03$; LSH $F_{1,63.08} = 93.28$, $P < 0.0001$) (Figure 6B, D). Although the stomatal conductance of nonsucculent individuals in the control groups exhibited different average stomatal conductance values between shadehouses (LSH 81.62 ± 9.46 mmol m⁻² s⁻¹ vs.

USH 27.83 ± 7.09 mmol m⁻² s⁻¹), the drought-treated nonsucculent individuals regulated gas exchange to a similar extent in both shadehouses (LSH 6.75 ± 0.79 mmol m⁻² s⁻¹ vs. USH 10.94 ± 1.71 mmol m⁻² s⁻¹) (Figure 6B, D).

In the LSH, LDMC was higher for both succulent and nonsucculent drought-treated individuals in both shadehouses. These differences were significant for the nonsucculent individuals in the USH and the succulent individuals in the LSH (nonsucculents in USH: $\chi^2 = 3.85$, $df = 1$, $P = 0.049$, succulents in LSH: $\chi^2 = 3.82$, $df = 1$, $P = 0.049$) (Figure 7B, D). Although on average, the nonsucculent individuals of both shadehouses produced structurally denser leaves during the drought, leaves produced by individuals in the LSH were substantially denser (0.44 ± 0.03 g/g) than those in the USH (0.17 ± 0.02 g/g). In the LSH, drought also led to a substantial reduction in the average SLA in both succulent (85.88 ± 10.96 cm²/g vs. 64.54 ± 8.32 cm²/g) and nonsucculent (181.49 ± 37.25 cm²/g vs. 86.96 ± 6.58 cm²/g) plants; however, due to large variation across species, these differences were not statistically significant (Figure 7C). In the USH, SLA was similar between nonsucculent and succulent functional groups

