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Primary nerve (vein) density influences spatial heterogeneity of photosynthetic response to drought in two *Acacia* species

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Abstract. We examined the relationship between variation in phyllode nerve density and the spatio-temporal response of the photosynthetic apparatus to water-stress in two *Acacia* s.str. species with contrasting nerve patterns: *Acacia floribunda* (Vent.) Willd and *Acacia pycnantha* Benth. *A. floribunda* had greater primary nerve density than *A. pycnantha* and also showed greater spatial homogeneity in photosynthetic function with drought than phyllodes of *A. pycnantha*. *A. pycnantha* had lower maximum quantum efficiency of PSII in phyllode tissue further from primary nerves consistent with its lower primary nerve density. Further, *A. floribunda* phyllodes maintained function of the photosynthetic apparatus with drought for longer and recovered more swiftly from drought than *A. pycnantha*. These findings suggest that greater primary nerve density may enhance drought tolerance and are consistent with the observed predominance of acacias with high primary nerve density in areas with lower precipitation.

Additional keywords: fluorescence, foliage, hydraulic architecture, photosynthesis, water, wattle.

Introduction

Climate change models suggest increasing drought frequency and severity in many regions of the world (Petit *et al*. 1999; Christensen *et al*. 2007; Hennessy *et al*. 2008). Given the adverse impact of drought on plant productivity and distribution (Hsiao 1973; Boyer 1982; Kramer 1983), there is a need to further elucidate plant physiological responses to water stress (Chaves *et al*. 2003). Recent work has highlighted the role of the plant hydraulic pathway in limiting plant gas exchange and, thus, plant growth with drought (Sperry *et al*. 2002; Brodribb and Holbrook 2005; Blackman *et al*. 2009; Resco *et al*. 2009). Within the plant hydraulic pathway, the leaf constitutes ~30% of all hydraulic resistance and so plays a vital role in constraining plant gas exchange (Sack and Holbrook 2006; Brodribb 2009). The present study examined the relationship between variation in the foliage vascular transport network and the spatio-temporal response of the photosynthetic apparatus to water-stress in the evergreen angiosperm genus, *Acacia* s.str.

Of the 969 *Acacia* species, 917 possess phyllodes as their principal foliage when mature. Although there is still debate about the developmental origin of the phyllode (Gardner *et al*. 2008), the structural differences between phyllodes and leaves are clear. In phyllodes there are always two layers of nerves (veins), whereas leaves possess only one vein layer. *Acacia* phyllodes also show a unique capacity to alter the number of primary (first order) nerves both within a species and even on different phyllodes of the same plant (Gardner *et al*. 2005; Gardner 2006). Where other taxa demonstrate a lower primary vein density as leaves become wider, wider *Acacia* phyllodes generally have additional primary nerves, thus, maintaining or even increasing primary nerve density. The pattern in *Acacia* is even more curious when one considers that higher phyllode primary nerve density is associated with decreased precipitation (Hnatiuk and Maslin 1988; Sommerville 2010). The advantage (if any) of such high primary nerve density in acacias receiving meagre and infrequent rainfall is unknown.

A greater primary nerve density may allow more even spatial distribution of water across the phyllode during drought. Greater primary vein density in leaves has been associated with increased tolerance of hydraulic disruption (Sack *et al*. 2008). Primary nerves act as major hydraulic supply lines from which smaller nerves receive water. These smaller nerves ultimately distribute water to the mesophyll tissue (Sommerville 2010). Where drought-induced vessel cavitation blocks or reduces supply of water from one primary nerve (Wheeler *et al*. 2005), additional primary nerves may allow the water requirements of mesophyll tissue to be met through alternate routes. High primary nerve density may also allow greater consistency of mesophyll hydration with drought. A more consistent mesophyll hydration may, in turn, allow continued photosynthetic carbon assimilation. A higher primary nerve density may also permit swifter upregulation of phyllode function with the return of precipitation following drought. The capacity to rapidly upregulate function may be critical in parts of Australia where

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rainfall is unpredictable and pulse-like, providing few opportunities for plant carbon and water gain (Byrne *et al*. 2008; Grigg *et al*. 2010). The comparative drought tolerance of mesophyll tissue in *Acacia* species with different nerve patterns remains untested and the mechanisms underlying any differences have not yet been explored.

A decrease in photosynthetic activity with drought is almost ubiquitous in plants (Boyer 1982; Chaves *et al*. 2003). An initial decrease in photosynthetic activity with mild water stress is most often due to decline in stomatal conductance, resulting in a decline in assimilation rate consistent with a decrease in the intercellular carbon dioxide concentration (Adams and Demmig-Adams 2004; Bukhov and Carpentier 2004). However, as water stress becomes more severe, the photosynthetic enzyme, Rubisco, may be inactivated and photoinhibitory damage to the photosynthetic apparatus may occur (Havaux 1992; Medrano *et al*. 1997).

