The influence of predawn leaf water potential on stomatal responses to atmospheric water content at constant $C_i$ and on stem hydraulic conductance and foliar ABA concentrations

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Abstract

Stomatal and photosynthetic responses to increasing leaf-to-air vapour pressure difference ($V$) were investigated in watered and droughted Eucalyptus tetrodonta when either ambient $CO_2$ ($C_a$) or internal $CO_2$ concentration ($C_i$) were constant. Stem hydraulic conductance and xylem and foliar abscisic acid levels were measured periodically during the drought period.

As $V$ increased, stomatal conductance ($g_s$) declined. Maintaining $C_i$ did not affect the response of $g_s$ to $V$ or predawn leaf water potential ($\psi_{pd}$). In fully watered plants the decline in $g_s$ was insufficient to prevent increased transpiration rates ($E_t$) with increasing $V$. In contrast, in droughted plants, stomatal closure was sufficient to prevent increasing $E_t$. Stomatal sensitivity to increasing $V$ was increased by drought. As drought developed, the three phases of stomatal responses to $V$ progressively collapsed to one where feedforward mechanisms result in decreasing $E_t$ with increasing $V$. Thus as drought developed the feedforward response of stomata to $V$ entirely dominated.

Net photosynthesis ($P_n$) and $g_s$ responded in unison to changes in $V$ and $\psi_{pd}$. The decline in $P_n$ as $V$ increased, despite $C_i$ being maintained constant, was observed in fully watered plants and in severely droughted plants. $P_n$ was most dependent on $g_s$ at large values of $V$ and in droughted plants.

As drought progressed and $\psi_{pd}$ declined, stem hydraulic conductance decreased and foliar ABA concentrations increased. The decline in maximum $g_s$ was correlated with foliar ABA levels. It is concluded here that hydraulic signals, be they atmospheric water or soil water in origin, and possibly also chemical signals regulate $g_s$, which in turn can limit assimilation rates in seasonally dry savannas.

Key words: Eucalyptus tetrodonta, stomatal sensitivity, transpiration, hydraulic conductance, ABA.

Introduction

The method by which stomata sense a change in the environment, particularly concerning the interactions between humidity, transpiration ($E_t$) and leaf water potential ($\psi_l$), is debated (Schulze, 1986, 1993; Grantz, 1990). High leaf-to-air vapour pressure differences ($V$) result in stomatal closure (Maroco et al., 1997; Meinzer et al., 1997). A decline in $E_t$ as $V$ increases is cited as evidence for a feedforward response of stomata to $V$ (Schulze, 1986; Grantz, 1990) as feedback control cannot account for a decrease in $E_t$ with further increases in $V$ (Farquhar, 1978). Mott and Parkhurst (1991) using ‘helox’ gas mixture showed $E_t$ controls $g_s$. A re-analysis of published data by Monteith (1995) also indicated $E_t$ might control $g_s$ in normal air, rather than $V$ per se, controlling $g_s$.

Reduced internal carbon dioxide concentration ($C_i$) enhances stomatal opening (see reviews by Morison, 1987; Mott, 1990). Increasing $V$ under constant ambient carbon dioxide concentration ($C_a$) reduces $C_i$ (Aphalo and Jarvis, 1993; Eamus et al., 1995) as the response of stomata to $V$ is larger than that of photosynthesis. Therefore, stomata receive two opposing signals as $V$ increases—increased $V$, which decreases stomatal conductance ($g_s$); and reduced $C_i$, which tends to increase $g_s$. Maintaining a constant $C_i$ with increasing $V$ allows the direct closing

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influence of $V$ on stomatal aperture to be measured without a conflicting $C_i$ control signal.

Loss of xylem hydraulic conductivity as soil dries is evident in many species (Tyree and Ewers, 1991, 1996). Reduced $g_s$ has been correlated with loss of hydraulic conductivity in Quercus petaea and Quercus robur (Breda et al., 1993) and in Acer grandidentatum (Alder et al., 1996). Greater sensitivity of $g_s$ than xylem cavitation to declining $\psi_i$ was proposed as a protective mechanism against further loss in hydraulic conductivity in Acer grandidentatum (Alder et al., 1996). Such protective mechanisms have not been investigated for Australian savanna species.