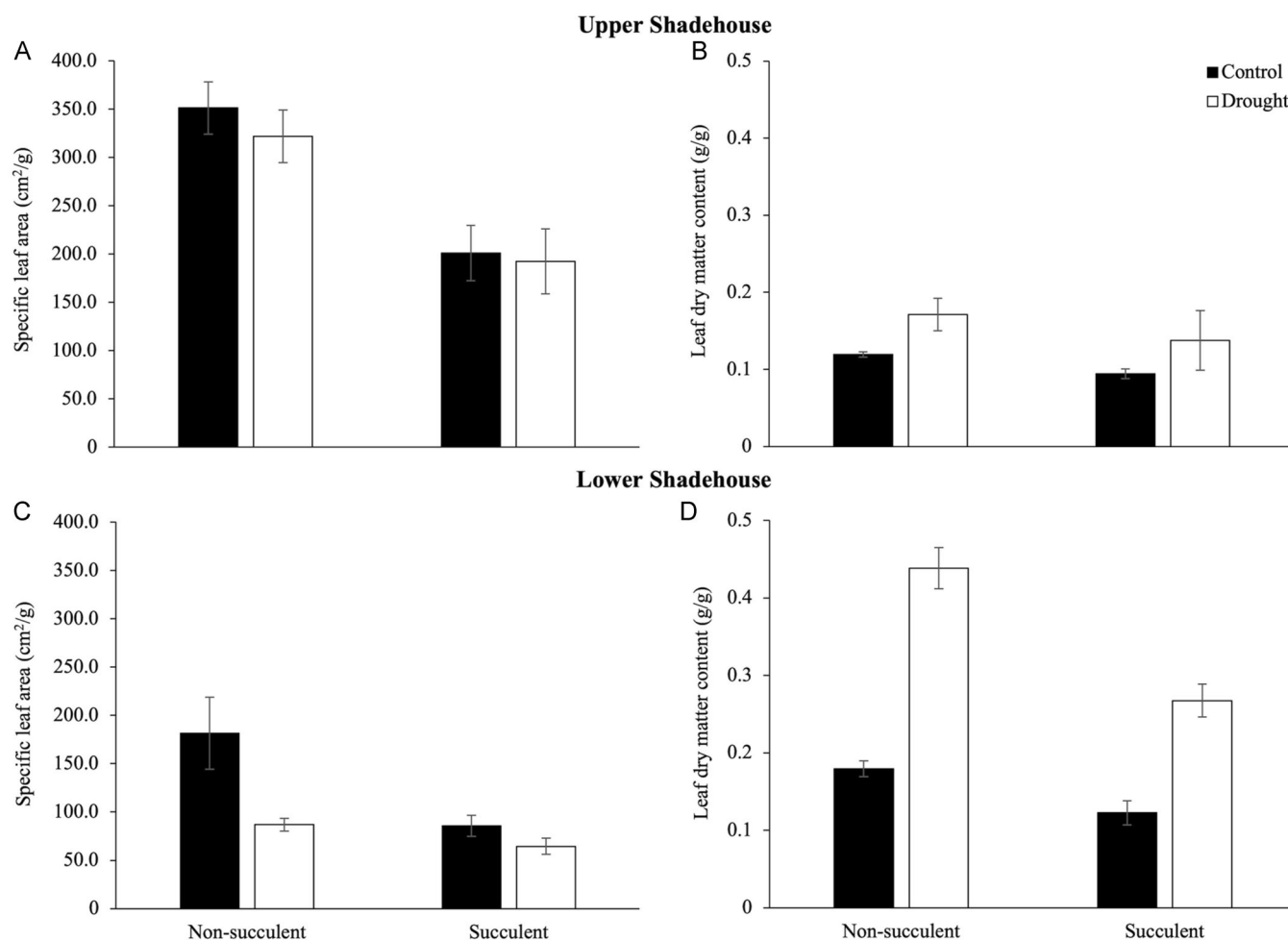


FIGURE 7 Mean specific leaf area (± 1 SE; A, C) and leaf dry matter content (± 1 SE; B, D) of nonsucculent and succulent epiphytes in the upper and lower shadehouses during the 10-week drought experiment. Traits of newly mature leaves that were produced after the start of the drought treatment were measured

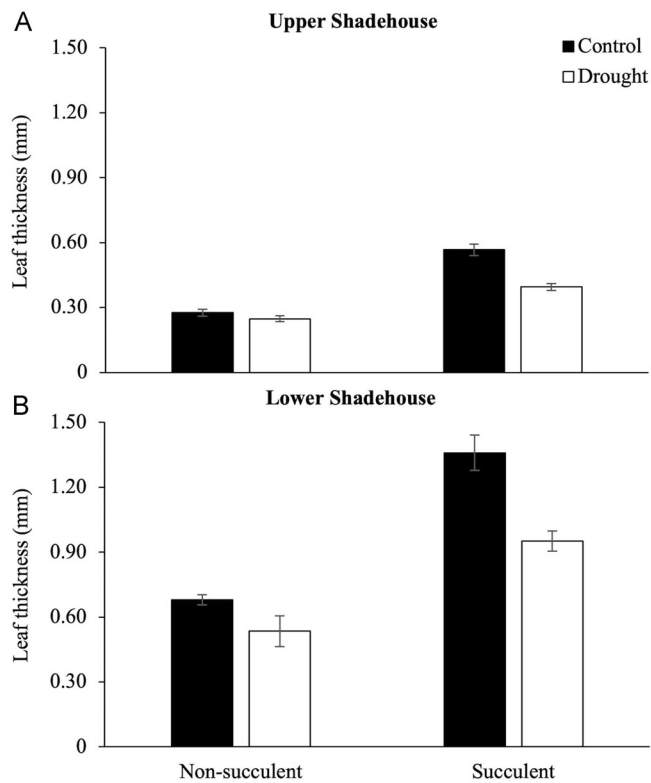


FIGURE 8 Mean leaf thickness (± 1 SE) of nonsucculent and succulent epiphytes in the upper (A) and lower (B) shadehouses measured at the end of a 10-week drought experiment

(Figure 7A). Neither stomatal density nor stomatal length were significantly different between treatments for either functional group in either shadehouse (data not shown).

