Published in the Australian Journal of Botany 2000, vol 48, p 143 – 151

Influence of Season, Drought and Xylem ABA on Stomatal Responses to Leaf-to-air Vapour Pressure Difference of Trees of the Australian Wet Dry Tropics

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Abstract

This paper reports the results of two experiments undertaken to investigate the influence of season and soil drying on stomatal responses to leaf-to-air vapour pressure differences (D). We examined the response of stomatal conductance (g) to increasing D, in the wet and dry seasons, of five tropical tree species. We also examined leaves of these species for anatomical differences to determine whether this could explain differences in stomatal sensitivity to D. Finally we conducted a split root experiment with one of those species to look for interactions between xylem abscisic acid concentration, pre-dawn water potential, leaf area to root mass ratio and stomatal responses to D.

Stomatal conductance declined linearly with increasing D in all species. Leaves expanded in the 'dry' season were more sensitive to D than those that had expanded in the 'wet' season. The value of D where 50% of extrapolated maximum stomatal conductance would occur was 5.5 kPa for wet season but only 3.4 kPa for dry season leaves. In the wet season, transpiration rate increased with increasing D in most examplesp; however in the dry season transpiration was constant as D increased in most cases. There were significant changes in the proportion of cell wall exposed to air space in leaves, between wet and dry seasons, in three of four species examined.

In the split root experiment, a very mild water stress increased stomatal sensitivity to D, and g declined linearly with decreased pre-dawn water potential. However levels of ABA in the xylem did not change, and stomatal sensitivity to

exogenous ABA did not change. The ratio of leaf area to root mass declined during water stress and was correlated to changes in stomatal sensitivity to D.

Introduction

Plants growing in the tropical savanna of Northern Australia experience diverse environmental conditions in a year. From May to October (dry season), leaf-to-air vapour pressure difference (D) can reach >5.0 kPa and leaf temperature (Tl) may exceed 35 (C. In contrast, from November to April (wet season), over 90% of the 1600 mm annual rain falls, D rarely exceeds 2.5 kPa and Tl is usually cooler than air temperatures (average maximum air temperature of 33 (C). Pre-dawn water potential of trees declines between wet and dry seasons as soil and atmospheric moisture content declines (Duff et al. 1997).

Field studies have shown daily maximum stomatal conductance is highly correlated with pre-dawn leaf water potential in a range of north Australian savanna species (Fordyce et al. 1997; Myers et al. 1997; Eamus and Cole 1997; Prior et al. 1997). These same studies showed stomatal conductance (g) was significantly and negatively related to D. However, these field data suffered from the lack of independent control of D and frequently utilised data collected on one occasion when water was least limiting in this environment (March). Therefore, to better understand the importance of D on g we used a laboratory based system where D, Tl and photosynthetic photon flux density (Q) could be controlled independently.

Comparisons of the long-term effect of D on stomatal sensitivity have focused on populations growing in different conditions, where both dew point and air temperature differ, and affect D (Roy and Mooney 1982; Mooney and Chu 1983). These studies concluded that differences in growth conditions did not affect sensitivity of g to D. In

contrast *Oryza sativa* grown at high D had a larger sensitivity of g to D than plants grown at low D, the sensitivity of net photosynthesis (A) to D was unaffected by growth conditions under different conditions of D (Kawamitsu et al. 1993). El-Sharkawy et al. (1985) reported that species with the largest stomatal density were most sensitive to D. However, there was no change in stomatal density in *O. sativa* associated with D during growth (Kawamitsu et al. 1993).

Abscisic acid concentration in xylem sap increases as soil moisture content declines (Loveys et al. 1987; Correia et al 1997). Split root systems, where a portion of the root is irrigated and another portion subject to drought, show that ABA derived from roots, rather than leaf water potential, causes g to decline during drought (Zhang et al. 1987; Zhang and Davies 1991). Tardieu et al. (1992) also suggest that D can influence the rate of supply of ABA to a leaf through dilution of xylem sap as transpiration increases. Finally, stomatal sensitivity to exogenous ABA can change following drought stress (Eamus 1986, 1987). Therefore, the second experiment in the paper investigates the influence of a mild water stress on stomatal sensitivity to D, xylem ABA levels, stomatal sensitivity to exogenous ABA and changes in leaf area to root mass ratio. *A priori* reasoning suggests that changes in the ratio of potential demand (leaf area) to potential supply (root biomass) of water could influence stomatal sensitivity to D (Norby and O'Neill 1991).

In this paper we address the following hypotheses: (a) stomata of leaves that have expanded during the wet season are less sensitive to D than leaves that expanded in the dry season; (b) differences in anatomy between wet and dry season leaves may explain observed differences in stomatal sensitivity to D; (c) drought increases stomatal sensitivity to D and this increase is the result of increased supply of ABA to the leaf; (d) stomatal sensitivity to exogenous ABA increases during a drought; and (e) changes in leaf area to root mass ratio correlate with changes in stomatal sensitivity to D.

Materials and Methods

Two experiments were conducted. The first was a comparison of stomatal responses to increased D of five tropical tree species. Comparisons were made among

leaves that had expanded in the dry season and those that had expanded in the wet season. The second experiment involved a split root experiment of just one species, (*Acacia auriculiformis*).

Experiment 1: Wet versus Dry Season

Plant material

The following five species were studied: *Acacia auriculiformis* Cunn., an evergreen species found widespread on coastal fringes and riparian sites inland; *Maranthes corymbosa* Blume, and *Myristica insipida* R.Br., evergreen rainforest species whose habitats include coastal and monsoon rainforests, riparian sites, sandstone country and dry vine thickets; *Syzygium eucalyptoides ssp eucalyptoides* (F.Muell.)B.Hyland, a semi-deciduous tree found in open woodland, permanent streams and monsoon rainforests; and *Terminalia platyphylla* F.Muell, a semi- to fully deciduous riparian species (Brock 1993). These species were chosen because they represent a range of phenologies and occur in a range of ecosystems, from open woodland to closed rainforest. Although not dominant, they are common species.