Differences in photosynthetic activity across the plane of the leaf blade have been widely observed (Bro *et al*. 1996; Baker *et al*. 2001). However, fewer studies have examined the effect of drought on photosynthetic activity in different regions of the leaf (Wise *et al*. 1992; Massacci *et al*. 2008) with most of these studies examining variation in stomatal responses across the leaf surface (Rezaei Nejad *et al*. 2006). To our knowledge, no study has examined the relationship between foliage hydraulic architecture and spatial heterogeneity of photosynthetic response to drought.

Where mesophyll areole regions are closer to a major supply line, a primary nerve, they may receive water from the vascular network more swiftly and steadily than those areole regions distant from primary nerves. Hence, phyllodes with greater density of primary nerves may demonstrate more uniform function across the lamina space than phyllodes with lower primary nerve density. In the present study, the effect of drought on the photosynthetic function of distinct mesophyll regions was examined in phyllodes of two *Acacia* species with contrasting nervation patterns. Fluorescence imaging was used in the present study to assess changes in the functional status of the photosynthetic apparatus in phyllodes experiencing severe drought (Omasa and Takayama 2003). It was hypothesised that the efficiency of PSII photochemistry (F_v/F_m) would be greater in areole regions near the central primary midnerve than in areole regions between secondary nerves with drought. In comparing two species with contrasting nervation patterns, it was hypothesised that F_v/F_m would be lower in tissue between secondary nerves in the species with lower primary nerve density, owing to greater distance from major hydraulic supply lines and, therefore, increased resistance in the path to the site of water use. Finally, it was hypothesised that F_v/F_m would recover faster from the effects of plant water deficit in the species with greater primary nerve density due to swifter uptake of water.

Materials and methods

Plants

Species studied were *Acacia pycnantha* Benth., with phyllodes having three primary nerves and secondary nerves paired oppositely, and *Acacia floribunda* (Vent.) Willd, with phyllodes having 3–5 primary nerves and 6–10 secondary nerves running in parallel (Chapman *et al*. 2001*a*, 2001*b*). The present study used the same plants and experimental infrastructure as Gimeno *et al*. (2010), although both studies were separate and independent in their nature.

Experimental design

Within a glasshouse, plants were organised according to a randomised split-plot design with four blocks. In each block plants were randomly allocated to either 'well watered' or 'drought' treatments with three plants per species allocated to the well watered treatment and four plants per species allocated to the droughted treatment. Of these plants, fluorescence was measured in one plant of each species in each treatment in each block (a total of 16 measured plants). During the experiment, plants in the well watered treatment were watered to field-capacity daily. Plants in the droughted treatment were deprived of water across consecutive days until phyllode wilting was observed (see Fig. S1 available as an Accessory Publication to this paper) (Engelbrecht *et al*. 2007). Droughted plants were then re-watered to field capacity daily and their recovery tracked for at least 4 days.

A primer drought cycle was imposed beginning November 2008 in order to allow plants to acclimate to dehydration (e.g. through osmotic adjustment) and reduce the likelihood of 'shock' responses in the subsequent measurement cycle. In the primer cycle, plants were exposed to a mild stress and re-watered as soon as wilting was observed. In the primer cycle, *A. pycnantha* plants wilted and were re-watered following measurement on day 5 and *A. floribunda* plants wilted and were re-watered following day 7 measurements. In the measurement cycle, *A. pycnantha* plants wilted and were re-watered following measurement on day 8 and *A. floribunda* plants wilted and were re-watered following day 10 measurements. Owing to the time taken to conduct measurements, the start day for each cycle was staggered such that plants in blocks 3 and 4 began and finished their cycle 1 day later than plants in blocks 1 and 2. In the measurement cycle, relative water content (RWC) was determined using phyllodes from a matched plant in each block. This was done to minimise the effect of phyllode removal on drought responses in measured plants. RWC was calculated as [(fresh mass – dry mass)/(fully hydrated mass – dry mass)] × 100. Fresh mass was obtained by weighing phyllodes immediately after removal from the plant. Phyllodes were then bagged, placed in the dark with their petioles in water and room temperature set at 21°C. Following 24 h of rehydration phyllodes were re-weighed and saturated mass determined. Finally, phyllodes were oven-dried at 68°C for 48 h to obtain the dry mass.

To aid in assessment of the extent of drought stress experienced by each species, five additional plants of each species were used to calculate phyllode water relations parameters. Plants were the same age and size, grown in the same pots, soil, and from the same seed as those used in the fluorescence measurements. Phyllodes were cut from plants, pressurised using a pressure chamber (Model 3005, Soil Moisture Equipment Corp., Santa Barbara, CA, USA) to obtain balancing pressure and immediately weighed for fresh mass determination. On average, eight measurements were made for each curve. Dehydrated phyllodes were bagged, placed in the dark with their petioles in water with room temperature set at

21°C. Following rehydration, phyllodes were weighed to obtain saturated mass, placed in an oven at 80°C for 48 h and then weighed again to obtain dry mass. RWC was then calculated.