Stomatal sensitivity of Commelina communis epidermal strips to abscisic acid (ABA) increased when the water potential of the incubation media was reduced (Tardieu and Davies, 1992), indicating interaction between ABA and $\psi_i$ can be important to stomatal control. Split-root experiments using whole plants indicate ABA rather than $\psi_i$ per se, controls $g_s$ (Zhang et al., 1987). However, field studies often fail to establish a causal correlation between xylem ABA concentration and $g_s$ (Loveys et al., 1987; Wartinger et al., 1990).

Eucalyptus tetrodonta F. Muell. is a dominant tree of savannas of Northern Australia (Wilson et al., 1990). It is an evergreen species which retains more than 75% of leaf biomass throughout the dry season (May to October) (Williams et al., 1997b), a period when less than 10% of the annual average 1651 mm of rainfall falls (Taylor and Tulloch, 1985). Therefore E. tetrodonta is a drought-tolerant species. Daily maximum $g_s$ of E. tetrodonta declines as predawn leaf water potential ($\psi_{pd}$) declines during the dry season (Prior et al., 1997). Stomata of E. tetrodonta seedlings respond more to $V$ at intermediate $\psi_{pd}$ (−0.5 to −1.5 MPa) than lower or higher values (Prior et al., 1997). This contrasts with Maroco et al. (1997) who showed drought-avoiding herbaceous species were insensitive to $V$, but drought-tolerant species responded to $V$. Korner and Cochrane (1985) showed that $V$ controlled stomata of Eucalyptus pauciflora independently of soil moisture.

The hypotheses is tested that (1) the response of the decline in $g_s$ as $V$ increases is larger as $\psi_{pd}$ declines during soil drought; (2) maintaining constant $C_i$ will increase the decline in $g_s$ as $V$ increases relative to when $C_i$ is kept constant; (3) hydraulic conductance of plant stems will decline as $\psi_i$ becomes more negative in droughted plants and this will correlate with reduced $g_s$; and (4) there will be an increase in xylem and foliar ABA concentration in droughted plants and this will correlate with reduced stomatal conductance. Tests of such hypotheses for tropical tree species will contribute significantly to our understanding of the ecophysiology of this major biome. Savannas cover approximately one quarter of the land area of Australia and represent sites of economic, cultural and social importance for Australia.

Materials and methods

Eucalyptus tetrodonta seeds were sown in PVC tubes (0.11 m diameter and 1.2 m length) containing fine sand in April 1996. The plants received irrigation to field capacity twice daily and were fertilized twice weekly with 150 ml full strength Aquasol. Leaves displayed no signs of nutrient toxicity or deficiency. Treatments were imposed on 22 August 1996. Several plants (control) continued to receive weekly nutrient solution and irrigation to free drainage twice daily. Treated plants received only the nutrient solution. Stomatal responses to $V$ were measured on four plants on a weekly or more frequent basis until 4 October 1996 as soil moisture and $\psi_{pd}$ declined.

Stomatal responses to $V$

The response of leaf gas exchange to $V$ was measured using a laboratory-based system previously described (Eamus et al., 1995) except a cooled mirror dew point hygrometer (Hygro-M1, General Eastern Instruments, Watertown, MA, USA) measured dew point of incoming and outgoing air. Four plants were moved to the laboratory the night before gas exchange measurements and remained in darkness. For $\psi_{pd}$, $\psi_i$ of the next leaf down (i.e. older) from those used for gas exchange measurements was measured using a Scholander-type pressure chamber while plants were in darkness. Photosynthetic photon flux density, provided by 400 W metal halide lamps, was increased from darkness at 07.30 h to the maximum of 750 μmol m$^{-2}$ s$^{-1}$ at the leaf surface by 09.00 h by removing neutral density filters.