All plants were grown in 6 L pots of sand:peat:vermiculite:perlite (2:2:1:1) and irrigated daily, in Darwin, NT, Australia. Plants were grown in a 30% light transmission shadehouse. Plants were regularly fertilised with liquid (Aquasol) and slow release fertiliser (Osmocote), with regular pest and disease control being undertaken when appropriate. Leaves that had expanded in the wet season (November onwards) were measured in January. Near the beginning of the dry season (May) approximately 25% of shoot biomass was removed. Leaves that expanded on new regrowth during the dry season were thus identified and measured in October.

Laboratory leaf gas exchange measurements

Gas exchange was measured using equipment described previously (Eamus et al. 1995), which allowed environmental conditions within each of the three or four leaf chambers to be controlled. Modifications to this equipment included use of a cooled mirror dewpoint hygrometer (Hygro-M1, General Eastern Instruments, Watertown,

MA, USA) to measure dew point of the incoming and outgoing air. Dew point of the incoming air was controlled by bubbling air through water at a specific temperature. Leaf temperature was controlled in water-cooled leaf chambers and measured by a leaf thermocouple adpressed to the abaxial leaf surface. By altering either Tl or the dew point of incoming air, the D of the air surrounding the leaf could be controlled. Input air had an ambient CO2 concentration of 360 ± 10 μmol mol-1. Photosynthetic photon flux density (Q) was provided by 400 W metal halide lamps.

Three or four replicate plants of a species were moved to the laboratory the day before measurements were taken. PPFD was increased from 0 to 1000 μmol m^{-2} s^{-1} over 90 minutes by removing neutral density filters. All of the plant except for the leaf being studied was maintained in low light conditions by sheltering the leaves from light by metal foil. All gas exchange measurements were collected between 1000 to 1600 hours as previous studies had shown that gas exchange was stable during these hours when environmental conditions were maintained constant. During the experimental period the plants were kept well watered by irrigating every 2 h and by maintaining a small reservoir of water under the pots.

Stomatal conductance was measured on 1 to 2 x 10-3 m^2 of a leaf from each plant near the central portion of mature leaves. Stomatal density was later determined on the central sections of these leaves using glue impressions of both leaf surfaces. Stomatal conductance responses to D at 33 ^{o}C was studied by increasing from lowest to highest value throughout the day (1.8 to 4.2 kPa in approximately 0.6 kPa steps). Equilibration of gas exchange to a new set of conditions typically took between 30 and 60 minutes. Stomatal conductance was calculated using the equations outlined in Long and Hallgren (1985).

Leaf anatomy

Leaf anatomy of leaves similar to those used for stomatal conductance measurements (young, fully expanded) were investigated using 2 mm2 leaf pieces from the central region of three replicate leaves. These segments were fixed in 2.5% glutaraldehyde in 50 mM phosphate buffer (pH = 7.0), dehydrated in an ethanol series

then embedded in Spurrs resin. Thin transverse sections were stained with toluidine blue before taking photomicrographs of areas of the leaf not situated near major vascular bundles. Photographs were scanned into an image processor (Vistascan then Adobe photoshop) and then analysed using NIH image, version 1.5. Cells were outlined to increase definition between cells and air spaces in leaf. The area and perimeter of individual cells and air spaces was calculated. The average cell area (mm2);, the ratio of cell to (cell+air) space and the ratio of air space perimeter to perimeter of cells; and average ratio of cell area to cell perimeter was calculated. These values were regressed against the calculated sensitivity of g to D.

Experiment 2: Split Root

Plant material

Acacia auriculiformis seeds were surface sterilised and germinated on sterile filter paper in petri-dishes. The terminal 10 mm of root tip was removed to encourage root branching and the seedling transplanted to a 0.75 L pot containing sand:peat:vermiculite: perlite (2:2:1:1) in May 1997. These plants were grown in a 30% light transmission shadehouse. In July 1997 these small pots were positioned over a 6.5 L pot constructed from two lengths of rectangular PVC pipe (100 (65 (500 mm) held together such that the roots could grow into either independent soil compartment. These pots contained 5.5 L soil (top soil:peat:coarse sand (2:1:1) with 6 kg m^{-3} osmocote (17 N: 2.6 P:10 K). Plants received regular additional liquid fertiliser (Aquasol) and pest and disease control was undertaken when appropriate. The plants were moved to 70% light transmission shadehouses and irrigated daily to field capacity until September 1997.

In September, the plants were randomly divided into three groups. Control plants received irrigation to field capacity twice daily in both soil compartments (FW); fully droughted plants received no irrigation to either soil compartment (FD); the third group received irrigation to field capacity in one compartment and no irrigation in the other compartment (PW). Plants received a maximum of 16 days drought and were measured periodically in this time period.

Stomatal sensitivity to phyllode-to-air vapour pressure difference

Initial experiments showed that it did not matter which side of the plant was chosen when selecting a leaf for study. Thus, we found phyllodes behaved similarly within a plant and therefore we measured one phyllode per plant in this second experiment.

The sensitivity of stomata of a young, fully mature phyllode to changes in D was measured, as described above, on four plants per day. One representative of each of the FW and FD treatments and two of the PW treatment were moved to the laboratory the night before measurements. The following morning pre-dawn phyllode water potential was measured on a more mature phyllode than that used in gas exchange measurements. Photosynthetic photon flux density at the phyllode surface was slowly increased to 1000 μmol m^{-2} s^{-1} over 1.5 h while phyllode temperature was maintained at 33 ^{o}C and D maintained at less than 2 kPa. Throughout the experiment phyllode temperature was maintained at 33^{o} C, and ambient carbon dioxide concentration was maintained at 360 μmol mol^{-1}. The stomatal response to D was measured while D was increased from 1.8 to 4.2 kPa in approximately 0.5 kPa steps.

