

Seasonal ABA concentration and stomatal conductance of evergreen and deciduous trees

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Seasonal patterns of xylem sap pH, xylem abscisic acid concentration, leaf water potential and stomatal conductance of six evergreen and deciduous Australian savanna tree species

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Abstract. Deciduous trees of Australia's northern savannas typically have less negative leaf water potentials than evergreen species, and their stomata are more sensitive to soil drought than evergreen species. This paper presents the first investigation of the role of xylem sap pH and abscisic acid content in explaining stomatal behaviour of Australian trees in the field. We measured stomatal conductance, leaf-to-air vapour pressure difference (D) and leaf water potential, xylem abscisic acid (ABA) concentration and xylem sap pH of evergreen, semi-deciduous and fully deciduous tree species in the field over a 15 month period. Measurements were made during both the wet and the dry seasons. Stomata closed in response to increasing D in both evergreen and deciduous species and were equally sensitive to increasing D or declining leaf water potential. Xylem ABA concentration increased with declining leaf water potential in evergreen and semi-deciduous species, but not deciduous species. Similarly, there was an inverse correlation between stomatal conductance and xylem ABA concentration. Xylem sap pH increased as leaf water potential declined from wet to dry season for evergreen and semi-deciduous species but not for deciduous species. Deciduous species had less negative water potentials and lower xylem ABA concentrations than evergreen species or semi-deciduous species. We conclude that changes in xylem sap pH and ABA content do occur seasonally in the wet-dry tropics of Australia and that these changes influence stomatal conductance, but only in evergreen and semi-deciduous species. Deciduous species do not appear to modulate either of these chemical signals.

Introduction

The seasonally dry tropics of northern Australia (monsoonal Australia) are characterised by a wet season (November-April) when over 95% of annual average 1651 mm rainfall falls (Anon 1999) and a dry season (May-September) when no effective rain (in the sense of significantly enhancing soil water stores) falls. During the wet season soil water is supra-abundant and vapour pressure deficits and leaf-to-air vapour pressure differences (D) are low (Duff *et al.* 1997; Prior *et al.* 1997a, 1997b). In the dry season soil water becomes progressively less available and D becomes much larger (Duff *et al.* 1997; Eamus and Cole 1997;

Prior *et al.* 1997a, 1997b; Thomas *et al.* 2000). Deciduous tree species avoid the impact of the dry season by dropping their leaves (Williams *et al.* 1997), while evergreen species utilise water stored at depths of up to 6-8 m (Williams *et al.* 1997; Cook *et al.* 1999; O'Grady *et al.* 1999; Eamus *et al.* 2000).

Pre-dawn leaf water potential of both evergreen and deciduous trees decline during the dry season and stomatal conductance (of evergreen, brevi- and semi-deciduous species) declines in response to reduced soil water availability and increased D (Myers *et al.* 1997; Prior *et al.* 1997a, 1997b; Fordyce *et al.* 1997; Eamus and Cole 1997; Thomas *et al.* 1999a, 1999b). Stomata of deciduous species are more sensitive to declining leaf water potential (Ψ_L) than stomata of evergreen species (Myers *et al.* 1997).

Exogenous abscisic acid (ABA) causes declining stomatal conductance when supplied through the transpiration stream (Eamus 1986, 1987) and increased concentrations of ABA in the xylem are correlated with reduced conductance in droughted plants growing in pots (Zhang *et al.* 1987; Loewenstein and Pallardy 1998). In contrast, the few field studies available are contradictory (Loveys *et al.* 1987; Liang *et al.* 1999), as are some pot studies (Coleman and Schneider 1996). More recently, interactions between hydraulic and chemical signals have been suggested as being responsible for declines in stomatal conductance (Tardieu and Davies, 1993; Tardieu *et al.* 1993; Thomas and Eamus 1999). In addition, drought can increase the pH of xylem sap by up to 3 units in tomato (Wilkinson *et al.* 1998) and 0.6 units in *Commelina communis* (Wilkinson and Davies 1997). Increased xylem sap pH can also cause increased apoplastic ABA by inhibiting ABA uptake into the symplast (Wilkinson and Davies, 1997) and by decreasing the metabolism of ABA (Zhang *et al.* 1997). Decreasing pH of ABA solutions supplied exogenously can also increase the effectiveness of ABA in causing a decrease in stomatal conductance and transpiration (Wilkinson and Davies 1997).

