Intracanopy adjustment of leaf-level thermal tolerance is associated with microclimatic variation across the canopy of a desert tree (*Acacia papyrocarpa*)

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Abstract

Tree crowns are spatially heterogeneous, sometimes resulting in significant variation in microclimate across the canopy, particularly with respect to temperature. Yet it is not known whether such localised temperature variation equates to intracanopy variation in leaf-level physiological thermal tolerance. Here, we studied whether microclimate variation across the canopy of a dominant desert tree equated to localised variation in leaf thermal thresholds (T_{50}) among four canopy positions: upper south, upper north, lower south, lower north. Principal component analysis was used to generate a composite climatic stress variable (C_{STRESS}) from canopy temperature, vapour pressure deficit, and relative humidity. We also determined the average number of days that maximum temperatures exceeded the air temperature equating to this species' critical threshold of 49 °C (AT_{49}). To estimate how closely leaf temperatures track ambient temperature, we predicted the thermal time constant (τ) for leaves at each canopy position. We found that C_{STRESS} and AT_{49} were significantly greater in lower and north-facing positions in the canopy. Differences in wind speed with height resulted in significantly longer predicted τ for leaves positioned at lower, north-facing positions. Variation in these drivers was correlated with significantly higher T_{50} for leaves in these more environmentally stressful canopy positions. Our findings suggest that this species may optimise resources to protect against thermal damage at a whole-plant level. They also indicate that, particularly in desert environments with steep intracanopy microclimatic gradients, whole-plant carbon models could substantially under- or overestimate productivity under heat stress, depending on where in the canopy T_{50} is measured.

Keywords Canopy microclimate · Desert plants · Heat stress · Leaf plasticity · Thermotolerance

Introduction

Temperature is one of the most influential climate variables driving the physiological responses of plants (Hikosaka et al. 2006; Teskey et al. 2015). Outside of their optimum

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thermal range, plants experience thermal stress, which can impair growth, survival and reproductive output (Bauerle et al. 2007; Bernacchi et al. 2009; Pearcy et al. 1987; von Caemmerer et al. 2009). In many regions, an increase in the frequency and intensity of maximum and minimum temperatures is expected under climate change (IPCC 2014). Conditions eliciting heat stress in plants are therefore likely to occur more frequently and will be longer lasting than current episodes of stress (Teskey et al. 2015). The photosynthetic machinery within the leaves of plants, in particular, photosystem II (PSII), is especially sensitive to thermal change (Georgieva and Yordanov 1994; Havaux et al. 1991; Schrader et al. 2004). Measures of a plant's photosystem thermal damage threshold can be a useful index for gauging high-temperature tolerance. One measure of the thermal damage threshold is the temperature causing a 50% decline in maximum quantum yield of PSII (T_{50}), corresponding to the onset of irreparable thermal damage to PSII (Curtis

et al. 2014; Knight and Ackerly 2003a). The critical thermal threshold of plants is highly dynamic and varies not only with species, but also through time and space (Curtis et al. 2016; Knight and Ackerly 2003a). Spatial variation reflects adaptation to a particular thermal environment represented by different biomes (Knight 2010; Knight and Ackerly 2003a, b) and microhabitats within biomes (Curtis et al. 2016). What is not known is whether leaf thermal thresholds are influenced by finer scale environmental changes; for example, within a single plant canopy.

Tree crowns are spatially heterogeneous, sometimes resulting in significant variation in the microclimate of individual leaves across a given canopy. Incident sunlight is highest for equatorial-facing foliage and it typically increases and humidity decreases along a vertical profile from the bottom to the top of a canopy (Niinemets and Valladares 2004; Russell et al. 1990). Given only these parameters, in the southern hemisphere, upper canopy and north-facing foliage might be expected to be exposed to higher average air temperatures and greater vapour pressure deficits (VPD) than foliage elsewhere in the canopy (Eamus 2006; Niinemets 2012; Niinemets and Anten 2009). Wind speed also strongly affects the thermal environment of a leaf (Niinemets et al. 1999). Even gentle wind speeds, e.g. above 0.5 m s^{-1}, are sufficient to disturb the leaf boundary layer and increase the rate at which heat is transferred away from the surface of a leaf via convection. Yet wind speed is highly dynamic, fluctuating on the order of seconds or minutes (Vogel 2009). When air movement drops, leaf temperatures may increase rapidly (Leigh et al. 2012). The latter scenario is more likely if the thermal time constant of a leaf is short and/or if a protracted lull in wind speed is coupled with conditions of high light and/or high temperature.