Abscisic acid collection and analysis

Abscisic acid was collected from the phyllode used in gas exchange measurements and from the severed stem immediately after collecting gas exchange data when D was largest (about 4.2 kPa) by pressurising these segments to pressures greater than xylem equilibrium pressure in a Scholander type pressure chamber. The samples were immediately frozen in liquid nitrogen before being freeze dried. Abscisic acid concentration was analysed by addition of an internal standard of deuterium labelled ABA [3',5',5',7',7',7'-D6 ABA] (usually 20 ng per sample). The samples were methylated with ethereal diazomethane, dried down and redissolved in approximately 50 μl acetone prior to GC/MS analysis. The HP 6890 MSD was operated in selected ion mode, monitoring 190, 194, 162, 166 ions. ABA was quantified by referring the sample 190/194 or 162/166 ratios to a calibration curve.

ABA - phyllode feeding

Stomatal responses to exogenous ABA were measured by the method of Eamus (1986, 1987). In summary, two plants from each treatment were moved into the gas exchange laboratory maintained at a constant temperature of 30 ºC and allowed to equilibrate to ambient temperature and PPFD conditions. PPFD of 1000 μmol m^{-2} s^{-1} was provided by metal halide lamps. Stomatal conductance of phyllodes was measured with a porometer (AP4, Delta-T devices, UK) prior to cutting them under solutions containing 10-5 mol m^{-3} ABA dissolved in 0.8 ml L^{-1} ethanol, 1 mol m^{-3} $CaCl_2$, pH 6.0 adjusted with NaOH, or control solutions not containing ABA. Stomatal conductance was measured periodically for up to 3 h. We compared the influence of presence or absence of ABA in the solution on the decline in stomatal conductance with time for the three irrigation treatments.

Plant and soil collection

Soil samples from both the upper and lower regions from both sides of the pots were collected, weighed, oven dried for 24 h at 105 ^{o}C, and reweighed in order to calculate the % soil moisture profile within the pots. Roots were collected from both halves of the pot. All plants were oven dried (80 ^{o}C) and dry weights of leaves, stems and branches, and roots measured. Leaf area was measured with an area meter (Delta-T devices, UK).

Statistical Analysis

Experiment 1

Linear regression analysis was used to determine the relationships between g and D using Statistica 5.0. Maximum stomatal conductance (g_{max}) (extrapolation of the g against D regression to D=O) and D_{50} (the value of D that gives half the g_{max}) were calculated for each leaf by fitting individual regression lines for each replicate leaf. Differences in calculated values of D_{50} between seasons (wet and dry) were analysed by t-tests. Anatomical differences were compared by ANOVA and where significant

($P<0.05$) differences existed the species means were separated by Tukeys' honest significant difference test.

Experiment 2

Spearman's rank correlations were used to test for correlations between ranked water availability and other 3 variables, namely, g_{max}, D_{50} and pre-dawn phyllode water potential. Linear functions to relationships were fitted by Statistica 5.0.

Results

Experiment 1

Stomatal conductance declined linearly with increasing D, in all species (Fig. 1). In two of the four possible comparisons, leaves that had expanded during the wet season had a smaller slope than those that had expanded in the dry season (Table 1). Taking a simple average of all species, a smaller slope (-28 mmol m^{-2} s^{-1} kPa^{-1}) was observed for leaves that had expanded in the wet season compared to leaves that had expanded during the dry season (slope = -39 mmol m^{-2} s^{-1} kPa^{-1}). The intercept (g_{max}) did not differ between the two sets of leaves. Leaves that had expanded during the wet season exhibited a significantly larger D_{50} (4.1 kPa) than those that had expanded during the dry season (3.4 kPa).

Stomatal density within a species did not differ significantly ($P>0.05$) between seasons (Table 1), nor was there a relationship between stomatal density and g_{max}, or D_{50}.

For leaves that had expanded in the wet season, transpiration increased markedly (eg. *Acacia auriculiformis, Syzygium eucalyptoides* and *Maranthes corymbosa* in the wet season) or only slightly (*Myristica insipida, Terminalia platyphylla*) as D increased (Fig. 2). For leaves that had expanded in the dry season, transpiration tended to increase minimally (*Acacia auriculiformis, Syzygium eucalyptoides*) or not at all (*Maranthes corymbosa, Myristica insipida*).

In most instances, and for much of the tested range of D, we observed that

transpiration (E) was kept within very narrow bounds despite large variations in g and D (Fig. 3). Our data could be categorised as typical of what Monteith (1995) classifies as regime A followed by regime B, that is, stomatal conductance decreasing linearly with increasing rate of transpiration and increasing D (regime A) followed by a constant rate or small decline in E at higher D, caused by further declines in g (regime B). Regime A can be observed in *Acacia auriculiformis* and *Maranthes corymbosa* in the wet season, and regime A followed by regime B can be observed in the remaining cases (Fig. 3).

Leaf morphology was very different between species with large differences in stomatal density (only *Acacia auriculiformis* and *Syzygium eucalyptoides* were amphistomatous) and leaf anatomical characterisics (Table 1). About 70% of the leaf volume in *Acacia auriculiformis* and *Syzygium eucalyptoides* was occupied by cells, with less than about 30% occupied by air spaces, whereas the other species were almost equally divided between air spaces and cellular material. The percentage of the total perimeter of cellular material in contact with an air space was significantly lower in *Acacia auriculiformis* and *Syzygium eucalyptoides* (approximately 25% compared with 40% for other species). *Maranthes corymbosa* and *Myristica insipida* leaves which expanded in the wet season had larger cells and less of the leaf was taken up by air spaces. This led to a reduced contact between air spaces and cells. In contrast leaves of *Acacia auriculiformus* and *Syzygium eucalyptoides* which had expanded in the wet season had a higher proportion of the cross-sectional area of the leaf taken up by air spaces and smaller or similar sized average cell areas. This resulted in Syzygium *eucalyptoides* having a greater proportion of the cell perimeter in contact with the air.