Savannas of northern Australia cover approximately 25 % of the Australian continent. They have conservation, economic, ecological and social value and contribute to determining regional climate, water cycling and national carbon budgets. Understanding the ecophysiology of savanna trees will aid their long-term sustainable management. While much is known about leaf-scale attributes (Eamus and Prior 2001), relatively little is known about mechanisms underlying leaf-scale behaviour.

The purpose of the work described in this paper is to answer the following questions. First, what are the seasonal patterns of xylem sap ABA concentration of evergreen and deciduous savanna tree species? Second, are any observed seasonal changes in sap ABA concentration and pH correlated with seasonal patterns of stomatal conductance? Finally, are there any differences in the interactions among ABA concentration, leaf water potential and stomatal conductance among deciduous and evergreen savanna tree species?

Materials and Methods

Study Site

The open savanna woodland study site was situated on the Palmerston campus Northern Territory University (15 km E from Darwin GPO). The site is dominated by *Eucalyptus tetradonta* F.Muell. and *Eucalyptus miniata* (Cunn. Ex Schauer). We studied two evergreen species, *Eucalyptus tetradonta* and *Acacia mimula* Pedley; two semi-deciduous species, *Alphitonia excelsa* (Fenzl) Reisseck ex Bench. and *Planchonia careya* (F.Muell.) Knuth; and two fully deciduous species, *Cochlospermum fraseri* Planchon, and *Terminalia ferdinandiana* Excell. These species are common to open woodland forest of northern Northern Territory (Brock, 1993). All species were abundant on the site. Trees used in the study were between 2 and 5 m in height.

Preliminary study - Xylem ABA concentration, stomatal conductance and leaf water potential

We undertook measurements in mid-March (wet season), late April (late wet season), early July (early dry season), late August (late dry season) and early December (early wet season) of 1997. Fully deciduous and semi-deciduous species were measured only in the wet season (measured three times), except *A. excelsa*, which was measured in July (early dry). The evergreen species were measured at all measurement times.

Xylem sap was collected using the method previously described (Thomas *et al.* 2000) from seven replicate trees in the morning (0900 – 1100 hours) and in the afternoon (1400 – 1600 hours). In summary, small branches containing several leaves, or individual *T. ferdinandiana* leaves, were over-pressurised in a Scholander type pressure chamber. The over-pressure was 1 MPa. Preliminary studies (repeated samples of two branches sampled from each of three plants of two species - *E. tetradonta* and *P. caryra*) indicated no significant ($P > 0.05$) difference in xylem ABA concentration when the branches were over-pressurised by 0.4 or 1.0 MPa. The initial xylem water potential (Ψ_L) was noted in all cases and used when interpreting stomatal conductance data. Xylem sap was stored in darkness before immersing the sample in liquid nitrogen in the field prior to freeze drying for quantitative analysis.

Abscisic acid concentration was quantified by addition of an internal standard of deuterium labeled 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 μ l 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.

Stomatal conductance was measured between 1130 and 1230 hours using a Li-Cor 6400 portable photosynthesis system on two or more leaves on different primary (sub-tending to the stem) branches of the same seven replicate trees from which xylem sap was collected. These measurements were averaged for each tree. All leaves were measured in full sunlight but were maintained in their natural orientation to sunlight during measurements. Leaf-to-air vapour pressure difference was calculated by measuring ambient vapour pressure with the Li-Cor 6400 with the chamber fully open and empty and recording leaf temperature immediately the leaf was placed in the Li-Cor leaf chamber.

Second study: Determination of xylem ABA concentration, xylem pH and leaf water potential

We sampled xylem sap and measured sap pH eight times from five or six replicate trees from each of the six species over a wet-dry-wet season in 1999/2000 using the method described above for sampling of xylem ABA. Sample dates were early April (late wet); early June (early dry); early July (mid dry); late

August (late dry); early October (dry-wet transition); early November (early wet); mid February 2000 (mid-wet); and early April 2000 (late wet). Different individual trees were sampled at each sample date but the same trees were sampled in the morning (0900 – 1100 hours) and in the afternoon (1400 – 1600 hours).