The diversity of fine-scale environmental conditions drives significant intracanopy variation in leaf morphology and can profoundly influence leaf-level physiological and developmental processes (Bauerle et al. 2007; Niinemets 2007; Zwieniecki et al. 2004). For example, due to microclimatic changes in light, temperature and VPD, leaf photosynthetic capacity can vary two- to four-fold along a vertical gradient within a canopy (Meir et al. 2002; Niinemets 2012) and transpiration rates can vary among branches (Frak et al. 2002; Zweifel et al. 2002). Documenting gradients in leaf-level responses to changes in the microclimate has contributed predictive insight into a range of processes that ultimately affect whole-plant productivity; for example, the influence of light on leaf development (Niinemets 2007), variability of water transport (Zwieniecki et al. 2004) and photosynthetic carbon gain and respiratory carbon release from leaves (Küppers and Pfiz 2009; Niinemets 2007; Niinemets and Anten 2009). Here, we extend these observations to leaf-level photosynthetic thermal tolerance.

Profiling of within-canopy variation in leaf traits is often done in vegetation communities with small inter-crown gaps (e.g. dense, closed forests) or contrasts inner and outer canopy positions. These studies frequently employ the change in light environments across a canopy to explain intracanopy leaf trait variation, such as for sun *versus* shade leaves (Niinemets and Anten 2009; Niinemets and Valladares 2004; Pearcy et al. 1990). In contrast to closed-canopy communities, in desert environments, individual trees tend to be widely spaced, resulting in a distinctly different canopy microclimate profile. In a desert environment, air temperature typically decreases rapidly with height above ground, due to intense, unabating solar radiation (Whitford 2002). For Australian deserts in summer, for example, mean maximum near-surface air temperatures of 65–70 °C have been recorded (Mott 1972; Cook et al. unpublished data), while being as much as 20 °C cooler two meters above the ground (Warner 2009).

The high thermal loading of exposed surface soils drastically alters the thermal environment for near-surface vegetation (Warner 2009) and can contribute as much as 10–30% of a canopy's total energy budget in hot, dry, arid environments (Eamus 2006). Also, in these environments, high surface temperatures often are coupled with naturally low soil water, leading to high VPD (Macinnis-Ng and Eamus 2009). A common consequence of these combined conditions is that desert plants will limit stomatal conductance, causing leaf temperatures to rise rapidly by restricting transpiration and influencing the energy budget of a leaf (Macinnis-Ng and Eamus 2009; Teskey et al. 2015). Thus, we might expect leaves near the surface and/or in north-facing portions of the canopy to have greater photosynthetic thermal tolerance. We addressed this question by studying one of the dominant tree species in Australia's Southern arid zone, *Acacia papyrocarpa* (Benth.).

Methods

Site and study species

The study site was located at the Australian Arid Lands Botanic Garden (AALBG) in Port Augusta, South Australia, within Australia's southern arid region (32°27′56.3″S 137°44′40.7″E). Sampling was conducted throughout the 2013/14 austral summer. The AALBG covers an area exceeding 250 hectares, of which a significant portion includes a natural stand of western myall (*Acacia papyrocarpa* Benth.). The mean annual rainfall is approximately 250 mm and the mean maximum summer temperature is approximately 31.3 °C, but maximum temperatures frequently exceed 45 °C (AGBoM 2013).

Acacia papyrocarpa is a large evergreen perennial desert shrub to small tree (3–8 m high), with a dense spreading canopy (8–10 m diameter), with foliage that consists of phyllodes rather than true leaves (Electronic Supplementary Material Fig. S1) (World Wide Wattle V2 2016), but hereafter referred to as 'leaves'. This species is slow-growing and long-lived, with lifespans exceeding 300 yrs. Although evergreen, foliage is lost cyclically, with new growth occurring in spring and summer and net leaf losses commencing in late summer (Maconochie and Lange 1970). Large expanses of mature *A. papyrocarpa* occur throughout Australia's southern arid region, where it forms sparse open woodlands with an understorey dominated by chenopod shrubs. In contrast to juvenile growth stages, mature *A. papyrocarpa* plants have a dense canopy with foliage that droops near to ground level and even rests on the ground in mature trees. For the current study, we selected five *A. papyrocarpa* plants categorised as mature (Lange and Purdie 1976), which had no visible signs of damage. All study plants experienced similar environmental conditions and grew on sandy soils surrounded by low growing herbs and shrubs, with no shading of the canopy by neighbouring plants.

Microclimate measurements

Temperature, humidity, VPD

In the outer canopy of five replicate plants, forty temperature/humidity (°C/%) data loggers (DS1923 iButtons®, Alfa-Tek Australia) were placed at one of four positions: the upper north-facing (UN), upper south-facing (US), lower north-facing (LN) and lower south-facing canopy (LS). Lower and upper canopy were defined here as a height of approximately 0.4 m and 2 m above ground level, respectively. Prior to canopy positioning, the data loggers were pre-programed using the Express Thermo 2007 Basic Software (http://www.eclo.pt/home), set to record ambient air temperature and humidity every 45 min for a period of 11 weeks between December and February (austral summer). Data loggers were individually attached to device mounts with recess (DM9000 Touch device mounts, Alfa-Tek Australia) and suspended inside a custom-built, white plastic housing that shielded the sensor from overhead and lateral light, whilst being shallow enough to allow adequate air flow around the sensor (Electronic Supplementary Material Fig. S1). To account for potential instrument failure, two data loggers were placed adjacent to one another at each of the four positions, equating to eight data loggers per replicate shrub. As all the devices remained functional throughout the measurement period, data points of both loggers at each canopy position were averaged. For each position, the maximum ambient air temperature and minimum per cent humidity were determined for each measurement

day ($n=80$ days). For each replicate plant, measurements from the 80 days were averaged to provide overall summer maximum ambient air temperature and minimum per cent humidity for the four canopy positions.