Gas exchange was measured over a 6 h period as $V$ was varied from less than 2.0 to greater than 4.5 kPa while maintaining leaf temperature at 33°C. Leaves were measured 30 min after $V$ (and $C_i$ if required) was altered, and checked for any further changes in gas exchange a further 10 min later. If no substantial change had occurred between the two measurement times both measurements were used in analysis. The influence of $CO_2$ concentration on the response of $g_s$ to increasing $V$ was measured over 2 d. On the first day, $C_i$ was maintained at 360 μmol mol$^{-1}$. On the second day, $C_i$ was changed as $V$ was altered such that $C_i$ was maintained constant. A value for the constant $C_i$ was chosen to correspond to that which exists at a $C_a$ of 360 μmol mol$^{-1}$ and the lowest achievable $V$ (typically 290 μmol mol$^{-1}$ although this value declined in more droughted plants). $C_a$ of up to 410 μmol mol$^{-1}$ was required to maintain a $C_i$ of 290 μmol mol$^{-1}$ at high values of $V$ (4.5 to 5 kPa).

A $C_a$ of 540 μmol mol$^{-1}$ was also used during examination of the response of stomata to changes in $V$ because it was found that there was minimal response of $g_s$ to $CO_2$ when $C_a$ was greater than 500 μmol mol$^{-1}$. Therefore, the response of $g_s$ to $V$ was observed without the impact of concomitant changes in $C_i$ because $C_i$ was saturating for $g_s$. Use of $C_a$ values that resulted in $C_i$ values lower than 200 μmol mol$^{-1}$ may have decreased the stomatal response to $V$ but caused error in interpretation due to photoinhibition and altered stomatal responses.

The stomatal density of the tested leaves was measured using glue imprints. The leaf chlorophyll concentration of the tested leaves was measured according to the method of Lichtenthaler and Wellburn (1983).
Leaf ABA content

Four 12 mm diameter leaf discs from the leaf used above were freeze-dried and specific leaf area calculated. This leaf material was used to determine the concentration of ABA within the leaf according to the method of Loveys and van Dijk (1988).

Stem hydraulic conductivity

$\psi_{pd}$ and water potential at noon ($\psi_y$) were measured on a subset of plants growing in shadehouses. $\psi_{pd}$ was measured to determine a common benchmark for the degree of plant drought and $\psi_y$ to determine a minimum (or close to minimum) value of $\psi_y$ that should indicate the severity of drought experienced by the plant. Stem hydraulic conductance and xylem ABA concentrations were measured periodically on these stems in the morning (09.00 h) and afternoon (14.00 h). Hydraulic conductance of the whole stem was measured using the method of Kolb et al. (1996). Briefly, shoots were placed in plastic bags before cutting under water in the field. These were subsequently removed to the laboratory where stems were re-cut under water before removing leaves under water. Stems were placed in a sealed PVC chamber (1.2 x 0.25 x 0.25 m) with their proximal ends protruding through a rubber seal. Tubes connected to pipettes were connected to these stem ends and a known constant vacuum applied to the chamber. The flow of filtered (0.2 µm) degassed acidified water (HCl, pH = 2.0) through the stems was measured at vacuum of 80, 50 and 30 kPa. Hydraulic conductance was calculated from the slope of the relationship between pressure gradient and flow rate. Stems were then pressurized at 175 kPa for between 30 and 60 min to remove xylem emboli from the stem before final hydraulic conductance was measured. Percentage loss of hydraulic conductance was calculated from the difference in final and initial hydraulic conductance. This method measures total stem conductance (main stem, nodes and branches). Stem diameter, main stem length, branch lengths, and leaf area were measured and related to final stem hydraulic conductance and percentage loss of hydraulic conductance.