In both shadehouses, drought and succulence had a significant impact on leaf thickness, and a significant interaction term indicated that succulence influenced the drought response (USH: $F_{1,949.2} = 103.14$, $P < 0.0001$; succulence $F_{1,29.96} = 10.36$, $P = 0.0031$; treatment \times succulence: $F_{1,41.4} = 21.93$, $P < 0.0001$. LSH: $F_{1,1550} = 406.19$, $P < 0.0001$; succulence $F_{1,29.96} = 10.36$, $P = 0.0031$; treatment \times succulence: $F_{1,1550} = 19.88$, $P < 0.0001$). Drought-treated succulents had leaves that were on average 30% thinner than the control groups in the USH (0.39 ± 0.02 mm vs 0.57 ± 0.03 mm) and the LSH (0.95 ± 0.05 mm vs 1.36 ± 0.08 mm) (Figure 8A, B). Drought-treated nonsucculent species had a 21% average reduction in leaf thickness in the lower shadehouse (Figure 8B; 0.53 ± 0.07 mm vs 0.68 ± 0.02 mm). In contrast, the leaves of drought-treated nonsucculent species in the upper shadehouse had only a 9% decrease in thickness (Figure 8A; 0.28 ± 0.02 mm vs 0.25 ± 0.01 mm).

DISCUSSION

Exposure of vascular epiphytes to a severe and extended dry period yielded insights regarding drought responses of different functional groups. Atmospheric moisture played a

substantial role in offsetting the effects of drought stress, and succulent epiphytes were more tolerant than nonsucculent epiphytes to prolonged drought. The phenotypic plasticity in the functional traits of the epiphytes may be important indicators of species-level success in the TMCF, where climate is changing rapidly. Drought led to substantial changes in LDMC in nonsucculent species, while succulent species exhibited minimal shifts in leaf traits.

The importance of atmospheric moisture in epiphyte resistance to drought

Drought-induced decreases in stomatal conductance and water potential occurred in both shadehouses, and the reductions were much greater for plants in the LSH (Figure 3). Since the epiphyte mats in both shadehouses were equally dry by the end of the experiment (Figure 2B), these differences indicate that plants in the USH were able to maintain water status with the moisture provided by low-lying clouds that occur frequently in the TMCF. In the LSH, plants experienced a warmer, sunnier, and drier atmosphere, resulting in higher evaporative demand and greater water stress.

In cloud forest communities, frequent interception of fog and mist can be a major source of water, both as inputs to the rooting substrate as well as via foliar water uptake (FWU). For example, cloud water contributed up to 31% of total water input in a spruce–fir dominated forest in the southern Appalachian Mountains and up to 34% of the total water input in a redwood-dominated forest in northern California (Dawson, 1998; Berry et al., 2014). In the TMCF, FWU is common in both trees as well as epiphytes, and this process is important for water balance and drought avoidance (Goldsmith et al., 2013; Gotsch et al., 2014, 2015; Darby et al., 2016). To date, all species of epiphytes tested have the capacity for FWU (Gotsch et al., 2015, Darby et al., 2016). Furthermore, FWU can lead to an average foliar reabsorption rate of 70% of the transpired water during periods when cloud cover and atmospheric moisture are high, suggesting that low-lying clouds are important to maintaining water balance in this community (Gotsch et al., 2015). Foliar water uptake is also associated with physiological benefits such as higher predawn water potentials and higher net photosynthetic rate (Carmichael et al., 2020). In the USH, frequent cloud immersion likely promoted FWU by aiding in the maintenance of water status during the drought.

In the USH, stomatal regulation presumably coupled with FWU led to a much smaller decrease in water potential (Figure 3A). Although drought-treated epiphytes in the USH maintained higher water potentials, stomatal conductance remained low throughout the experiment (Figure 3B). This finding indicates that regardless of water status, epiphytes during drought may respond with actions that conserve water instead of acquiring carbon (Williams et al., 2020). The consequences of long-term stomatal

closure have not yet been established for this community but is likely to have important implications for growth and development. Although drought clearly triggered stomatal closure, stomatal conductance was lower even for the control in the USH. These results indicate that cloud forest epiphytes maintain water status during drought by a combination of reduced photosynthetic demand due to lower light conditions and foliar uptake of atmospheric moisture, which can directly offset water loss (Gotsch et al., 2015; Bittencourt et al., 2019). Another possible explanation for the reduction in stomatal conductance is a switch to CAM photosynthesis since the C_3 -CAM intermediate photosynthetic pathway has been documented in some epiphytes (Silvera et al., 2010), although we did not assess photosynthetic pathways in this study. Intermediate pathways are not likely to be pervasive in this community, however, due to the presence of high atmospheric moisture (Silvera et al., 2010; S. G. Gotsch, unpublished data).