We chose to measure canopy air temperatures instead of leaf temperatures for several reasons: (1) Leaf temperatures are inherently quite variable for the reasons discussed here, which would require an even greater level of replicate measurements to adequately sample leaf temperatures at these canopy positions, (2) the most reported measurements of local or regional temperature are air temperatures at 1.5 m; leaf temperatures are rarely reported, thus, our reports of physiological responses to differences in air temperature have greater currency, (3) placement of the greater number of leaf temperature sensors was cost and time prohibitive.

Average summer maximum vapour pressure deficit (VPD_{max}) was estimated for the four canopy positions from ambient air temperature and humidity data using the equation:

$$VPD \text{ (kPa)} = e_s - e_a, \tag{1}$$

where e_s and e_a are the saturated and actual vapour pressure of air, respectively, estimated here from the following equations:

$$e_s = 0.6108 e \frac{17.27 \times T}{T + 237.3}, \tag{2}$$

and,

$$e_a = e_s(T) \times \frac{RH}{100}, \tag{3}$$

where T is a point measurement of ambient temperature at the location of interest and RH is a point measurement of relative humidity corresponding to the time of T (Murray 1967; Walter et al. 2005). VPD was first determined for each measurement point corresponding to those for temperature and humidity. Subsequently, the daily maximum VPD was determined ($n=80$ days), followed by averaging daily measurements for a summer maximum VPD for the four canopy positions for each replicate plant.

Another indicator of stressful microclimatic conditions is the frequency with which air temperature at a given canopy position reaches a known critical threshold. For each canopy position, we determined the mean number of days that maximum ambient air temperature exceeded the previously recorded T_{50} threshold of 49 °C for north-facing *A. papyrocarpa* foliage (Curtis et al. 2014), here termed AT_{49}.

Wind speed

Long-term meteorological records for the Port Augusta region indicate that at a height of 7 m above sea level summer winds prevail from a southerly direction and that

winds originating from the south frequently exceed speeds reached in any other direction (AGBoM 2016). Therefore, in this desert community, the leeward side of plants is generally north. However, in addition to the prevailing wind direction, we also were interested in canopy scale wind speed data. Near to the ground, wind speeds tend to approach zero and increase approximately logarithmically with height above the canopy (Warner 2009). Here, of interest were the potential differences that may arise in wind speed with small scale (< 2 m) changes in height and aspect across the canopy of a plant. We recorded wind speed adjacent to the canopy and at a height corresponding to positions where leaves were sampled: 0.4 m and 2 m above the ground. Measurements were obtained using a Testo 435 multifunction anemometer with hot wire probe attachment (m s^{-1}, °C) positioned adjacent to the foliage of a representative *Acacia papyrocarpa* tree. Measurements were recorded at one second intervals for a period of 5 min between 1600 and 1700 h on each of 3 days in late summer. From these data, a mean wind speed for the 3 days was calculated, as well as the proportion of time wind speed dropped below 0.5 m s^{-1} at each canopy position.

Thermal response indices

Leaf thermal damage thresholds

To assess variation in leaf-level physiological thermal protection across positions of the canopy, T_{50} was measured at each position for each plant following the protocol of Curtis et al. (2014). This method uses chlorophyll *a* fluorescence to measure the temperature at which F_V/F_M drops to 50% of pre-stress levels using a pulse-modulated chlorophyll fluorometer with fibre optics and leaf-clip attachment (MINI-PAM; Heinz Walz, Effeltrich, Germany). Briefly, for each canopy position ten fully expanded, healthy leaves of a similar age were detached and treated with one of four heat treatments using temperature-controlled water baths, accurate to ± 0.2 °C (60 leaves per canopy position). Of the four temperature treatments, one was a control treatment (28 °C) and the other three were heat stress treatments, increasing by 2 °C increments: 50, 52 and 54 °C. This range of treatments encompassed the temperatures that bracketed the lowest and highest T_{50} for all canopy positions across all replicates. Leaves were exposed to a subsaturating light level of ca. 280 μmol photons m^{-2} s^{-1} throughout the treatment process. F_V/F_M was recorded after 30 min of dark adaptation. Leaf physiological and morphological measurements were made over a period of 12 days during the peak of summer beginning in late January.