Xylem abscisic acid concentration

Xylem exudate was collected by pressurizing two or more leaves in a Scholander pressure chamber to approximately 1 MPa above $\psi_y$. The sap was freeze-dried and stored in the dark at −4 °C until analysed. Two replicates per plant were collected. The sap samples were analysed by addition of an internal standard of deuterium labelled ABA [3',5',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, 162, 166 ions. ABA was quantified by referring the sample to changes in $\psi_{pd}$, as $V$ increased (Fig. 1a). The response was not affected by maintaining either constant $C_i$ or constant $C_s$. Maximum $g_s$ measured at the lowest $V$ used declined from 430 mmol m⁻² s⁻¹ in plants having high (least negative) $\psi_{pd}$, to close to zero in plants having the lowest (most negative) $\psi_{pd}$ (Figs 1a, 2a). The decline in $g_s$ as $V$ increased (\(\Delta g_s/\Delta V\)) was negatively related to maximum $g_s$ (Fig. 3a). The response of $g_s$ to changes in $V$ decreased from −96 to −3 mmol m⁻² s⁻¹ kPa⁻¹ as $g_s$ declined from 430 to < 10 mmol m⁻² s⁻¹ (Fig. 3a). Maintaining constant $C_i$ or constant $C_s$ as $V$ declined did not affect this relationship (Fig. 3a). The response of $g_s$ to changes in $V$ became less negative as $\psi_{pd}$ declined (Fig. 3b).

$C_i$ declined from 290 to 250 µmol mol⁻¹ as $V$ increased.
when $C_a$ was maintained at 360 μmol mol$^{-1}$ and $C_n$ had to be increased to 400 μmol mol$^{-1}$ when $C_i$ was maintained at 290 μmol mol$^{-1}$ (data not shown). The CO$_2$ response of stomata ($C_n$ from 800 to 100 μmol mol$^{-1}$) at low $V$ (2 kPa) showed $g_s$ changed little when $C_n$ increased from 360 and 400 μmol mol$^{-1}$ but stomata did close at higher $C_n$ and open at higher $C_s$ (Fig. 4a). The response of $g_s$ to $V$ when $C_n$ was 540 μmol mol$^{-1}$ was similar to when $C_n$ was 360 μmol mol$^{-1}$ (Fig 3a).

**Leaf transpiration**

Maximum $E_t$, like maximum $g_s$, declined as $\psi_{pd}$ declined (Fig. 2b). Maintaining constant $C_i$ or constant $C_n$ while $V$ varied did not influence the response of $E_t$ to $V$. $E_t$ increased as $V$ increased, although the response of $E_t$ to $V$ (i.e. the initial slope of the relationship) decreased at higher $V$ or in the most droughted plants (Fig. 1b). Plants experiencing moderate to severe drought ($\psi_{pd} < -0.6$ MPa) showed a smaller response of $E_t$ to changes in $V$ (Fig. 1b) and a smaller initial incline in $E_t$ as $V$ increased at low $V$ (data not shown).

Figure 5 shows that $V$ and leaf water potential determined the response of $g_s$ to $E_t$. When plants were well watered ($\psi_{pd} > -0.05$ MPa), stomata are either (a) unresponsive to $E_t$, when $V$ was low (corresponding to region C of Monteith, 1995); (b) show feedforward responses so that $g_s$ declines at intermediate values of $V$ and $E_t$ remains essentially constant (region A of Monteith, 1995); or (c) stomata close more extensively in a feedforward response to cause a large reduction in $E_t$ at high values of $V$ (region B of Monteith, 1995).

As leaf $\psi_{pd}$ declined during drought, region C became progressively lost and eventually region A was also lost so that only region B remained in the response curve. The range of $V$ experienced in these experiments was the same for fully watered, moderately stressed and highly water-stressed plants. Therefore the collapse of the response curve so that all responses to $E_t$ occur on region B, indicates that there was a substantial decline in the value of $V$ associated with transition from C to A and from A to B.
Stomatal and photosynthetic responses in Eucalyptus

Net photosynthesis

Drought and a larger $V'$ significantly ($P \leq 0.05$) decreased maximum $P_n$ (Fig. 1c, 2c). The slope of the relationship between $P_n$ and $g_s$ increased as $\psi_{pd}$ declined (Fig. 6). As water became less available, as measured by increased $V'$ or declining $\psi_{pd}$, $P_n$ and $g_s$ declined and this relationship was unaffected by maintaining constant $C_a$ or constant $C_i$ (Fig. 6). The CO$_2$ response of carbon assimilation ($C_a$ from 800–100 μmol mol$^{-1}$) showed $P_n$ responded very little when $C_a$ ranged from 360–400 μmol mol$^{-1}$ (comparable to $C_i$ of 280 to 330 μmol mol$^{-1}$) (Fig. 4b).