Despite anatomical differences between species and seasons there were no significant relationships between anatomical measurements of percentage cellular cross sectional area to total cross sectional area, or average cell perimeter exposed to air space and stomatal sensitivity to D.

Experiment 2

Spearman's rank correlation showed a significant correlation between ranked

irrigation treatments and several measured variables. In the following description, Treatment 1 refers to the control (FW - fully watered in both soil compartments), Treatment 2 refers to irrigation supplied in one side only (PW) and Treatment 3 refers to no irrigation supplied to either side (FD).

The soil was drier in the unirrigated soil compartments, but no differences existed in the wet compartments of FW and PW, or the dry soil compartments of FD and PW (Table 2). As plant available moisture declined (in the sequence FW > PW > FD), the ratio of leaf area to root dry weight decreased in the same order (Table 2). The linear relationship between D_{50} and leaf area to root dry weight ratio was significant (r^2 = 0.63; P <0.05).

There was a significant correlation between ranked water availability and pre-dawn water potential from Treatment 1 through to Treatment 3 (Table 2). Similarly, maximum stomatal conductance (g_{max}), calculated from the extrapolation of the linear regression of g against D showed a significant correlation with ranked water availability, in the order Treatment 1 > 2 > 3. There was a linear decline in g_{max} as pre-dawn phyllode water potential declined (Table 2, Fig. 4). Finally D_{50} declined in the order 1 > 2 > 3 (Table 2, Fig. 4). In contrast, there was no treatment effect on xylem sap abscisic acid (Table 2).

When ABA was supplied to excised leaves, g declined with time (Fig. 5 as one example). The slope of the response of stomata to exogenous ABA did not differ between watering treatments.

Discussion

For two of four species for which data were available, stomata of leaves which had expanded in the dry season were more sensitive to D than those which had expanded in the wet season. Similarly, using simple means of all species tested, the slope of the decline in g with increasing D, was larger for dry season leaves compared to wet season leaves (Table 1). Furthermore, D_{50} (D where 50% of g_{max} occurs), was smaller in leaves that had expanded in the dry season leaves compared to wet season leaves (Table 1). In this respect the stomatal response to D was similar to those species

where D was varied independently of air temperature (Kawamitsu et al. 1993), as opposed to species where both air temperature and relative humidity were altered (Roy and Mooney 1982; Mooney and Chu 1983). We did not find any differences in responses sensitivity, between the closed canopy, rainforest, species (*Maranthes corymbosa, Myristica insipida*), and the more open canopy, riparian/woodland species of either evergreen (*Acacia auriculiformis, Syzygium eucalyptoides*) or deciduous (*Terminalia platyphylla*) guilds. This agrees with the results of Myers et al. (1997) who examined g and D relationships between phenological guilds in savanna woodland species in the wet season.

Why Were Stomata of Dry-season Leaves and Water Stressed Plants more Sensitive to D than Wet Season or Well Watered Leaves?

The differences in responses of g to D of leaves expanded in the two seasons cannot be explained by changes in stomatal density, which, within a species, were not significantly different ($P>0.05$) between seasons (Table 1). Three alternative hypotheses can be considered briefly. First, changes in xylem anatomy caused a decline in hydraulic conductivity of *Acer grandidentatum* plants growing in lower soil moisture (Alder et al. 1996) and such a mechanism may operate in response to atmospheric moisture deficits. Thus, a reduced g in the dry season may, in part, be a consequence of anatomical changes to the pathways of water flow. Second, in two species (*M. corymbosa and M insipida*) there was a significant decline in the average cell wall area exposed to air space in leaves that developed within the dry season compared to leaves that developed in the wet season. In a third species (*S. eucalyptoides*) there was a significant increase in the amount of cell wall exposed to air space and in the fourth (*A. auriculiformis*) there was non-significant increase in the area of cell wall exposed to air space. In all species, stomata of dry-season leaves were significantly more sensitive to D than wet-season leaves. It is interesting to note that the two species showing a significant decline in area exposed to air space are both hypostomatous, whilst the remaining two species, which showed an increase in area of cell wall exposed to air space, are amphistomatous. We can only speculate that these differences in stomatal distribution, and the fact that the direction of change of the amount of cell wall exposed to air space between seasons were opposite for the two groups, are related. Evaporation from mesophyll cell walls may provide the

coupling between transpiration rate and assimilation, whereby increasing transpiration causes a decline in assimilation rate independent of stomatal aperture (Sharkey 1984; Bunce 1988). It is possible that changes in stomatal sensitivity to D between wet and dry seasons are, in some manner, connected to changes in leaf anatomy. Finally, Kerstiens (1996) reports that in two of three examples a higher permeability of water through the cuticle was significantly ($P<0.05$) correlated with greater sensitivity of stomata to D. This could explain why dry-season leaves have greater sensitivity to D.

Abscisic Acid and Leaf Area to Root Biomass Ratios

The concentration of ABA in xylem sap increases with soil drying and such increases in xylem ABA concentration are negatively correlated with g (Correia et al. 1997; Loewenstein and Pallardy 1998). Drought also influences stomatal sensitivity to exogenous ABA (Eamus 1986, 1987). We applied only a very mild water stress, even in treatment 3, where both soil compartments were allowed to dry out. Thus pre-dawn water potential declined to only –0.75 MPa (average of final 3 days of treatment) in Treatment 3. In Treatment 2, pre-dawn water potential did not differ from that of the control. Thus, xylem levels of ABA did not differ between treatments, and pre-dawn water potential and g_{max} were negatively correlated only in treatments 2 and 3, further highlighting that the drought stress was very mild. However, in both experimental treatments, stomatal sensitivity to D increased as observed by the changes in D_{50} and slope of the decline in g as D increased (Table 1, 2). It cannot be changes in xylem ABA content that caused the increase in stomatal sensitivity to D. In addition, it cannot be changes in stomatal sensitivity to ABA that increased sensitivity to D since the exogenous supply of ABA induced the same closing response in all three treatments (Fig. 5).