Pressure-volume curves were created from plots of the inverse of phyllode balancing pressure and RWC. We used the regression fitting method by Schulte and Hinckley (1985) to calculate linear regressions for the last three points on the curve, adding previous points until the percentage variance explained by the fitted regression was maximised. The fitted regression was then extrapolated back to the intercept. Using this method, the RWC at the turgor loss point (RWC_0) and phyllode water potential at the turgor loss point (Ψ_0) were estimated (Turner 1981).

Fluorescence measurements

Plants were dark adapted for 30 min before measurement with a Heinz Waltz GmbH (Effeltrich, Bavaria, Germany) IMAGING-PAM chlorophyll fluorometer with standard head. Phyllodes were exposed to a weak modulated measuring beam to obtain the minimal fluorescence (F_o) where all PSII reaction centres are open. Phyllodes were then given a short pulse of light (2400 µmol m^{-2} s^{-1} for 0.8 s) to obtain the maximal fluorescence (F_m) where all PSII reaction centres are closed. The maximum quantum efficiency of PSII photochemistry was then calculated: $F_v/F_m = (F_m - F_o)/F_m$. The same area of the same phyllode on each plant was measured throughout both cycles. Images were analysed using the software ImagingWin ver.2.32 (Heinz Waltz GmbH) with the same areas on each phyllode followed on each day. Six areas on each phyllode were selected, three areole regions adjacent to the mid-nerve (referred to hereafter as 'adjacent') and three areole regions near secondary nerves (referred to hereafter as 'peripheral') (Fig. 1).

Fig. 1. Location of six measured areas on each phyllode, three areole regions adjacent to the mid-nerve (circles) and three areole regions between secondary nerves (squares); (*a*) *Acacia floribunda* and (*b*) *Acacia pycnantha*. Phyllodes cleared in sodium hydroxide to show nerve patterns. Scale bar = 1 cm.

Nerve density

Following conclusion of the drought experiment, phyllodes were excised from plants and cleared with 15% w/v sodium hydroxide in ethanol for 3 weeks, washed in water, bleached and stored in lactic acid. Cleared phyllodes were stained with Safranin O (0.01%) and imaged at ×1, ×12.6 and ×64 magnification using a Wild M400 photomacroscope with Spot Flex CCD camera (Leica-Wild, Heerbrugg, Switzerland). Primary (first order), secondary and minor nerve density (third order and above if present) were measured using ImageJ (Bethesda, MD, USA) software. Primary nerve density was calculated as the number of nerves running from the base to the tip of the lamina per mean phyllode width. Secondary nerve density was calculated as the number of second order nerves (nerves branching from a primary nerve) per mean phyllode width. Minor nerve density was calculated as length of minor nerve per lamina area. Total nerve density was determined as the sum of minor, secondary and primary nerve densities.

Statistical analysis

To test for differences in nerve density, two-way ANOVA comparing species and treatment were conducted. Multiple

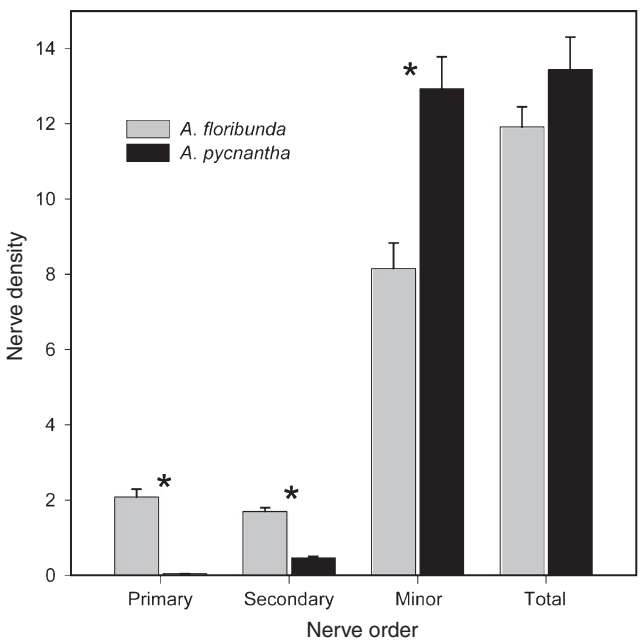

Fig. 2. Differences in nerve density of different nerve orders between *Acacia floribunda* and *Acacia pycnantha*. Primary and secondary nerve density (nerves mm^{-1}); minor nerve density (mm nerve mm^{-2}). Asterisks indicate significant differences between species ($P < 0.05$).