Xylem pH was measured using a micro-combination pH electrode (Lazer PHR-146, Lazer research laboratories, CA) before the samples were stored in liquid nitrogen for later analysis of xylem ABA. Leaf xylem water potential (Ψ_L) was recorded prior to over-pressurisation to obtain xylem sap.

The fully deciduous species *C. fraseri* and *T. ferdiandiana* were not sampled in the dry season (July, August or October); and the semi-deciduous species *P. caryra* was not sampled in late August as these species were leafless at these times. *P. caryra* had leaf flushes in late September prior to any rainfall, while *C. fraseri* flushed after small amounts of rain in mid-October and *T. ferdiandiana* flushed about one week later. The semi-deciduous species *A. excelsa* had a major leaf flush in late October.

Statistical analyses

Data were categorised into three seasons (wet, early plus mid-dry combined and dry-wet transition) for analyses. Differences in gas exchange, Ψ_L , xylem pH and ABA concentration between phenological guilds, and diurnal and seasonal changes were analysed using analysis of variance. Diurnal change in xylem abscisic acid concentration, xylem pH and Ψ_L were analysed using paired t-tests. The effect of Ψ_L on pH between phenological guilds, different seasons and times of day were examined by analysis of covariance or linear regression.

Results

Stomatal conductance and hydraulic signals

Pre-dawn leaf water potential of all species decreased (became more negative) as the dry season progressed, decreasing from -0.1 MPa in March to almost -0.9 MPa in July, indicative of a declining soil water content (Table 1). An atypical and small amount of rain occurred at the site in late August, causing a

small increase in pre-dawn water potential earlier than is usual at this time of year (Table 1). No significant ($P > 0.05$) differences in pre-dawn leaf water potential between species or phenological guilds were observed and data are presented as means of all species (Table 1). Leaf water potential (Ψ_L) measured in the morning and afternoon showed similar seasonal changes as pre-dawn water potential (Table 1). Afternoon values of Ψ_L were significantly lower than those observed in the morning for all three phenological guilds. Leaf water potential was significantly lower (more negative) in evergreen and semi-deciduous species than fully deciduous species in both the morning and afternoon, for all periods (Table 1).

Stomatal conductance declined significantly ($P \leq 0.05$) as Ψ_L became more negative (Fig. 1). There was no difference among phenologies in sensitivity of stomata to declining Ψ_L (Fig. 1).

Leaf-to-air vapour pressure difference (D), measured in the morning when stomatal conductance was measured, increased from the wet season into the dry season and declined once again by early December (wet season) (Table 1). Stomatal conductance declined as D increased (Fig. 2). It should be noted this variation in D reflects annual patterns of change in D and not diurnal patterns of variation. The range of D experienced by fully or partially deciduous species was smaller than that experienced by evergreen species because deciduous species have lost their leaves by the mid-dry season. Stomata of evergreen and deciduous species were equally sensitive to D (Fig. 2).

Xylem sap abscisic acid concentration and stomatal conductance

Xylem abscisic acid (ABA) concentration of evergreen species increased significantly ($P < 0.05$) from $0.074 \text{ mmol m}^{-3}$ in mid-March to $0.270 \text{ mmol m}^{-3}$ in early December (Table 2). Xylem ABA concentration was always higher in the afternoon than in the morning in evergreen species. The pattern of change was more complicated in semi-deciduous and deciduous species. In the morning, for semi-deciduous and deciduous species, there was minimal change in xylem sap ABA concentration between March and July. However, there was a large (approximately 6-8 fold) increase between March/April/July values and early December (Table 2). In the afternoon, there was little change in ABA concentration for semi-deciduous

and deciduous species during March and April, but a large increase in July, for semi-deciduous and December for semi-deciduous and deciduous species.

For evergreen and semi-deciduous species, as leaf water potential declined between wet and dry season, xylem sap ABA concentration increased (Fig. 3). No relation could be found for deciduous species, primarily because the range of leaf water potentials measured was low as they were confined to the wet season (deciduous species lose their leaves rapidly once the wet season ends). As xylem ABA concentration increased, stomatal conductance declined for deciduous and semi-deciduous species (Fig. 4).