The microclimate in the LSH can be seen as a model for the projected changes expected in the TCMF. This region is experiencing longer dry periods and increases in the average cloud base height (Pounds et al., 1999; Still et al., 1999; Helmer et al., 2019). In this study, drought was greatly exacerbated by the warmer and drier atmosphere of the LSH, which induced large reductions in water potential and overall health (Figures 3A, 5). Although epiphyte health will likely be affected by any microclimatic changes leading to drought, our results indicate that a rising cloud base may be particularly challenging to epiphyte communities in the TCMF.

Trait plasticity in epiphytes

In addition to altering water relations traits, the prolonged drought, coupled with the drier conditions in the LSH, also led to substantial increases in LDMC (Figure 4). An increase in LDMC may be due to additional allocation to cell walls, which has been associated with a higher water-use efficiency and would have been beneficial in the warmer and drier environment of the LSH (Wilson et al., 1999; Garnier et al., 2001; Devi et al., 2011; Wellstein et al., 2017).

In the USH, atmospheric moisture inputs combined with stomatal closure may have been sufficient to mitigate the effects of drought. This response may explain why we documented no difference in SLA and only a modest increase in LDMC in plants that underwent drought in comparison with individuals in the LSH (Figure 4). Since SLA and LDMC tend to be correlated, the increase in LDMC in the USH, in the absence of a change in SLA, is likely indicative of less water being stored in the leaf rather than a structural change to the leaf. Changes in leaf thickness, a trait related to leaf water storage in vascular epiphytes (Gotsch et al., 2015), can help explain the differences in trait plasticity between the two shadehouses. In the USH, the leaf thickness of succulent individuals that experienced drought was 30% less than that of leaves on

control plants, indicating a large reduction in stored water, which would have also led to an increase in LDMC (Figure 8B). It is likely that the mild water stress of the USH was not severe enough to trigger changes in leaf morphology and that the modifications to water potential and stomatal conductance were sufficient to mitigate drought effects.

These findings indicate that epiphytes may avoid or tolerate water stress via the sequential deployment of traits and behaviors depending on drought severity. Under mild drought conditions, stomatal regulation and the release of stored water may be sufficient to maintain water status, whereas under more severe conditions, shifts in structural traits such as SLA may be needed to minimize water loss. The production of smaller and denser leaves seems to be a response to drought in this community. We did not find evidence of plasticity in stomatal traits in this experiment.

Variation in drought responses of succulent and nonsucculent epiphytes

Access to atmospheric moisture may have been the most important driver of epiphytic responses to drought in this experiment. However, we also found that the magnitude and type of response depended on leaf succulence, especially in the absence of consistent cloud immersion.

In the LSH, succulent epiphytes exhibited a 2-fold increase in LDMC and a 35% reduction in leaf thickness but no change in SLA (Figures 7C, 7D, 8B). Leaves of control plants of these species were on average more than two times thicker than the leaves of nonsucculent individuals, indicating their capacity for substantial leaf water storage. A comparison of water loss in the tissues of a succulent plant species found significantly greater water loss from hydrenchymal tissue in comparison with chlorenchymal tissue, which allowed for continued photosynthetic activity (Schmidt and Kaiser, 1987). In our study, we found significant decreases in leaf thickness of succulent epiphytes, possibly indicating a similar phenomenon of internally stored water being released to the more chlorophyll-rich cells to maintain water status during drought. In fact, the water potential of drought-treated succulent species in the LSH reached an average of only -1.7 MPa in comparison with -3.7 MPa for nonsucculent epiphytes. By the storage and release of water within the leaf and reducing leaf water loss, succulent plants could maintain sufficient turgor without additional changes to leaf structure (Figures 6 and 7).

In contrast, nonsucculent epiphytes exhibited a distinct structural response to drought. While nonsucculents also closed stomata to avoid drought (Figure 6D), they additionally exhibited significant changes in leaf structure (Figure 7C, D). Leaf thickness of these individuals also decreased; but the reduction was modest and insufficient to offset water loss, which led to a large reduction in leaf water potential (Figure 6C). Newly produced leaves of these

species were smaller and denser, which may have conferred either higher water-use efficiency or enhanced tolerance to lower water potentials (Wellstein et al., 2017). Dry season osmotic adjustment in TMCF epiphytes can depress the turgor loss point to as low as -3.5 MPa, and this shift appears to be more pronounced in drier microclimates (Gotsch et al., 2018). In the LSH, average leaf water potential of nonsucculents fell below -3.6 MPa, suggesting that the prolonged drought in our experiment may have pushed these nonsucculent epiphytes beyond turgor loss and potentially close to the point of hydraulic failure. As a result of such extreme water stress, structural shifts in leaf traits may have been triggered to avoid mortality. Together, these results indicate that leaf water storage is likely a key trait in determining drought resistance in the vascular epiphyte community of this TMCF.