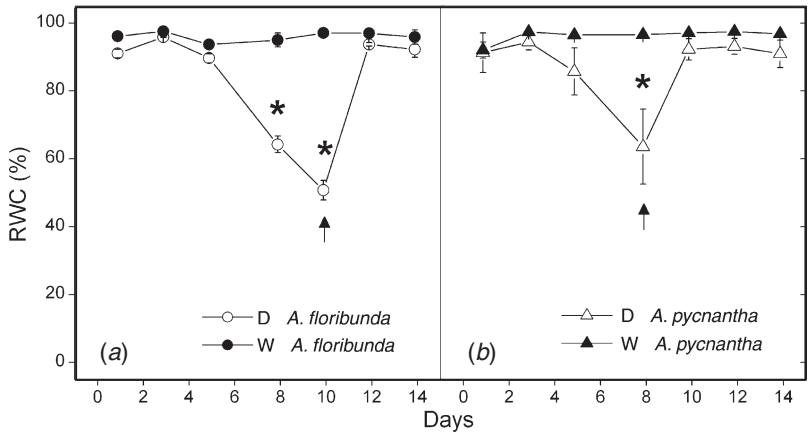

Fig. 3. Impact of watering regimes on phyllode relative water content (RWC ± s.e., $n = 4$) through time in the measurement cycle for *Acacia floribunda* (circles) and *A. pycnantha* (triangles). Closed symbols: well watered plants (W), open symbols: droughted plants (D). Significant differences in RWC identified using repeated-measures ANOVA with Tukey's test for individual comparisons indicated by asterisks. Arrows indicate rewatering following measurement. Adapted from Gimeno *et al.* (2010).

pairwise comparisons were tested using a *post hoc* Tukey's test (SigmaPlot ver. 11, Systat Software, Hounslow, London, UK). To determine the impact of watering treatment, RWC data from the measurement cycle were analysed for each species on each day using repeated-measures ANOVA; differences between particular days and treatments were explored using a Tukey's test (SigmaPlot ver. 11, Systat Software). Normality was tested using the Kolmogorov–Smirnov test and homogeneity of variance was checked using Levene's test (Quinn and Keough 2002). RWC_0 and Ψ_0 for each species were compared using a two-sample *t*-test. To determine the effect on fluorescence variables of differences between species with watering treatment and position on the phyllode, repeated-measures linear mixed models (uniform correlation, split-plot in time) were used with day and individual plant forming the random model (GENSTAT ver.12.1, VSN International Ltd, Hertz, UK). Within each species in each treatment, repeated-measures ANOVA were used to compare fluorescence measurements at given phyllode positions for different days; differences between particular days and treatments were explored using a *post hoc* Tukey's test (SigmaPlot ver. 11, Systat Software). Differences were considered significant at $P < 0.05$.

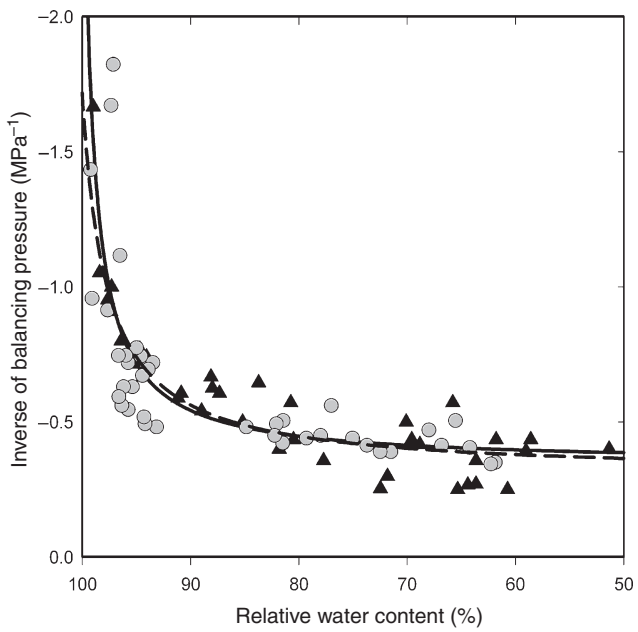

Fig. 4. Pressure-volume curves fitted with Rational 3 parameter functions for *Acacia floribunda* (grey filled circles, dashed line, $R^2 = 0.55$) and *Acacia pycnantha* (black filled triangles, solid line, $R^2 = 0.88$). Data merged for each species for display purposes.

Results

Nerve density

Acacia floribunda had significantly higher primary and secondary nerve density than *A. pycnantha* (Fig. 2). Conversely, *A. pycnantha* had significantly higher minor nerve density than *A. floribunda.* As a result, there was no significant difference in total nerve density between species.