Stem hydraulic conductance

Stems used to measure hydraulic conductance had leaf water potentials ranging from $-0.2$ to $-4.5$ MPa. The water potential of stems measured predawn were on average 0.70 MPa higher than those measured at noon. As drought progressed the percentage loss of hydraulic conductance increased (Fig. 7). The relationships were similar in the morning and afternoon. No significant relationships ($P > 0.05$) existed between the maximum stem hydraulic conductance (post-pressurization to remove emboli) and leaf area, stem diameter or stem and branch length.

ABA in xylem and in leaf tissue

Foliar ABA concentrations of leaves covering the range of $\psi_{pd}$ ranged from 1–5 nmol g$^{-1}$ dry weight. Maximum

Fig. 4. Relationship between calculated internal CO$_2$ concentration ($C_i$) and (a) stomatal conductance (mmol m$^{-2}$ s$^{-1}$) and (b) net photosynthesis (μmol m$^{-2}$ s$^{-1}$) when ambient CO$_2$ concentration was varied between 800 to 100 μl l$^{-1}$ and leaf-to-air vapour pressure difference was 2.0 kPa. Data are means and SE of six plants.

Fig. 5. Stomatal conductance as a function of transpiration rate for several representative plants. Each graph represents one plant. The arrows represent the progression from low to high leaf-to-air vapour pressure difference. The three regimes (A, B, C) of the relationship between stomatal conductance, transpiration and leaf-to-air vapour pressure difference as described by Monteith (1995) are indicated on each graph. Predawn leaf water potentials for each plant are shown.
As watered and droughted plants, as observed previously in plants are shown. of sensitivity to potential and $V$ significantly ($P_g △$). Linear regressions for each $Y_d$ range are $P_y = 7.9 \pm 0.015 \times g_y$; $P < 0.001$ (○); $P_y = 2.2 \pm 0.027 \times g_y$; $P < 0.001$ (□); $P_y = -0.2 \pm 0.048 \times g_y$; $P < 0.001$ (△). Data from nine representative plants are shown.

measured $g_y$ was negatively correlated ($P \leq 0.05$) with foliar ABA concentration (Fig. 8). Xylem ABA concentrations averaged 0.24 mmol m$^{-3}$ and did not differ significantly ($P > 0.05$) with $ψ_l$ (range = $-0.05$ to $-4.2$ MPa).

Discussion

Stomatal responses to $V$ as function of $E_p$ predawn water potential and $C_i$

As $V$ increased, stomatal conductance declined for fully watered and droughted plants, as observed previously in $E.$ tetrodonta (Berryman et al., 1994; Prior et al., 1997) and many other species (Morison and Gifford, 1983; Turner et al., 1984; Maroco et al., 1997). In addition, maximum $g_y$ declined as predawn water potential declined during drought, as previously observed (Gollan et al., 1985; Turner et al., 1985; Prior et al., 1997). However, several important features are apparent in our data; namely, how the decline in $g_y$ was a function of tree water status, how effective the decline in $g_y$ was in regulating transpiration and whether $C_i$ was important in modulating the response of $g_y$ to increasing $V$.

Stomatal conductance increases as $C_i$ declines (Berryman et al., 1994; Bunce, 1997). It is therefore expected that the closing response of stomata as $V$ increased would be reduced when $C_a$ remained constant (and hence $C_i$ allowed to decrease) relative to when $C_i$ was maintained at a constant value. However, there was no difference in $δg_y/δV$ when $C_i$ was maintained constant compared to when $C_a$ was constant and $C_i$ allowed to vary (Fig. 3a, b). This does not differ from that of Morison and Gifford (1983) who observed that a low $C_i$ increased stomatal sensitivity to $V$. However as Morison and Gifford (1983) note, if $δg_y/δV$ is used as a measure of sensitivity to $V$ and $δg_y/δV$ is linearly related to $g_y$, then because a high $C_s$ results in a small $g_s$, $δg_y/δV$ must be reduced by a large $C_a$. It is proposed that $δg_y/δV$ is not a valid measure of stomatal sensitivity to $V$ because $V$ is not the correct measure of signal strength for changes in $g_y$. Rather, it is the change in transpiration rate that would occur in response to the increase in $V$ that is the signal to which stomata respond (Mott and Parkhurst, 1991; Monteith, 1995). This is further discussed below.