Xylem sap pH

For evergreen and semi-deciduous species, xylem sap pH increased significantly as leaf water potential decreased between wet and dry seasons (Fig. 5a, b). Over the range of leaf water potentials experienced (between -0.5 and -3.5 MPa), pH increased by approximately 0.7 pH units. In contrast, xylem sap pH of deciduous species declined slightly with decreasing leaf water potential (Fig. 5c).

Discussion

Leaf water status, D and stomatal behaviour

Leaf water potential was significantly lower (more negative) in evergreen and semi-deciduous species than fully deciduous species in both the morning and afternoon, for all periods (Table 1). Evergreen species tend to be taller (hence larger path for water flow from root to leaf) and have larger canopies, than deciduous species and may have a smaller vulnerability to xylem cavitation than deciduous trees (Sobrado 1997). Furthermore they tend to have smaller stem hydraulic conductivity (Sobrado 1993; Eamus and Prior 2001), indicative of a smaller xylem pit pore membrane and/or xylem diameter. These observations suggest that a lower leaf water potential will be observed for evergreen than deciduous species, at a given soil water potential, as observed in the present study.

No significant ($P > 0.05$) differences in pre-dawn leaf water potential between species or phenological guilds were observed and data are presented as means of all species in Table 1. Pre-dawn water potential is a surrogate measure of soil water availability and the lack of difference between phenological guilds suggests that roots of all guilds were accessing the same soil profile. Although it has generally been concluded that deciduous trees have shallower roots than evergreen trees (Sobrado 1986), this is rarely tested in the field. Recent data for north Australian savannas shows no difference in an index of shallow rootedness for deciduous and evergreen species (Chen *et al.* 2002).

Stomatal conductance declined significantly ($P \leq 0.05$) as Ψ_L became more negative, with no difference observed between the three phenologies. This is in contrast to Myers *et al.* (1997) who found that stomata of deciduous species were more sensitive to pre-dawn water potential than evergreen species. There may be two reasons to explain this difference. First, Myers *et al.* (1997) examined mature (tall) trees 8-12 m in height. The present study used younger trees 2-5 m in height. Tree height influences the water relations and canopy behaviour of trees in these savannas (Prior and Eamus 1999) probably because mature tall trees access groundwater in the dry season. Therefore, stomatal responses to water status may differ with height. Second, pre-dawn water potential was used in the study of Myers *et al.* (1997) but morning and afternoon values were used in the present study.

Stomata of evergreen, semi-deciduous and deciduous species were equally sensitive to D. Thomas *et al.* (2000) similarly concluded that stomata of two evergreen rainforest and two riparian/open woodland species and one open woodland deciduous species were equally sensitive to leaf-to-air vapour pressure difference. These results are in contrast to previous results that showed evergreen species to have stomata that are more sensitive to D than deciduous species (Myers *et al.* 1997). Again, the principal difference between the two studies was the size of the trees. Myers *et al.* (1997) studied mature, 8-12 m trees while the present study was restricted to trees less than 5 m tall. It is likely that smaller trees, with small root systems, will exhibit different stomatal responses to D as they experience different water relations (Prior *et al.* 1997) and exhibit larger degrees of leaf loss in the dry season than mature trees (Prior and Eamus 2000).

Abscisic acid and pH

Abscisic acid concentration in the xylem sap ranged from less than 0.02 to 0.6 mmol m⁻³ (all species and all times). This range is typical of woody species (Loveys *et al.* 1987; Waringer *et al.* 1990; Correia *et al.* 1995; Correia *et al.* 1997; Fort *et al.* 1997). Xylem sap abscisic acid (ABA) concentration of evergreen species increased significantly ($P < 0.05$) from 0.074 mmol m⁻³ in mid-March to 0.270 mmol m⁻³ early December. This is presumed to result from declining soil water content (as shown by the decline in pre-dawn water potential) and concomitant increased root synthesis of ABA (Zhang and Davies 1991). (Table 2). Xylem ABA concentration was always higher in the afternoon than in the morning in evergreen species, presumably reflecting the impact, on root ABA synthesis, of diurnal patterns of soil drying around roots throughout the day. This increase in seasonal xylem sap ABA content was correlated with declines in stomatal conductance in the dry season in leaves of evergreen trees, a result consistent with many studies of annual plants (Zhang *et al.* 1987; Zhang and Davies 1991) and trees measured in summer or winter (Bertrand *et al.* 1999).