Overall, the warmer and drier environment of the lower shadehouse induced phenotypic changes in the nonsucculent epiphytes, which lack the buffering capacity to maintain high water potentials. For succulent individuals, the ability to store water in hydrenchymal cells and then release stored water to more vital components of the leaf during drought represents an important strategy to mitigate water limitation. However, these species could still be vulnerable to conditions when both drought and a high VPD are combined. Such climatic conditions can cause widespread mortality in the epiphyte community, including a number of succulent species as documented in an experimental drought using TMCF epiphytes by Darby et al. (2016).

CONCLUSIONS

The prolonged drought induced by our experiment caused substantially different effects on epiphytic species depending on their ability to store water. The most striking result was the dependence on access to direct contact with low-lying clouds, rather than precipitation. Drought-treated epiphytes in the USH remained healthy due to a lower evaporative demand and presumably by using atmospheric inputs of water. This location currently supports extremely high biomass and diversity of epiphytes but is within 100 m of the height of the current cloud base and will likely be among the sites impacted by the projected contraction of TMCFs in the neotropics (Helmer et al., 2019). In the LSH, the absence of cloud immersion led to a warmer and drier microclimate, which elicited responses that differed based on leaf succulence. Nonsucculent epiphytes in the LSH are likely more susceptible to water stress even though structural traits shifted, since the leaf water potentials measured in this study were at or near the turgor loss point measured in a previous study (Gotsch et al., 2018). Succulent epiphytes, however, better withstood the effects of drought by virtue of internal leaf water stores. While this study highlights variation in drought responses, most drought-treated plants in the LSH showed visible signs of water stress, indicating that both groups are vulnerable to

extended drought. All epiphytes are likely vulnerable to substantial changes in climate, but nonsucculent epiphytes may be more vulnerable, and their loss from the community may occur with more subtle changes in precipitation patterns or atmospheric humidity. Nonsucculent epiphytes represent hundreds of species in this system and comprise a substantial component of the leaf area and biomass in the canopy (Haber, 2000, Amici et al., 2020). A loss of this group would have considerable ramifications for cloud water interception as well as water and nutrient cycling.

ACKNOWLEDGMENTS

We thank Rikke Reese Næsborg, Keylor Muñoz, and Riley Fortier for assisting with data collection and fieldwork and the anonymous reviewers for their thoughtful comments. We also thank the following individuals and institutions for hosting the shadehouse as well as access to the properties: The Monteverde Institute (Deborah Hamilton, Former Executive Director) and Centro Científico Tropical (Yoryi Mendez, Director of Research). Funding for this project was provided by the National Science Foundation and Franklin and Marshall College (Gotsch IOS Award #1556289, Nadkarni IOS Award #1556319, Dawson IOS Award #1557333).

AUTHOR CONTRIBUTIONS

S.G.G., T.E.D., and N.N. secured funding for the research. C.B.W. and S.G.G. designed the experiment. C.B.W. managed the data collection and fieldwork. C.N.B. and H.W. collected data and performed the initial analyses. B.N.F. and S.G.G. performed the final analyses and wrote and revised the manuscript. All authors provided feedback on the manuscript prior to submission.

DATA AVAILABILITY STATEMENT

Data are available on Figshare at <https://doi.org/10.6084/m9.figshare.16823362>.

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How to cite this article: Ferguson, B. N., S. G. Gotsch, C. B. Williams, H. Wilson, C. N. Barnes, T. E. Dawson, and N. M. Nadkarni. 2022. Variation in cloud immersion, not precipitation, drives leaf trait plasticity and water relations in vascular epiphytes during an extreme drought. *American Journal of Botany* 109(4): 1–14.

<https://doi.org/10.1002/ajb2.1833>