The ratio of potential demand to water supply potential (leaf area to root biomass ratio) has been used as an indicator of plant vulnerability to drought (Norby and O'Neill 1991; Duff et al. 1994). We now suggest that a change in this ratio also causes changes in stomatal sensitivity to D. If leaf water potential (or turgor potential) are to be regulated by a plant as soil and atmospheric water content vary, changes in stomatal sensitivity to D in response to changes in leaf area to root biomass ratio may represent an efficient optimisation response to couple the potential for water

loss to the potential for water uptake. In field observations of root growth, Bowman (pers. comm) has seen reductions in fine root biomass in the upper 1.5 m of soil in north Australian savannas at the same time as large reductions in leaf canopy (Williams et al. 1997). We suggest that such differences may contribute to changes in stomatal sensitivity to D between wet and dry seasons (Prior et al. 1997).

In conclusion, stomatal sensitivity to D differs between wet and dry seasons for two species of savanna trees. Changes in leaf anatomy were also observed but these were not correlated with observed differences in sensitivity to D. In a split root experiment in which only mild soil drying was allowed, changes in stomatal sensitivity to D occurred without significant changes in xylem ABA or pre-dawn water potential. We suggest that changes in leaf area to root biomass ratio may couple the stomata to changes in soil and atmospheric water potential so that leaf water potential is tightly regulated.

Acknowledgments

This research was supported by Australian research council grant A19532684. We thank B. Loveys (CSIRO, Adelaide) for analysis of abscisic acid samples.

References

Alder, N.N., Sperry, J.S., and Pockman, W.T. (1996). Root and stem xylem embolism, stomatal conductance, and leaf turgor in *Acer grandidentatum* populations along a soil moisture gradient. *Oecologia* **105**, 293-301.

Brock, J. (1993). 'Native Plants of Northern Australia.' (Reed publishers: Chatswood.)

Bunce, J. A. (1988). Non-stomatal inhibition of photosynthesis by water stress. Reduction in photosynthesis at high transpiration rate without stomatal closure in field grown tomato. *Photosynthesis Research* **18**, 357-362.

Correia, M.J., Rodrigues, M.L., Ferreira, M.I., and Pereira, J.S. (1997). Diurnal

change in the relationship between stomatal conductance and abscisic acid in the xylem sap of field-grown peach trees. *Journal of Experimental Botany* **48**, 1727-1736.

Duff, G.A., Berryman, C.A., and Eamus, D. (1994). Growth, biomass allocation and foliar nutrient content of two *Eucalyptus* species of the wet-dry tropics of Australia grown under CO2 enrichment. *Functional Ecology* **8**, 502-508.

Duff, G.A., Myers, B.A., Williams, R.J., Eamus, D., O'Grady, A.O., and Fordyce, I.R. (1997). Seasonal patterns in soil moisture, vapour pressure deficit, tree canopy cover and pre-dawn water potential in a northern Australian savanna. *Australian Journal of Botany* **45**, 211-224.

Eamus, D. (1986). The responses of leaf water potential and leaf diffusive resistance to abscisic acid, water stress and low temperature in *Hibiscus esculentus*; the effect of water stress and ABA pre-treatments. *Journal of Experimental Botany* **37**, 1854-1862

Eamus, D. (1987). The influence of pre-conditioning upon changes in leaf conductance and leaf water potential induced by chilling, water stress and abscisic acid in soybean. *Australian Journal of Plant Physiology* **14**, 331-339.

Eamus, D., and Cole, S. (1997). Diurnal and seasonal comparisns of assimilation, phyllode conductance and water potential of three *Acacia* and one *Eucalyptus* species in the wet-dry tropics of Australia. *Australian Journal of Botany* **45**, 275-290.

Eamus, D., Duff, G.A., and Berryman, C.A. (1995). Photosynthetic responses to temperature, light flux-density, CO_2 concentration and vapour pressure deficit in *Eucalyptus tetrodonta* grown under CO_2 enrichment. *Environmental Pollution* **90**, 41-49.

El-Sharkawy, M.A., Cock, J.H., and Pilar, A.D. (1985). Stomatal response to air humidity and its relation to stomatal density in a wide range of warm climate species. *Photosynthesis Research* **7**, 137-149.

Fordyce, I.R., Duff, G.A., and Eamus, D. (1997). The water relations of Al*l*osyncarpia

ternata (Myrtaceae) at contrasting sites in the monsoonal tropics of northern Australia. *Australian Journal of Botany* **45**, 259-274.

Kawamitsu, Y., Yoda, S., and Agata, W. (1993). Humidity pretreatment affects the responses of stomata and CO2 assimilation to vapour pressure difference in C3 and C4 plants. *Plant Cell Physiology* **34**, 113-119.

Kerstiens, G. (1996). Cuticular water permeability and its physiological significance. *Journal of Experimental Botany* **47**, 1813-1832.

Loewenstein, N.J., and Pallardy, S.G. (1998). Drought tolerance, xylem sap abscisic acid and stomatal conductance during soil drying: a comparison of young plants of four temperate deciduous angiosperms. *Tree Physiology* **18**, 421-430.

Long, S.P., and Hallgren, J.E. (1985). Measurement of CO2 assimilation by plants in the field and the laboratory. In 'Techniques in Bioproductivity and Photosynthesis'. (Eds J. Coombs, D.O. Hall, S.P. Long and J.M.O. Scurlock.) pp. 62-94. (Pergamon Press: Oxford.)

Loveys, B.R., Robinson, S.P., and Downton, W.J.S. (1987). Seasonal and diurnal changes in abscisic acid and water relations of apricot leaves (*Prunus armeniaca* L.). *New Phytologist* **107**, 15-27.

Monteith, J.L. (1995). A reinterpretation of stomatal responses to humidity. *Plant, Cell and Environment* **18**, 357-364.