Relative water content and phyllode turgor dynamics

In both *A. floribunda* and *A. pycnantha* there was a significant interaction between treatment and day for RWC. Examination of

Table 1. Mean maximum quantum efficiency of PSII (F_v/F_m ± s.e., $n = 4$) for species in each treatment (well watered (W) and droughted (D)) at each phyllode position for all days combined estimated using repeated-measures linear mixed models

Species	Treatment	Position	
		Adjacent	Peripheral
Acacia floribunda	W	0.79 ± 0.00	0.80 ± 0.00
	D	0.77 ± 0.01	0.77 ± 0.01
Acacia pycnantha	W	0.79 ± 0.00	0.80 ± 0.00
	D	0.77 ± 0.01	0.73 ± 0.01

individual comparisons revealed that RWC was significantly lower in droughted plants than in well watered plants on day 8 in *A. pycnantha* whereas RWC was significantly lower in *A. floribunda* on both days 8 and 10 (Fig. 3). Note that the mean RWC of droughted *A. pycnantha* (63.41 ± 11.06%) and *A. floribunda* (64.01 ± 2.47%) on day 8 were very similar.

A comparative pressure-volume analysis of the phyllodes (Fig. 4) revealed a slightly higher RWC_0 (90.6 ± 1.2% *v.* 89.0 ± 2.6%) and a less negative Ψ_0 (–1.88 ± 0.06 MPa *v.* –1.97 ± 0.38 MPa) in *A. floribunda* than *A. pycnantha*, respectively. However, differences between species in RWC_0 ($t = 0.60$, $P = 0.56$) and Ψ_0 ($t = -0.90$, $P = 0.83$) were not significant.

Differences between species, with treatment and position

There was a significant interaction between species, treatment and position for F_v/F_m ($P = <0.001$, $F = 21.02$). Both species showed an overall decline in F_v/F_m with drought (Table 1).

Differences within species with treatment and position

F_v/F_m was not significantly different with day in either well watered *A. floribunda* ($P = 0.852$, $F = 0.439$) or well watered *A. pycnantha* ($P = 0.998$, $F = 0.0816$). Equally, there was no significant difference between adjacent and peripheral positions in well watered *A. floribunda* or well watered *A. pycnantha* (Fig. 5). By contrast, there was a significant interaction between day and position in droughted *A. pycnantha* ($P \leq 0.001$, $F = 5.778$). Pairwise multiple comparisons using Tukey's test revealed F_v/F_m was significantly lower on days 8 (the day of lowest RWC) and 10 in droughted *A. pycnantha*, but that on these days, F_v/F_m was significantly lower in peripheral positions as opposed to adjacent ones (Figs 5, 6). In droughted *A. floribunda* plants there was no significant difference in F_v/F_m with position on phyllode ($P = 0.970$, $F = 0.00145$) despite these plants showing a significant decrease in F_v/F_m with day ($P = <0.001$, $F = 12.645$). Multiple pairwise comparisons using Tukey's test exposed day 10, the day of lowest phyllode RWC, as having significantly lower F_v/F_m compared with all other days ($P = 0.011$) in *A. floribunda* droughted plants. In comparing the two species, *A. floribunda* appeared to show a slower decline of F_v/F_m with drought and a swifter recovery following rewatering (Fig. 5).

Examining fluorescence variables in droughted plants on the day of least stress (day 3) compared with the day of