Morison and Gifford (1983) concluded from a comparison of $C_3$ and $C_4$ species that although stomata were more responsive to changes in $C_i$ or $V$ when $g_s$ was larger, the relative responses of $g_y$ to either $C_i$ or $V$ were unaffected by the other variable. Like Morison and Gifford (1983) it was also observed that $δg_y/δV$ was larger when $g_s$ was larger (Fig. 3a).

Fig. 8. Maximum measured stomatal conductance (mmol m$^{-2}$ s$^{-1}$) as a function of foliar ABA concentration (mmol g$^{-1}$ dry weight). The fitted line is $400 \pm 25.88 \pm 9 \times$ ABA concentration. $P = 0.009$. Each point is the mean of two replicates. SE are shown.
Stomatal closure, tree water status and stomatal sensitivity

The slope of the relationship between \( g_s \) and \( V \) increased (became less negative) as tree water status declined (became more negative) (Fig. 1). This could be interpreted (incorrectly) as indicating that stomatal sensitivity to \( V \) decreased with declining tree water status. However, as has been previously shown, it is \( E_t \) that determines changes in \( g_s \) (Mott and Parkhurst, 1991; Monteith, 1995). Therefore the size of the signal is large when \( g_s \) is large and small when \( g_s \) is small and the use of \( \delta g_s / \delta V \) as a measure of sensitivity is incorrect. In Fig. 1a and b, when \( g_s \) and \( E_t \) are smallest (droughted leaves), as \( V \) increases, \( E_t \) did not increase and therefore the stomata exhibited the largest control and are therefore the most sensitive to increasing \( V \). A similar conclusion was reached by Turner et al. (1984).

Further support of the view that drought increased stomatal sensitivity to \( V \) is given by the fact that in well-hydrated plants, the decline in \( g_s \) as \( V \) increased was insufficient to prevent an increase in \( E_t \) for the lower range of \( V \) (Figs 1b, 5). Only when drought had progressed so that predawn water potential was \(-0.6\) MPa or lower was the response of the stomata sufficient to prevent \( E_t \) increasing as \( V \) increased. The absolute magnitude of \( E_t \) declined with the development of drought, but regulation of \( E_t \) increased with increasing drought. A possible mechanism for this is that the dosage (concentration \times volume) of ABA received by the stomata, rather than the concentration of ABA in the xylem sap, determines stomatal response to ABA (Gowing et al., 1993). The xylem ABA concentration obtained in this experiment may be underestimated owing to the dilution of xylem sap resulting from too high an overpressurization of stems in order to express sufficient quantities of exudate (Else et al., 1995) thus rigorous conclusions cannot be drawn from these data nor a flux of ABA to the leaves calculated. However, increases in foliar ABA concentration in droughted plants were related to lower maximum measured \( g_s \) (Fig. 8).