Mooney, H.A., and Chu, C. (1983). Stomatal responses to humidity of coastal and interior populations of a Californian shrub. *Oecologia* **57**, 148-150

Myers, B.A., Duff, G.A., Eamus, D., Fordyce, I.R., O'Grady, A., and Williams, R.J. (1997). Seasonal variation in water relations of trees of differing leaf phenology in a wet-dry tropical savanna near Darwin, northern Australia. *Australian Journal of Botany* **45**, 225-240.

Norby, R.J., and O'Niell, E.G. (1991). Leaf area compensation and nutrient interactions in CO_2 enriched seedlings of yellow poplar (*Liriodendron* L.). *New Phytologist* **117**, 515-528.

Prior, L.D., Eamus, D., and Duff, G.A. (1997). Seasonal and diurnal patterns of carbon assimilation, stomatal conductance and leaf water potential in *Eucalyptus tetrodonta* sapling in a wet-dry savanna in northern Australia. *Australian Journal of Botany* **45**, 241-258.

Roy, J., and Mooney, H.A. (1982). Physiological adaptation and plasticity to water stress of coastal and desert populations of *Heliotropium curassavicum*. Oecologia 52, 370-375.

Sharkey, T.D. (1984). Transpiration-induced changes in the photosynthetic capacity of leaves. *Planta* **160**, 143-150.

Tardieu, F., Zhang, J., and Davies, W.J. (1992). What information is conveyed by an ABA signal from maize roots in drying field soil? *Plant, Cell and Environment* **15**, 185-191.

Williams, R.J., Myers, B.A., Muller, W.J., Duff, G.A., and Eamus, D. (1997). Leaf phenology of woody species in a north Australian tropical savanna. *Ecology* **78**, 2542-2558.

Zhang, J., Schurr, U., and Davies, W.J. (1987). Control of stomatal behaviour by abscisic acid which apparently originates in the roots. Journal of Experimental Botany 38, 1174-1181.

Zhang, J., and Davies, W.J. (1991). Abscisic acid produced in dehydrating roots may enable the plant to measure the water status of the soil. *Plant, Cell and Environment* **12**, 73-81.

Figures

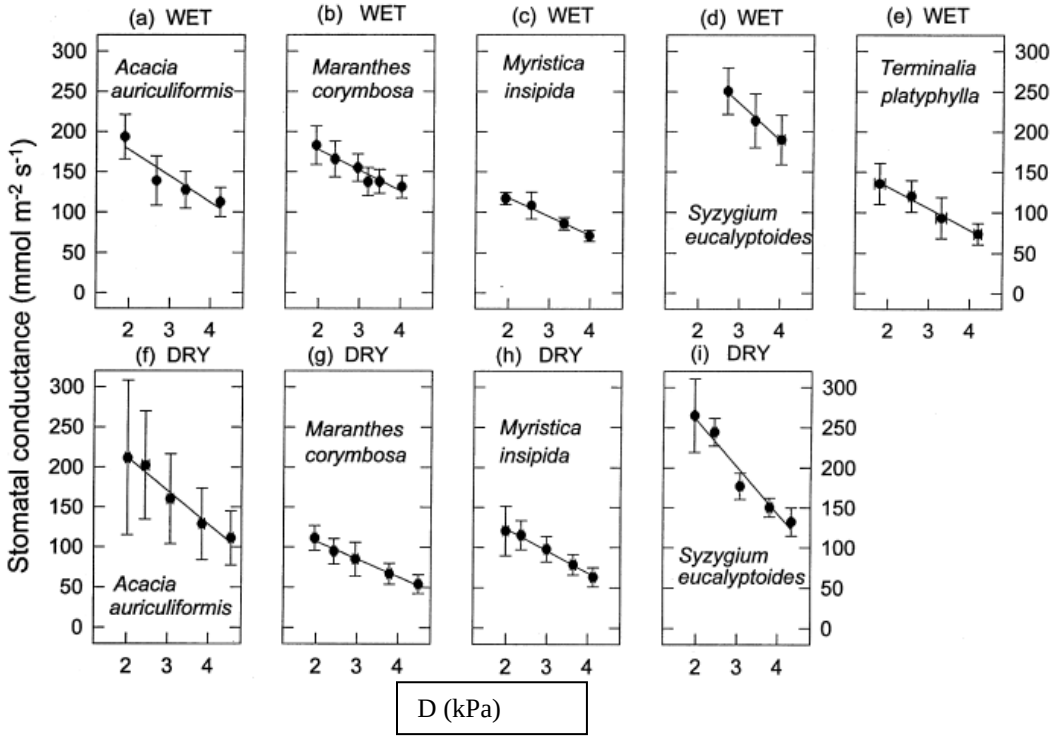

Fig. 1. Stomatal conductance (mmol m^{-2} s^{-1}) as a function of leaf-to-air vapour pressure difference (kPa) when leaf temperature was maintained at 33 ^{o}C for leaves expanded in the WET (a-e) and DRY (f-i) seasons. *Acacia auriculiformis* (a and f); *Maranthes corymbosa* (b and g); *Myristica insipida* (c and h); *Syzygium eucalyptoides* (d and i); and *Terminalia platyphylla* (e). Means of 3 or 4 replicates and s.e. are shown.