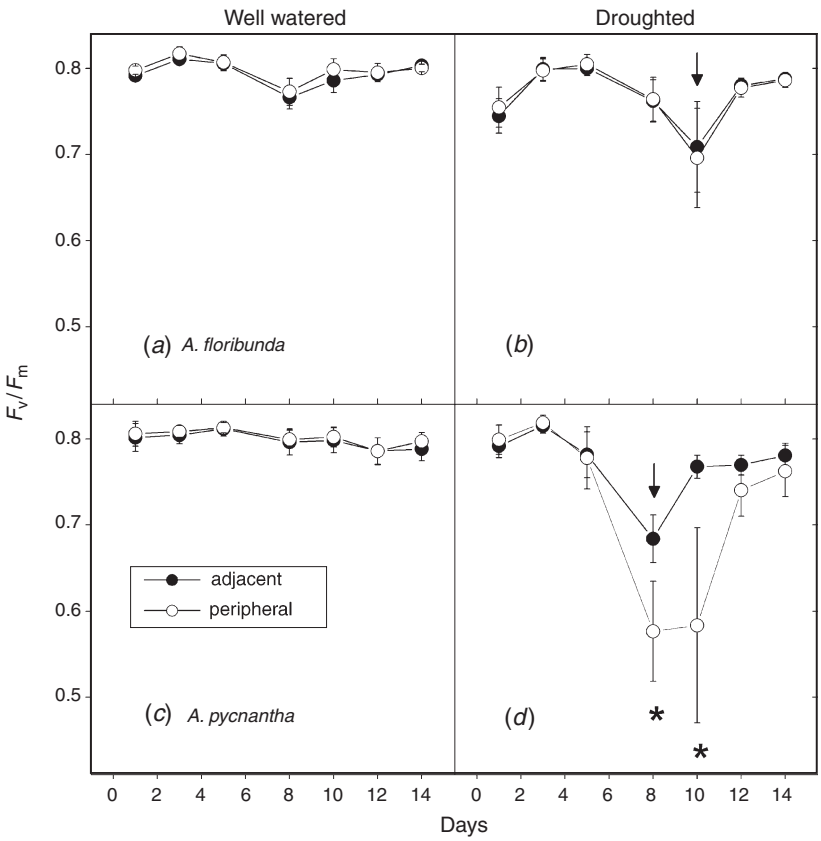

Fig. 5. Changes in the maximum quantum efficiency of PSII (F_v/F_m ± s.e., $n = 4$) with time and position on the phyllode in the measurement cycle for (*a*, *b*) *Acacia floribunda*, (*c*, *d*) *Acacia pycnantha*, well watered (*a*, *c*), droughted (*b*, *d*) plants. Closed circles indicate phyllode regions adjacent to a primary nerve, open circles indicate peripheral phyllode regions near a secondary nerve. Asterisks indicate significant differences ($P < 0.05$) as identified using repeated-measures ANOVA with Tukey's test for individual comparisons. Arrows indicate re-watering following measurement.

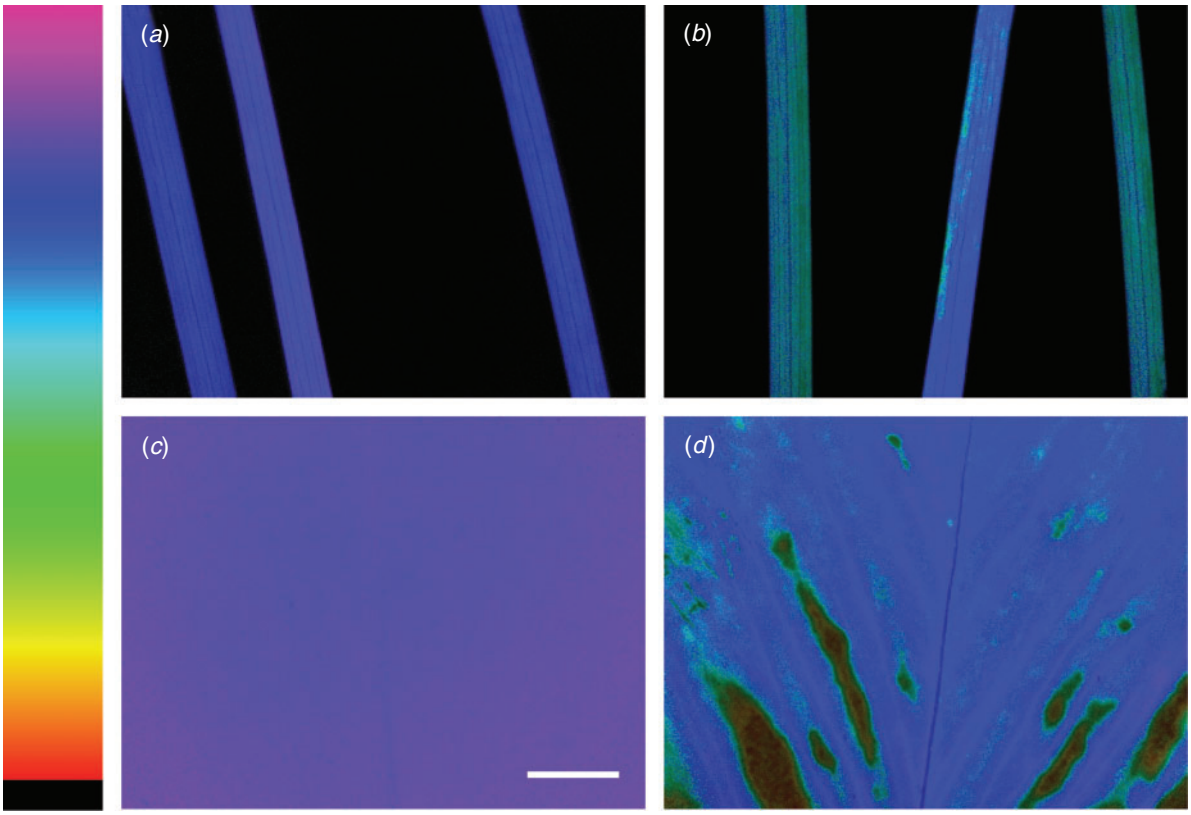

Fig. 6. Spatial variation in maximum quantum efficiency of PSII photochemistry (F_v/F_m) in phyllodes of (*a*, *b*) *Acacia floribunda* and (*c*, *d*) *A. pycnantha*. Phyllodes (*a*, *c*) before the measurement cycle and the same phyllodes (*b*, *d*) at the day of greatest water stress. Colour scale at left of image ranges from $F_v/F_m = 0$ (black) to 1 (pink). Spatial scale bar = 0.5 cm.

greatest stress (day 8 for *A. pycnantha* and day 10 for *A. floribunda*) there was a marked difference in the response of each species to drought (Fig. 7). Although *A. floribunda* showed a decline in F_m and a concomitant decline in F_v/F_m with stress, there was no significant difference between any of these variables on the day of greatest stress compared with the day of least stress. By contrast, *A. pycnantha* showed a significantly higher F_o at both the adjacent and peripheral positions on the day of greatest stress compared with the day of least stress. The higher F_o (and the markedly lower F_m) was reflected in a significant decrease in F_v/F_m in *A. pycnantha* on the day of greatest stress.