Predicted leaf thermal dynamics

Using leaves immediately adjacent to those used to measure thermal thresholds, a series of morphological measurements were made to estimate leaf boundary layer thickness and subsequent thermal time constants. A leaf boundary layer is defined as the still air situated adjacent to the surface of a leaf (Nobel 2012). The estimated boundary layer thickness (δ) can be used to predict the thermal time constant (τ) of leaves. Here, the average thickness of the leaf boundary layer was estimated for leaves at each of the four canopy positions using the following equation for a flat leaf presented in Leigh et al. (2017, and refs within):

$$\delta = 4.0\sqrt{(w_e/\mu)}, \tag{4}$$

where δ is the average boundary layer thickness in mm, the factor 4.0 is a constant, with units of mm s$^{-0.5}$; μ is the average wind speed in m s^{-1} recorded for that canopy position; w_e is the effective leaf width in unit meters. Effective leaf width, the diameter of the largest circle that can be placed within the leaf margin (Leigh et al. 2017), was measured for ten leaves and then averaged for each canopy position. Effective leaf width and leaf area (for Eq. 5, see below) were obtained from scanned images using the graphic software program ImageJ (version 1.50a, United States National Institute of Health, Bethesda, MD, USA). This process was repeated for each of the five plant replicates. Estimated thickness of the boundary layer for leaves at each position in the canopy was subsequently used to predict τ using the following equation (Leigh et al. 2017):

$$\tau = C\delta/2_k, \tag{5}$$

where C is the heat capacity of the leaf per unit area, obtained by multiplying the water content (g) per unit area for each leaf by the heat capacity of water (4.18 J g^{-1} °C^{-1}); k is the thermal conductivity coefficient of air (2.6×10^{-2} Joules °C^{-1} m^{-1} s^{-1}); the multiplier 2 accounts for the two sides of the leaves. Leaf water content was measured as: ((fresh weight–dry weight)/leaf area). All fresh and dry weights were measured using a precision analytical balance sensitive to 0.001 g (Mettler Toledo, city).

Data analyses

Temperature, vapour pressure deficit and relative humidity usually vary in tandem and have compounding effects on leaf physiology. We therefore used principal component analysis (PCA) with direct oblimin (oblique rotation) to extract a single composite variable from the canopy microclimatic variables (Garson 2013a): average summer maximum temperature, maximum VPD, and minimum RH. Each variable

was standardised using z transformation prior to the PCA (Abdi and Williams 2010; Jongman et al. 1995). Component scores for the composite variable were extracted and used as an index of climatic stress (C_{STRESS}) in subsequent analyses comparing differences among canopy positions. Note that wind speed does not necessarily vary concomitantly with temperature, VPD or RH, so was not included as part of this composite climatic stress variable.

Individual two-factor ANOVA were conducted to investigate differences in C_{STRESS}, predicted τ, wind speed, and AT_{49} among the four canopy positions of *A. papyrocarpa*. Aspect (north and south) and height (upper and lower) were considered as fixed factors. A generalised linear model (GzLM) approach with Gaussian distribution and identity link function was applied to investigate the influence of height and aspect and microclimatic covariates on T_{50}. Specifically, C_{STRESS} and predicted τ were included as a covariate in two separate models: Model 1 and Model 2, respectively. To simplify the models, and being already captured by C_{STRESS} and predicted τ, temperature, RH, VPD and wind speed were not included in these models. Initially, models consisted of the full factorial design, including all main effects and interaction terms. Models were reduced by eliminating all non-significant interaction terms until only significant interactions remained (Engqvist 2005). The

goodness-of-fit for each model was assessed using Akaike's information criterion corrected for small sample sizes (AICc, with low AICc indicating a better model fit) (Garson 2013b). The most parsimonious reduced models consisted of all main effects and the height $\times$ C_{STRESS} interaction for Model 1, or aspect $\times$ predicted τ interaction for Model 2.

Due to the high number of zero values, a nonparametric Kruskal–Wallis test with Dunn–Bonferroni post hoc tests was used to evaluate differences in AT_{49} among the four canopy positions. To evaluate the effects of only height (with aspect pooled) or aspect (with height pooled), individual nonparametric Mann–Whitney U tests were used. For all analyses, differences were considered significant at $\alpha = 0.05$. All data analyses were carried out using the statistical software IBM SPSS® (v23).