Xylem sap ABA concentration did not decline in December even after significant rainfall and a subsequent increase in Ψ_L in all trees (Tables 1, 2). We suggest that this represents a field test of the classic split root experiments (Zhang *et al.* 1987; Zhang and Davies 1991), whereby a portion of roots in dry soil can maintain increased xylem sap ABA levels despite another portion of roots being in wet soil and supplying water to leaves and thereby maintaining a high leaf water potential. At the very start of the wet season the first month of rainfall is sufficient to wet the upper soil profile but not the deeper soil profile. Therefore a portion of roots are in wet and a portion in dry soil.

Xylem sap pH of evergreen species increased from the wet to dry season, but declined during the late dry season prior to any rainfall and while Ψ_L remained very low. Regression analysis showed xylem sap pH increased significantly as Ψ_L declined in evergreen and semi-deciduous species, but not in fully deciduous species. As the dry season progressed soil water availability declined and pre-dawn leaf water potential (Prior *et al.* 1997; Myers *et al.* 1997) or leaf water potential measured during daylight, declined substantially, as was observed in the present study. Concomitant with this decline in soil water availability

or leaf water potential, xylem sap pH increased by approximately 0.7 pH unit. In several species of annual crops, drought resulted in an increase of between 0.2 and 2.0 pH units (Wilkinson and Davies 1997; Bacon *et al.* 1998; Wilkinson *et al.* 1998). Such changes in sap pH are increasingly viewed as a mechanism for signaling between roots and shoots (Wilkinson 1999). In grape vines, large increases in xylem pH (2.5 pH units) are highly correlated with declines in xylem sap flux (Campbell and Strother 1996). This increase in pH was suggested to result from a reduced organic acid content of the sap.

The pH of xylem sap modulates the impact of xylem sap ABA on stomatal conductance (Thompson *et al.* 1997; Hartung *et al.* 1998). Increased pH of an ABA solution increases the efficacy of ABA in causing stomatal closure (Wilkinson 1999). The increase in xylem pH would have enhanced the decline in stomatal conductance by modifying the sensitivity of stomata to ABA (Zhang *et al.* 1997) or as a consequence of altered compartmentalisation of ABA within the leaf (Hartung *et al.* 1998). Xylem pH affects the disassociation of ABA from ABAH. A rise in pH of just 0.5 units would alter the ratio of ABA to ABAH by a factor of 3, thus substantially more ABA may be physiologically active and available at the guard cell (Hartung *et al.* 1998; Slovik *et al.* 1992).

An increase in xylem sap pH of fully deciduous species with declining leaf water potential (Fig. 5c) was not observed. Similarly there were only minimal changes in xylem sap ABA with declining leaf water potential. Therefore we conclude that in deciduous species, which have leaves only during periods of plentiful water, neither ABA nor pH are used to modulate stomatal behaviour.

Stomatal behaviour, ABA, pH and leaf water potential

Tardieu and Davies (1992) and Tardieu *et al.* (1993) showed that stomata were more sensitive to abscisic acid concentration when Ψ_L was more negative. Similarly, Correia *et al.* (1995) and Correia *et al.* (1997) observed stomata were more sensitive to xylem abscisic acid concentration during afternoons than mornings (when Ψ_L would be more negative). Stomata conductance is always lower in the afternoon than the morning in the dry season in evergreen and semi-deciduous species (Myers *et al.* 1997; Prior *et al.* 1997). In the present study, leaf water potentials were always significantly ($P < 0.04$) lower in the

afternoon than the morning and for all species stomatal conductance is always lower in the afternoon than the morning in the dry season in evergreen and semi-deciduous species (Myers *et al.* 1997; Prior *et al.* 1997). It is possible, therefore, that increased stomatal sensitivity to ABA in the afternoon contributes to the reduced conductance observed in the afternoon in these.