Discussion

To our knowledge, this is the first study to show a different spatial response in photosynthesis with drought in two species with contrasting hydraulic architecture. We hypothesised that areole regions near primary nerves would show a smaller decline in the maximum efficiency of PSII photochemistry with drought compared with regions between secondary nerves and that the difference between areole regions would be smaller in phyllodes with higher primary nerve density. Indeed, the phyllodes of *A. floribunda* were found to have both greater primary nerve density and show greater spatial homogeneity in photosynthetic function with drought compared with the phyllodes of *A. pycnantha*. *A. floribunda* phyllodes also maintained function of the photosynthetic apparatus with drought for longer and recovered more swiftly from drought than *A. pycnantha*. These findings are consonant with the premise that greater primary nerve density in *Acacia* phyllodes may provide improved hydration of phyllode mesophyll where rainfall is meagre and infrequent (Sommerville 2010).

The observed spatial patterns in photosynthetic function with drought suggest that greater primary nerve density may allow more even spatial distribution of water across the phyllode or greater tolerance of hydraulic disruption in primary supply lines. Other studies have shown heterogeneity in photosynthetic activity related to leaf venation (Siebke and Weis 1995; Bro *et al*. 1996; Walter *et al*. 2004). However, to our knowledge, this is the first study to test a specific hypothesis regarding how contrasting nerve (vein) patterns may impact spatial heterogeneity in photosynthetic function with drought. The finding of significantly lower maximum quantum efficiency of PSII in peripheral phyllode regions with drought in the species with significantly lower primary nerve density (and higher minor vein density) has several implications. Minor veins may have more negative water potentials than the rest of the vascular network of a transpiring plant and therefore may be more likely to suffer hydraulic dysfunction with drought (Salleo *et al*. 2001). With a higher density of minor nerves, *A. pycnantha* may be more likely to exhibit lower F_v/F_m with drought in peripheral mesophyll tissue due to loss of supply from