Results

Drivers of thermal stress

Temperature, VPD, and humidity fluctuated greatly over the study period, both daily and among canopy positions (Fig. 1; Electronic Supplementary Material Fig. S2). When average daily summer temperatures increased, VPD also increased
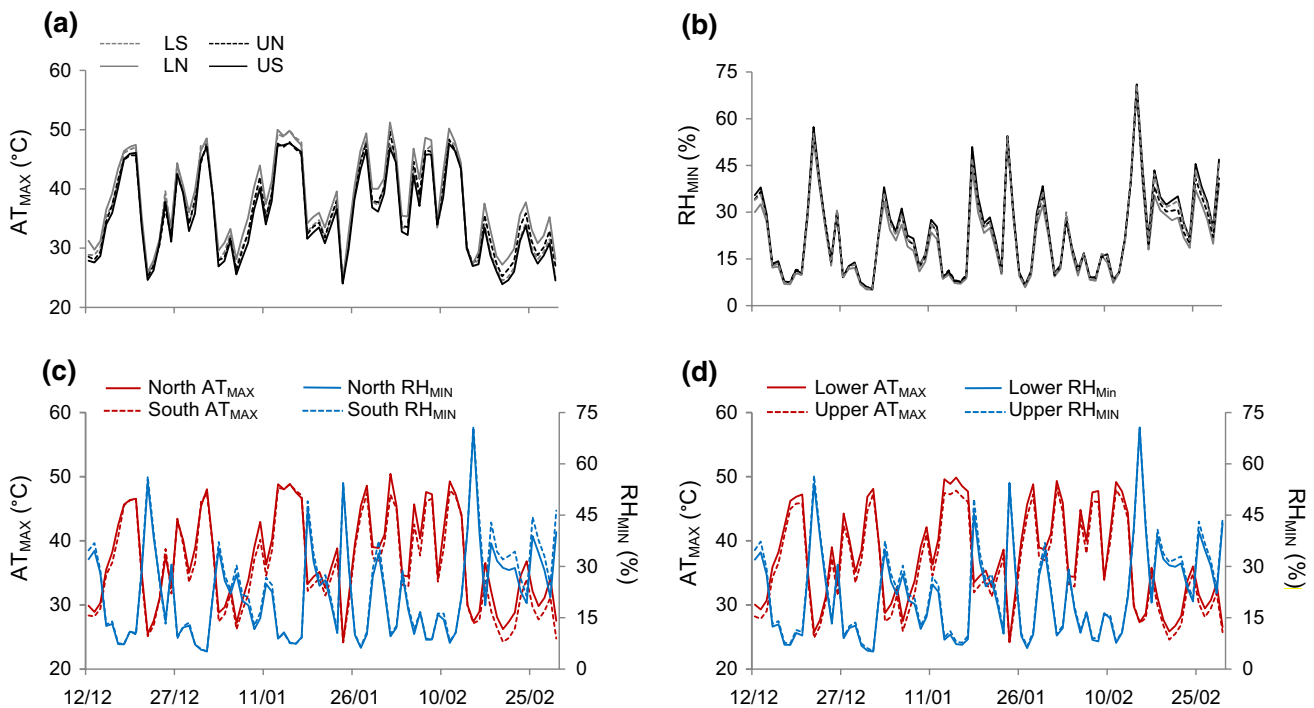

Fig. 1 Mean daily maximum ambient temperature (AT_{MAX}, °C) (**a**) and daily minimum per cent relative humidity (RH_{MIN}, %) in the outer canopy of five replicate *Acacia papyrocarpa* trees (**b**) at four positions in the outer canopy: upper north, lower north, upper south, and lower south (UN, LN, US, LS) ($n = 5$). Data also presented as

north- and south-facing positions combined (**c**) and upper and lower positions combined (**d**). Mean maximum daily vapour pressure deficit is not presented, but followed temperature trends. This figure is available in colour in the online version of the journal

and average daily summer humidity declined. PCA produced a single principal component, C_{STRESS}, which explained 80.2% of the total variability of the original microclimatic data. High positive component scores along the C_{STRESS} axis represented higher average maximum ambient summer temperatures and VPD, and lower average minimum summer humidity (Fig. 2a). Results of the two-factor ANOVA indicated that C_{STRESS} was significantly higher in the lower than upper canopy and in the north-facing than south-facing canopy; the interaction between height and aspect was non-significant (Table 1). For predicted τ, height, aspect and the interaction between height and aspect had a significant influence, such that overall, τ was predicted to be longer for