Like Monteith (1995), three different response phases of stomata to increasing \( V \) were recognized. Region C (Fig. 5) occurs at the lowest values of \( V \), where transpiration rates are limited primarily by \( V \) and not \( g_s \). As \( V \) increases in this low range, \( E_t \) increases and stomata do not actively limit water loss. It is presumed that this is because the availability of water in the soil and hydraulic architecture (Tyree and Ewers, 1996) of the plant are sufficient to supply water to the leaf at rates sufficient to reduce loss as transpiration. In region A, in the mid-range of \( V \) values, a transition occurs in the stomatal response. As \( V \) increases, stomata respond such that the increase in driving force for \( E_t \) is equalled by the decrease in \( g_s \) and hence \( E_t \) is constant. Finally, at the highest values of \( V \), feedforward (Farquhar, 1978; Schulze, 1986) stomatal responses are such that \( E_t \) declines as \( V \) increases (region B). It is presumed that this is because the hydraulic architecture of the plant and/or soil water availability is unable to supply water at rates sufficient to support the rates of \( E_t \) that would occur if \( g_s \) did not decline. In support of this view is the fact that as soil water availability declines, regions C and A are progressively lost and the entire stomatal response pattern is confined to region B—namely that region that is most able to prevent increasing \( E_t \) as \( V \) increases. It should be noted that in Fig. 5g and h, the range of \( V \) to which leaves were exposed is the same as that in Fig. 5a–f. Because the range of \( E_t \) experienced by the leaf in Fig. 5g and h is only 25% of that experienced in Fig. 5a–f, it is concluded that either it is not the absolute value of \( E_t \) per se that determines the response of stomata to \( V \) or, as drought progresses, the threshold value of \( E_t \) at which transitions in stomatal behaviour occur, decreases. This latter conclusion is supported by the fact that as drought progressed, region A, representing the intermediate behaviour of stomata between no regulation (region C) and complete feedforward regulation (region B) occurs are progressively smaller values of \( E_t \) (Fig. 5).

Stem hydraulic conductance and \( g_s \)

The hydraulic conductance of stems and branches were similar to those reported by Williams et al. (1997a), who used similar methodology to measure hydraulic conductance. Stem hydraulic conductance decreased (\( P \leq 0.05 \)) as leaf water potential declined (Fig. 7). An increase in xylem cavitation, and hence a loss of stem hydraulic conductivity as plant water potential declines has been shown in many species (Breda et al., 1993; Franks et al., 1995; Williams et al., 1997a). Similarly, a loss of stem hydraulic conductance can reduce \( g_s \) (Sperry et al., 1993). The increased tension within the xylem vessels caused by decreasing soil water content and/or increased demand for water would cause cavitations thereby restricting water movement and leading to reduced conductance. Thus, it is possible that increased embolism and decreased stem hydraulic conductance as predawn water potential declined (Fig. 7) is a mechanism by which stomatal conductance can respond to both the decline in soil water availability and increasing \( V \).

Photosynthetic responses to increasing \( V \)

The relationship between \( g_s \) and \( P_n \) was unaffected by maintaining a constant value of \( C_o \) or \( C_i \) when \( V \) was increased (Fig. 6). However, the slope of the relationship between \( P_n \) and \( g_s \) increased as \( \psi_{pd} \) decreased (Fig. 6), suggesting that the decline in \( g_s \) with increasing \( V \) or the declining \( \psi_{pd} \) was not the sole cause of the decline in \( P_n \). Sharkey (1984), Melzack et al. (1985) and Graan and Boyer (1991) have also concluded that declining leaf...
water potential or high transpiration rates decrease \( P_n \) primarily through a decline in \( g_s \) (and hence \( C_i \); Chaves, 1991). However, in these data, when \( C_i \) was maintained, at both high and low \( \psi_{pw} \) and at both high and low transpiration rates, \( P_n \) declined as \( V \) increased (Figs 1c, 2c). Therefore, neither low water potential nor high transpiration were required to cause \( P_n \) to decline as \( V \) increased.

In conclusion, the sensitivity of \( g_s \) to \( V \) declined as the severity of soil drought increased, but was unaffected by maintaining constant \( C_i \) compared to maintaining constant \( C_i \). The lower \( g_s \) in droughted plants could be due to reduced water supply to the leaves resulting from decreased stem hydraulic conductance as \( \psi_{pw} \) became more negative. There was also an increased foliar ABA concentration in droughted plants that was correlated with lower \( g_s \). This suggests that an increased ABA supply in droughted plants could also be contributing to the decline in \( g_s \), although values for instantaneous ABA supply from the roots to the leaves, in the form of xylem ABA concentration, showed no relationship with \( g_s \) or stomatal sensitivity to soil drought. It is therefore apparent that in a seasonally dry savanna environment, where leaf water potentials routinely decline to \(-2.0 \text{ MPa} \) or lower (Myers et al., 1997) in the dry season and \( V \) can increase to almost 6.0 kPa (Prior et al., 1997), both hydraulic and chemical signals may regulate \( g_s \).

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