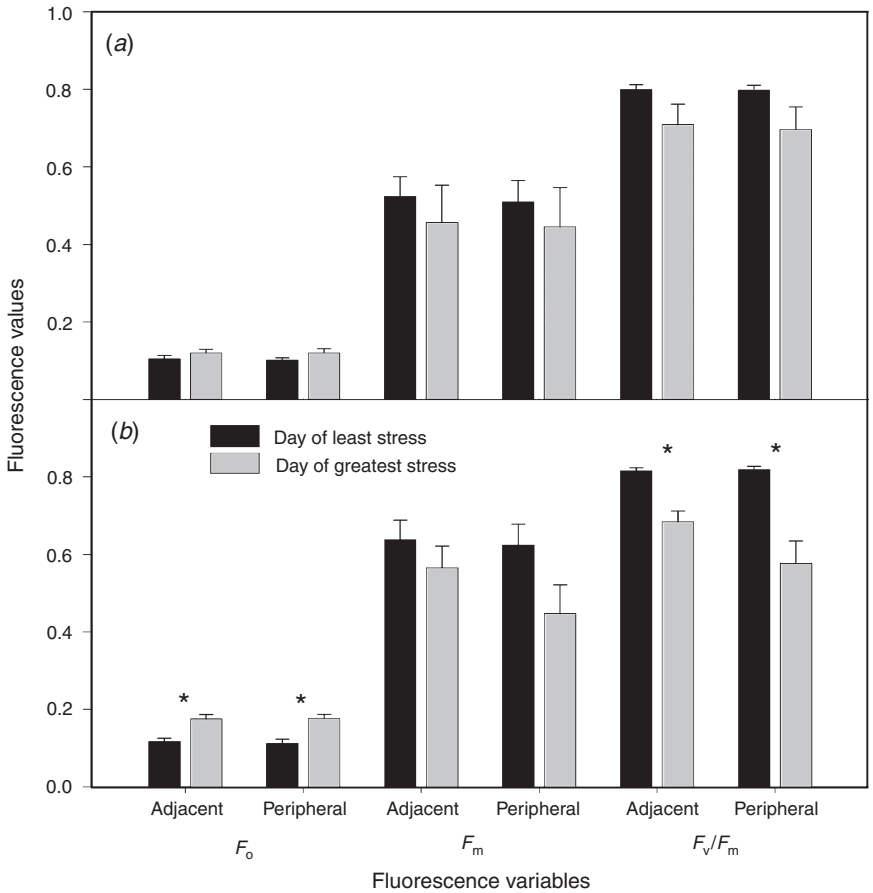

Fig. 7. Differences in the minimal fluorescence ($F_o \pm$ s.e., $n = 4$), maximal fluorescence ($F_m \pm$ s.e., $n = 4$) and maximum quantum efficiency of PSII ($F_v/F_m \pm$ s.e., $n = 4$) for drought plants on the days of least and greatest stress with position on phyllode in the measurement cycle for (*a*) *Acacia floribunda*, (*b*) *Acacia pycnantha*. Asterisks indicate significant differences ($P < 0.05$) between days of least and greatest stress as identified using repeated-measures one-way ANOVA with Tukey's test for comparisons of individual days (including those not displayed here).

minor nerves. It could also be that, where xylem cavitation occurs in one primary nerve, blocking the flow of water downstream, mesophyll regions can be supplied with water through alternate primary nerves where present (Sack *et al*. 2008), thus, explaining the higher F_v/F_m in peripheral mesophyll of *A. floribunda* with its higher primary nerve density. Whether it is through reduced hydraulic dysfunction in minor nerves, or availability of alternate supply routes, higher primary nerve density appears associated with greater tolerance of the photosynthetic apparatus to dehydration across the plane of the phyllode.

The maximum quantum efficiency of PSII in *A. floribunda* appeared to recover faster than in *A. pycnantha* despite *A. floribunda* phyllodes reaching lower RWC than in *A. pycnantha*. The RWC_0 and Ψ_0 were not significantly different between species suggesting both species experienced similar severity of drought stress at turgor loss. On day 8, when RWC for *A. floribunda* and *A. pycnantha* were similar, *A. floribunda* droughted plants showed no significant difference in maximum quantum efficiency of PSII compared with well watered plants. By contrast, *A. pycnantha* droughted plants showed a significant decline in maximum quantum efficiency of PSII, which, despite rewatering, did not recover to pre-drought levels for a further 6 days. In the present study, faster recovery from lower RWC may indicate greater tolerance of dehydration in *A. floribunda* phyllodes. More drought tolerant cells could be expected to respond more rapidly to rewatering than those that may have been damaged. However, the faster recovery from lower RWC in *A. floribunda* may also indicate faster rehydration through greater primary nerve density. Slatyer (1962*a*, 1962*b*) demonstrated that the arid zone species, *Acacia aneura*, remains dormant when drought occurs but can resume growth within 4 days after rainfall despite phyllodes persisting at a RWC of only 45% before water became available. The capacity to take advantage of water when it becomes available may assist the survival of plants in dry environments. It is possible that greater primary nerve density provides acacias with the architecture to quickly upregulate activity and maximise the potential for growth. Further work examining responses of tissue in spatially distinct positions on a phyllode to changes in phyllode water potential and conductance is required to elucidate the underlying physiological responses to drought and recovery observed in the present study.

In *A. pycnantha* droughted plants there was a significantly higher F_o on the day of greatest water stress compared with the day of least stress. This, in turn, was associated with a significantly lower F_v/F_m. By contrast, *A. floribunda* showed only a slight increase in F_o with a marked though non-significant decrease in F_m with stress. In plants, a decrease in F_m may be due to an increase in non-photochemical quenching perhaps due to photoinactivation of PSII reaction centres, which then dissipate excess energy as heat rather than through photochemistry (Osmond 1994). Photoinactivation can also lead to oxidative damage and loss of PSII reaction centres causing an increase in F_o (Bradbury and Baker 1986). However, changes in F_m and F_o must be interpreted with caution as these changes may have been the result of changes in the optical properties of the phyllode with changes in phyllode water content (Baker 2008). Changes in thylakoid membrane structure and organisation with dehydration can occur, which may alter measured F_m and F_o independent of fluorescence quenching or photodamage. Thus it is difficult to determine whether observed changes in F_m and F_o were due to photo-oxidative damage, quenching processes, or modification of phyllode optical properties. Nevertheless, following the interpretation by Franklin *et al*. (1992) the higher F_o coincident with the lower F_v/F_m in *A. pycnantha* droughted plants may indicate damage to PSII consistent with the slower rate of recovery of F_v/F_m following rehydration in these plants.

Conclusion

In *Acacia*, greater primary nerve density may ensure more even phyllode hydration with drought and swifter recovery following precipitation, thereby protecting photosynthetic function across the phyllode plane. The unique capacity of phyllodinous *Acacia* to alter the number of primary nerves may increase phyllode drought tolerance and possibly contribute to phyllode productivity following drought. This finding may explain, in part, the predominance of acacias with high primary nerve density in areas with lower precipitation. As drought frequency and intensity are predicted to increase in many parts of Australia (CSIRO and BOM 2007), traits that enhance drought tolerance, such as greater primary nerve density in *Acacia*, may become increasingly important.

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