north-facing and lower canopy foliage, but within height, the effect of aspect on predicted τ was only significant for the lower canopy positions (Table 1; Fig. 2b). Of the variables comprising τ, leaf area and wet weight did not differ among canopy positions (results not shown); the variation in predicted τ, therefore, was due to intracanopy variation in wind speed. Wind speed was significantly higher in upper canopy positions than lower canopy positions, but there was no significant effect of aspect and the interaction between height and aspect was non-significant (Table 1; Fig. 2c). In contrast to average wind speed results, the proportion of time that wind speed dropped to ≤ 0.5 m s^{-1} was not significantly influenced by height or aspect as main effects,
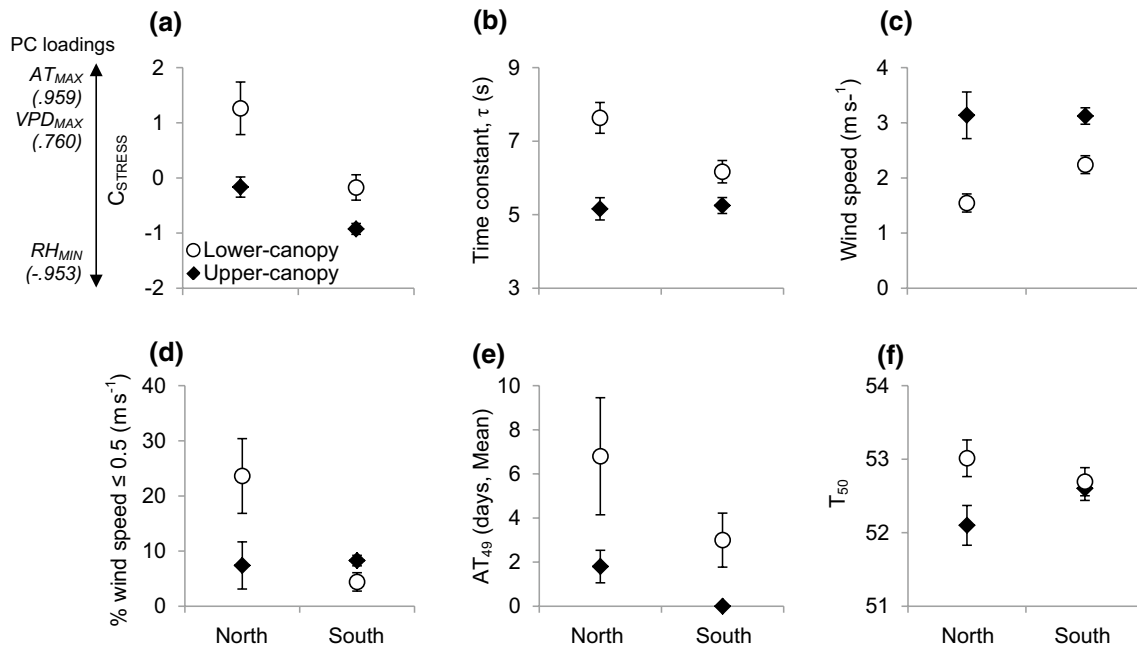

Fig. 2 Effect of within-canopy height and aspect on a range of microclimatic indicators and leaf physiological response: the climatic stress index (C_{STRESS}), as determined by Principle Components Analysis (PCA), where AT_{MAX} is the mean daily maximum ambient temperature (°C), VPD_{MAX} is the mean daily vapour pressure deficit (kPa) and RH_{MIN} is the mean daily minimum relative humidity (%) (PCA variable loadings shown adjacent to C_{STRESS} indicate a more positive or negative influence of that variable) (**a**); predicted thermal time

constant in seconds (τ) (**b**); wind speed (m s^{-1}) (**c**); frequency with which wind speeds drop ≤ 0.5 (m s^{-1}) (**d**); average number of days that maximum temperatures exceeded the critical threshold temperature of 49 °C (AT_{49}) (**e**); and thermal damage threshold (T_{50}) (**f**) for outer canopy leaves at four positions, upper north-facing (UN), lower north-facing (LN), upper south-facing (US) and lower south-facing canopy (LS). Error bars represent the standard error of the mean on all graphs. See Tables 1 and 2 and text for statistical results

Table 1 Summary of two-way ANOVA tests for the effect of canopy position on the climatic stress index (C_{STRESS}), wind speed (m s^{-1}), frequency with which wind speeds drop ≤ 0.5 m s^{-1} (arcsin transformed) and predicted leaf time constant (τ) in five replicate *Acacia*

papyrocarpa plants. The canopy positions were: upper north-facing, lower north-facing, upper south-facing and lower south-facing canopy. Where main effect differences within height or aspect were significant, the directions of effects are indicated with arrows

Variable	Height	Height effects	Aspect	Aspect effects	Height × aspect
C_{STRESS}	$F_{(1,16)}=14.68$ $(P<0.001)$	Upper < Lower	$F_{(1,16)}=14.95$ $(P<0.001)$	South < North	$F_{(1,16)}=1.42$ $(P=0.250)$
Wind speed (m s^{-1})	$F_{(1,8)}=24.05$ $(P<0.001)$	Upper > Lower	$F_{(1,8)}=1.83$ $(P=0.213)$	–	$F_{(1,8)}=1.97$ $(P=0.198)$
% Wind speed ≤ 0.5 m s^{-1}	$F_{(1,8)}=1.92$ $(P=0.203)$	–	$F_{(1,8)}=4.31$ $(P=0.072)$	–	$F_{(1,8)}=7.54$ $(P=0.025)$
Predicted time constant, τ (s)	$F_{(1,16)}=28.44$ $(P<0.001)$	Upper < Lower	$F_{(1,16)}=4.65$ $(P=0.047)$	South < North	$F_{(1,16)}=5.95$ $(P=0.027)$

but the interaction between these two factors was significant (Table 1). That is, in the north-facing canopy, lower positions reached wind speeds of ≤ 0.5 m s^{-1} proportionally more often than did upper positions (Fig. 2d).