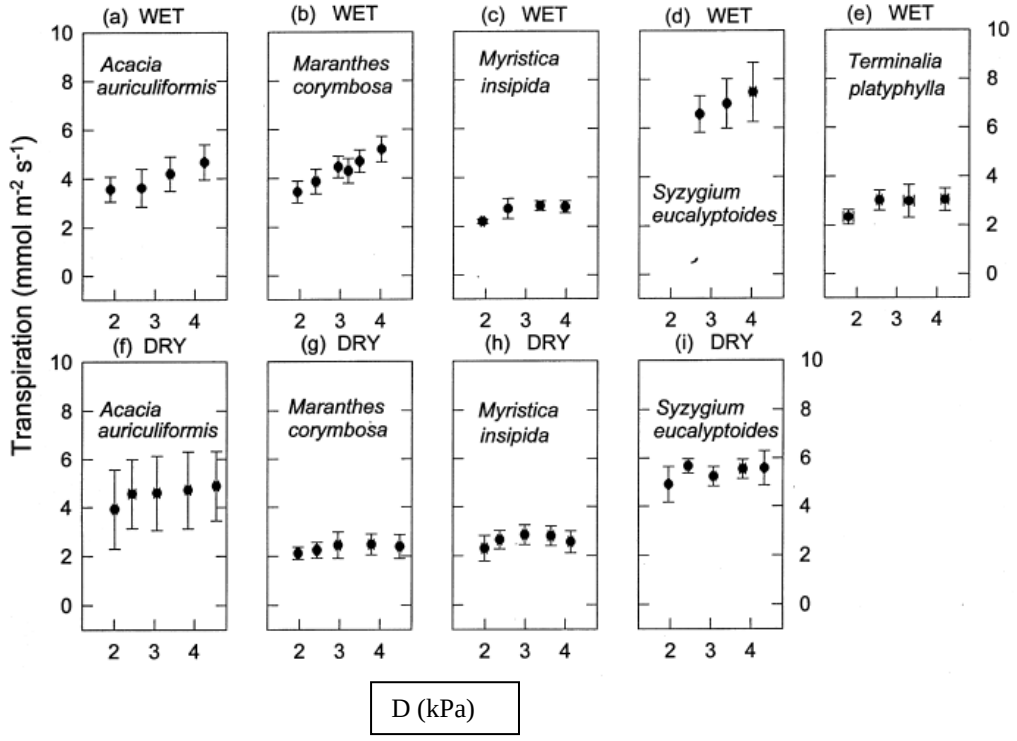

Fig. 2. Transpiration rate (mmol m^{-2} s-1) as a function of leaf-to-air vapour pressure difference (kPa) when leaf temperature was maintained at 33 ^{o}C for leaves expanded in the WET (a-e) and DRY (f-i) seasons. *Acacia auriculiformis* (a and f); *Maranthes corymbosa* (b and g); *Myristica insipida* (c and h); *Syzygium eucalyptoides* (d and i); and *Terminalia platyphylla* (e). Means of 3 or 4 replicates and s.e. are shown.

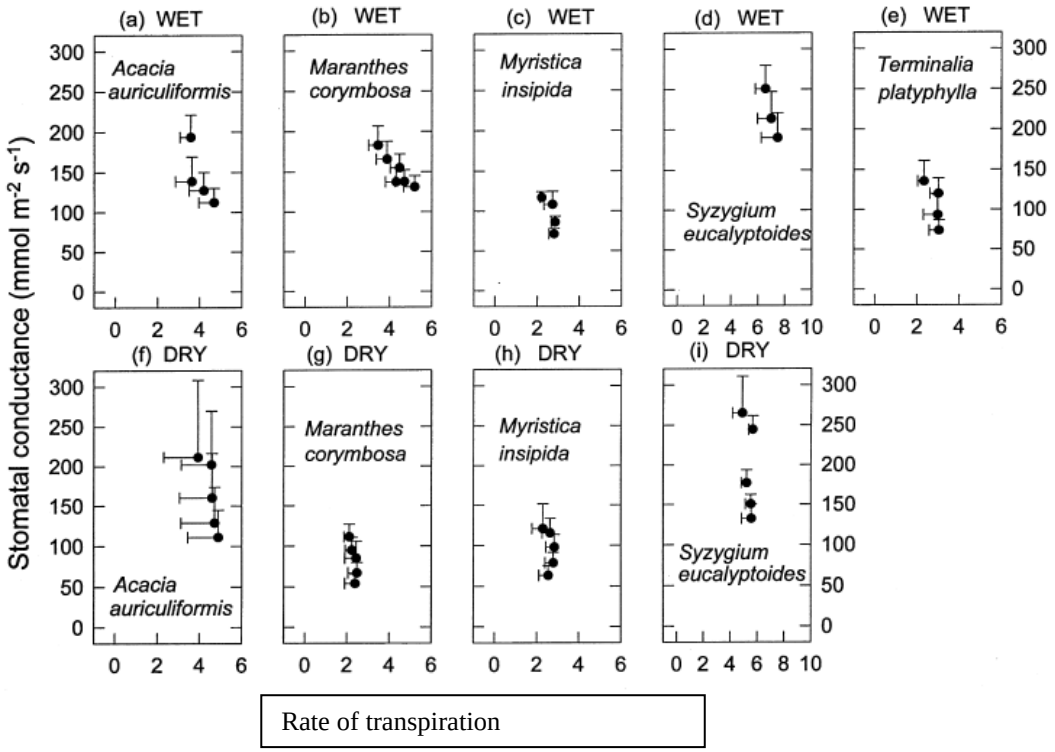

Fig. 3. The relationships between the rate of transpiration (mmol m^{-2} s^{-1}) and stomatal conductance (mmol m^{-2} s^{-1}) when leaf-to-air vapour pressure difference (D) (kPa) was varied at a leaf temperature of 33 ^{o}C on leaves which had expanded in the WET (a-e) and DRY (f-i) seasons. *Acacia auriculiformis* (a and f); *Maranthes* corymbosa (b and g); *Myristica insipida* (c and h); *Syzygium eucalyptoides* (d and i); and *Terminalia platyphylla* (e). Means of 3 or 4 replicates and s.e. are shown.