In conclusion, we have shown that changes in xylem sap pH and ABA concentration occur in response to soil drying for evergreen and semi-deciduous trees measured in the field. Thus, xylem ABA concentration increased with dry season declines of leaf water potential in evergreen and semi-deciduous species, but not in deciduous species. Similarly, there was an inverse correlation between stomatal conductance and xylem ABA concentration. Xylem sap pH increased as leaf water potential declined from wet to dry season for evergreen and semi-deciduous species but not for deciduous species. Concomitant with changes in xylem sap pH and ABA content, changes in stomatal conductance occurred and we suggest that changes in both ABA concentration and pH contribute to observed changes in stomatal behaviour for evergreen and semi-deciduous species only. Different stomatal sensitivities to D and leaf water potential among phenologies were not observed and could not be explained on the basis of differences in chemical signalling.

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Seasonal ABA concentration and stomatal conductance of evergreen and deciduous trees

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

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Table 1. Leaf water potential (MPa) of three phenological guilds: Evergreen (E), Semi-deciduous (SD), and Fully deciduous (FD) at three times during the day; and average leaf-to-air vapour pressure difference (kPa) during leaf gas exchange measurements (1130 – 1230 hours)

Data are presented for five measurement times throughout the year. Leaf water potential measurements in the morning (0900 – 1100 hours) and afternoon (1400 – 1600 hours) were collected in conjunction with xylem sap subsequently measured for abscisic acid concentration. No significant ($P > 0.05$) difference existed among pre-dawn leaf water potential of different phenological guilds. NA designates data not collected

Date		Leaf water potential (MPa)						Leaf-to-air vapour pressure difference (kPa)	
		Pre-dawn	Morning			Afternoon			
			E	SD	FD	E	SD	FD	
Mid-March	Wet	-0.10	-0.61	-0.55	-0.44	-1.35	-1.34	-1.07	1.41
Late April	Wet	-0.33	-0.90	-0.74	-0.67	-1.84	-1.61	-1.57	2.05
Early July	Dry	-0.87	-1.57	-2.30	NA	-2.30	-2.25	NA	2.86
Late August	Dry	-0.66	-1.32	NA	NA	-2.43	NA	NA	3.64
Early December	Wet	-0.17	-0.74	-0.55	-0.46	-1.08	-1.08	-0.88	1.27
lsd ($P = 0.05$)		0.09	0.15	0.21	0.07	0.18	0.21	0.17	0.15

Table 2. Abscisic acid concentration in xylem sap (mmol m^{-3}) of evergreen (E), semi-deciduous (SD), and fully deciduous (FD) species measured five times throughout the year in the morning (0900 – 1100 hours) and afternoon (1400 – 1600 hours)

NA indicates data not collected

Date		Xylem abscisic acid concentration (mmol m^{-3})					
		Morning			Afternoon		
		E	SD	FD	E	SD	FD
Mid-March	Wet	0.074	0.062	0.041	0.120	0.097	0.055
Late April	Wet	0.055	0.048	0.050	0.235	0.097	0.079
Early July	Dry	0.135	0.034	NA	0.468	0.353	NA
Late August	Dry	0.100	NA	NA	0.126	NA	NA
Early December	Wet	0.270	0.269	0.313	0.283	0.213	0.168
lsd ($P = 0.05$)		0.036	0.068	0.068	0.035	0.079	0.012

Figure legends

Fig. 1. Stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$) declined as leaf water potential (MPa) became more negative. Data are presented for all three phenological guilds with a single regression as it was not possible to show any difference in the response between guilds.

Fig. 2. Stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$) declined as leaf-to-air vapour pressure difference (kPa) increased. Data are presented for all three phenological guilds with a single regression as it was not possible to show any difference in the response between guilds.

Fig. 3. Xylem sap ABA concentration increased as leaf water potential declined in evergreen and semi-deciduous species.

Fig. 4. As xylem sap ABA content increases, stomatal conductance declined for evergreen and semi-deciduous species.

Fig. 5. As leaf water potential declined, xylem sap pH increased for (a) evergreen and (b) semi-deciduous species, but decreased for deciduous species (c).