The four canopy positions could be ranked, from the highest to lowest frequency with which AT_{49} was exceeded, as: LN > LS > UN > US. A Kruskal–Wallis test indicated a significant difference among the four canopy positions in the median number of days that the maximum temperature breached the critical threshold temperature of 49 °C [$\chi^2_{(3, 20)} = 8.354$, $P = 0.039$, Fig. 2e]. Subsequent post hoc pairwise comparisons signified that only differences between lower north and upper south were statistically different ($P = 0.036$). Nevertheless, the average frequency that the lower north position exceeded AT_{49} was more than twice that of lower south, more than three times upper north and more than six times the frequency of the upper south position, which did not exceed AT_{49} during the 11-week study period. Pooling positions and using Mann–Whitney U tests to compare differences in height and aspect indicated that AT_{49} was significantly greater at lower positions than upper positions ($U = 78.00$, $z = 2.197$, $P = 0.035$), whereas differences in AT_{49} between north and south-facing positions were non-significant ($U = 28.500$, $z = -1.687$, $P = 0.105$).

Thermal damage thresholds

Thermal damage thresholds, T_{50}, varied significantly among canopy position (Fig. 2f). Leaves at lower elevations had greater T_{50} at northern canopy positions but did not differ from upper leaves in the cooler south-facing canopy. There was a significant interaction between height and C_{STRESS} for predicting variation in T_{50} (Table 2, Model 1). When accounting for the influence of predicted τ on the variation in T_{50}, the main effect of aspect and the interaction between

aspect and predicted τ were statistically significant (Table 2, Model 2).

Discussion

Climate change is increasing the frequency and intensity of high-temperature days in many regions globally. Further, atmospheric 'stilling', or the slowing of wind speeds, is increasing at a continental scale (McVicar et al. 2008); still air can exacerbate the damaging effects of high temperature on plants, particularly those that occur in already harsh environments such as deserts (Leigh et al. 2012). Variation in wind speed, combined with leaf traits such as size and position, can lead to significant variation in leaf temperature. As expected, we found that the microclimate of leaves varied considerably in the canopy of a dominant shrub in the southern arid zone of Australia, *Acacia papyrocarpa*. Plants in desert systems are sparsely spaced, with the majority of their canopies exposed. As expected for an open-canopy community, we found that intracanopy temperature and relative humidity varied markedly with height above ground and differences in aspect (Fig. 1a; Electronic Supplementary Material Fig. S2). Compared with patterns observed in closed-canopy vegetation communities, the magnitude of microclimate variability with height above ground was marked. For example, we observed a 1.3 °C average increase in air temperature over 1.5 m between upper and lower canopy positions, which is large compared to a 2.2 °C difference in ambient temperature over 34 m in a mixed deciduous forest (Stiegel et al. 2017) or no discernible difference in temperature or humidity with tree height for northern temperate *Quercus rubra* (Zwieniecki et al. 2004). Along with greater climatic stress (measured as C_{STRESS} here), our desert plants experienced the lowest wind speeds at lower canopy positions on the northern, leeward side of plants, resulting

Table 2 Generalised liner models predicting the influence of four canopy positions and one of two covariates on the thermal damage threshold (T_{50}) of *Acacia papyrocarpa* leaves. Height and aspect were factors and the climatic stress index, C_{STRESS} (Model 1), and predicted thermal time constant of a leaf, predicted τ (Model 2), were covariates. Results are for the most parsimonious models, removing non-significant interaction terms, and assuming Gaussian distributions with identity link functions. Omnibus tests confirmed that each fitted model was significantly different from its null model

Model parameters	Model 1: C_{STRESS}			Model 2: predicted τ		
	df	Wald χ^2	P	df	Wald χ^2	P
Main effects						
Height	1	8.969	0.003	1	2.677	0.102
Aspect	1	0.271	0.603	1	4.944	0.026
Covariate	1	2.025	0.155	1	0.049	0.825
Interactions						
Height × covariate	1	5.830	0.016	–	–	–
Aspect × covariate	–	–	–	1	4.539	0.033

in significantly longer predicted thermal time constants for leaves at these positions. The co-occurrence of high-stress micrometeorological conditions with significantly slower thermal response times suggests that leaves in lower north-facing positions are more likely to experience extreme high temperatures than are leaves elsewhere in the canopy (Leigh et al. 2012; Niinemets and Valladares 2004; Vogel 2009).