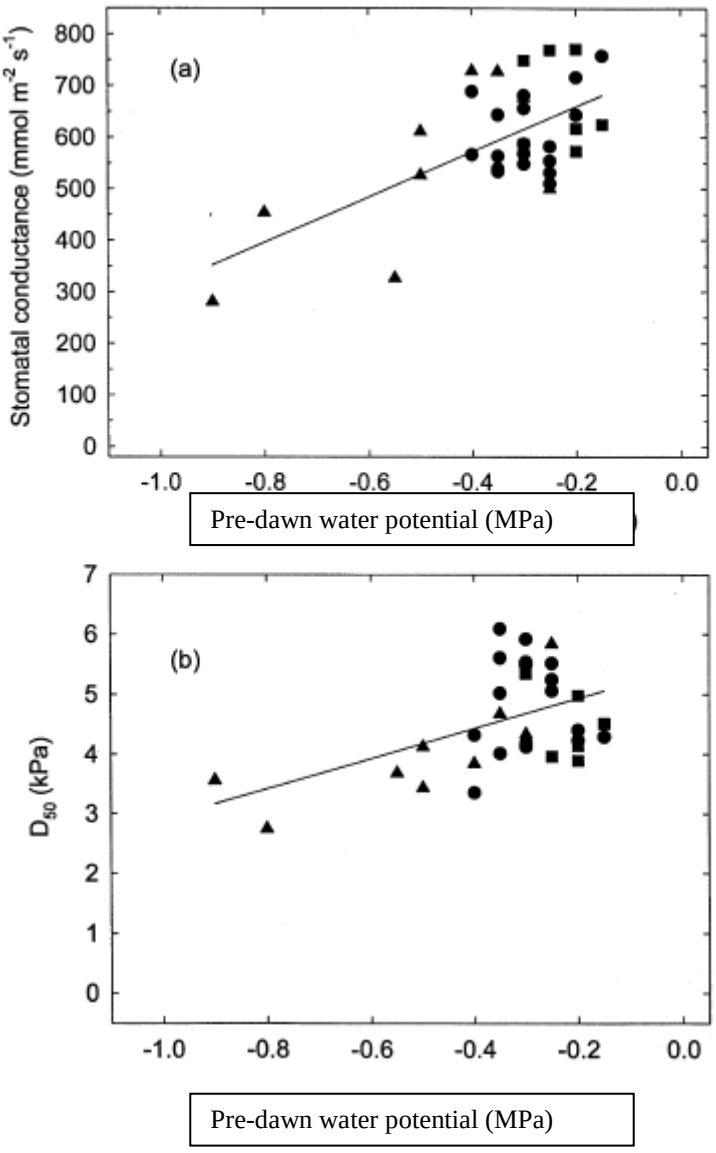

Fig. 4. a) Extrapolated maximum stomatal conductance (mmol m^{-2} s^{-1}) when leaf-to-air vapour pressure difference (D) equals 0 kPa and b) D where 50% of maximum stomatal conductance would occur (D_{50}) (kPa) as a function of pre-dawn phyllode water potential (MPa). Regression lines are for all data.

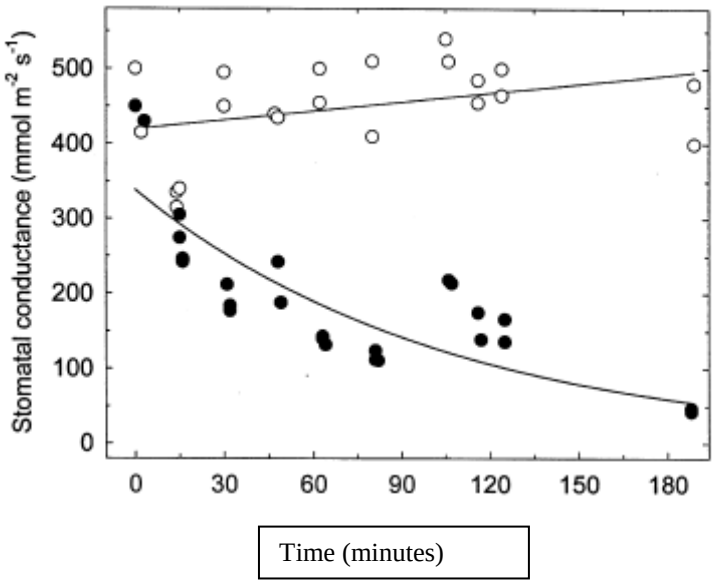

Fig. 5. Stomatal conductance over time when the phyllode was supplied via the xylem with a solution containing 10^{-5} mol m^{-3} ABA dissolved in 0.8 ml L^{-1} ethanol, 1 mol m^{-3} $CaCl_2$, pH 6.0 adjusted with NaOH (•) or control solutions not containing ABA (o).

Table 1. Characteristics of stomatal conductance (g) responses to leaf-to-air vapour pressure difference (D) and leaf morphology of wet and dry season expanded leaves.

Means of 3 or more replicates. D_{50} represents the value of D where 50% of extrapolated maximum g would occur. Similar letters within a species designate means not significantly different ($P>0.05$) between seasons with Tukeys HSD test.

Characteristic	Season	*Acacia auriculiformis*	*Maranthes corymbosa*	*Myristica insipida*	*Syzygium eucalyptoides*	*Terminalia platyphylla*
Linear decline in g with increasing D (mmol m^{-2} s^{-1} kPa^{-1})	Wet	-39 a	-34 a	-22 a	-43 a	-25
	Dry	-62 b	-22 a	-28 a	-53 b	
D_{50} (kPa)	Wet	3.8 a	4.1 a	3.8 a	4.2 a	4.4
	Dry	3.2 a	3.4 b	3.4 a	3.4 b	
Stomatal density (mm^{-1})	Wet	343 a	107 a	110 a	291 a	129
	Dry	330 a	113 a	114 a	290 a	
% cellular content to total cross	Wet	88.1 a	45.6 a	51.3 a	80.3 a	54.9
	Dry	75.4 a	67.1 b	55.6 a	64.5 a	

sectional area (%)						
Cell area to cell perimeter (mm^{-1})	Wet	0.953 a	0.699 a	0.755 a	2.110 a	0.599
	Dry	0.774 a	0.977 a	0.848 a	1.851 a	
Average cell perimeter exposed to air space (%)	Wet	25.8 a	44.4 a	46.7 a	19.2 a	50.3
	Dry	28.4 a	35.6 b	40.4 b	43.9 a	