In response to spatially patchy environmental conditions within a canopy, we expected that leaf thermal tolerance would adjust at a local scale within a single canopy. Indeed, we found that the more thermally stressful conditions experienced by leaves in the lower north-facing canopies of *A. papyrocarpa* equated to significantly higher T_{50} than for leaves elsewhere on the plant (Fig. 2; Table 2). Summed across days and seasons, these differences in leaf-level photosynthetic thermal tolerance could have significant effects on overall canopy carbon balance (Küppers and Pfiz 2009; Niinemets and Anten 2009). Consider that under heat stress, processes associated with protein synthesis and replacement vary exponentially with temperature, with the maintenance component of dark respiration being especially sensitive (Ryan 1991). An increase in maintenance respiration diverts carbohydrates from growth, significantly affecting the total carbon budget of a plant (Amthor 1984). Because the temperature at which the maximum dark respiration is reached corresponds with the critical temperature for PSII stability (Knight and Ackerly 2002, 2003a; Katja et al. 2012; Lin 2012; O'Sullivan et al. 2013; Marias et al. 2016), T_{50} thus can serve as a proxy for the leaf temperature at which both the photosynthetic apparatus and respiratory processes are disrupted by heat stress (O'Sullivan et al. 2013). Therefore, even if actual leaf temperatures are not known, small differences in leaf physiological responses within a canopy could provide a meaningful basis for estimating whole-plant performance.

The higher T_{50} for leaves nearest the ground in our study suggests that the thermal response pathways of *A. papyrocarpa* operate effectively to manage the higher risk of thermal damage at these positions through localised acclimatisation. Pre-exposure of leaves to sub-lethal temperatures can trigger a stream of protective pathways, including the synthesis of heat-shock proteins (Knight and Ackerly 2003b; Knight 2010; Bita and Gerats 2013) and reactive oxygen species accumulation (Gechev et al. 2006; Larkindale et al. 2005; Miller et al. 2008; for review, Suzuki and Mittler 2006). However, upregulating the physiological processes associated with damage prevention and repair can be costly (Hoffmann 1995; Leroi et al. 1994; Loeschcke and Hoffmann 2002). By limiting thermal damage in high-risk positions, while maintaining lower thresholds in cooler regions of the canopy, whole-plant carbon gain would be maximised, as occurs with intracanopy variation of key functional traits in other species (Sack et al. 2006). Our findings, therefore,

point to an important thermal optimisation strategy for this and potentially many other species.

Notwithstanding the idea of whole-plant optimisation of thermal protection in *average* conditions, during severe thermal events, such as a sudden heat wave, lethal thermal damage will inevitably occur. For example, during a recent summer heatwave event at our study site, where maximum temperatures exceeded 46 °C (AGBoM 2016), leaf temperatures of 50 °C were recorded (Cook et al. unpublished data). For the current species, such a scenario might on one hand be expected to cause most damage to the leaves with lowest critical thermal limits, which were found in the upper canopy (Fig. 2f). On the other hand, air temperatures at lower canopy positions were more than five times as likely to exceed pre-recorded thermal limits for this species (AT_{49}) than in the upper canopy (Fig. 2e). Little is known about the effect of repeated, frequent excursions to critical temperatures on leaves (Leigh et al. 2012). Given that photosystem repair occurs on the order of minutes to hours (for reviews, see Aro et al. 1993; Melis 1999) and that even moderate heat stress inhibits the repair of damaged PSII (Murata et al. 2007), a five-fold difference in intracanopy high temperature extremes could result in an equivalent degree of cumulative damage and, therefore, reduced carbon assimilation (Farquhar and Sharkey 1982). If the variation in leaf-level physiological responses with vertical temperature gradients is disregarded, canopy flux models may overestimate carbon uptake by as much as 25% (Bauerle et al. 2007). In line with this, our finding of significantly higher thermal thresholds for lower, north-facing canopy leaves in desert conditions demonstrates that estimates of productivity under heat stress could be under- or overestimated by a similar margin, depending on where in the canopy leaves are sampled.

Summary

In the present study, we have demonstrated that leaf-level thermal tolerance of *Acacia papyrocarpa* may be significantly influenced by localised canopy microclimatic conditions. This is the first study to document intracanopy variation in T_{50}, thereby contributing new insight into within-plant thermal tolerance dynamics. Because T_{50} represents a leaf-level thermal response index relating to the functional state of the photosynthetic machinery, this result reveals the potential implications of intracanopy variation in temperature stress on whole-plant productivity and growth. The work presented in this study prompts the need for further research to quantify the relationship between T_{50} and respiratory processes as well as cost–benefit analyses for a range of functional types and species. Such research would enhance our understanding of the functional importance of within-canopy T_{50} variation and could help in generalising

present findings across species to elucidate the resource implications of maintaining a higher thermal tolerance.

Author contribution statement AL and EMC generated hypotheses and designed the thermal tolerance work; EMC collected and analysed the data; CAK provided advice and contributed fundamental intellectual input; EMC led the writing, with AL revising the final text.

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