Variability among species of stomatal control under fluctuating soil water status and evaporative demand: modelling isohydric and anisohydric behaviours

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Abstract

Stomatal control of species with contrasting stomatal behaviours have been investigated under natural fluctuations of evaporative demand and soil water status. Sunflower and barley (anisohydric behaviour) have a daytime leaf water potential ($\psi_l$) which markedly decreases with evaporative demand during the day and is lower in droughted than in watered plants. In contrast, maize and poplar (isohydric behaviour) maintain a nearly constant $\psi_l$ during the day at a value which does not depend on soil water status until plants are close to death. Plants were also subjected to a range of soil water potentials under contrasting air vapour pressure deficits (VPD, from 0.5 to 3 kPa) in the field, in the greenhouse or in a growth chamber. Finally, plants or detached leaves were fed with varying concentrations of artificial ABA. Stomatal conductance of well-watered plants had no response to VPD when plants were grown in natural soils, suggesting that the opposite result observed in many laboratory experiments might be linked to the low unsaturated hydraulic conductivity of usual potting substrates. The response of stomatal conductance of all studied species to the concentration of ABA in pressurized xylem sap ([ABA]$_{xyl}$) was the same whether ABA had an endogenous origin (droughted plants) or was artificially fed. However stomatal response of maize and poplar to [ABA]$_{xyl}$ markedly changed with varying evaporative demand or $\psi_l$, whereas this was not the case in sunflower or barley. This suggests that isohydric behaviour is linked to an interaction between hydraulic and chemical information, while anisohydric behaviour is linked to an absence of interaction. In all cases, [ABA]$_{xyl}$ was related to soil water status with common relationships for different experimental conditions, but with markedly different responses among species. Diurnal variations of [ABA]$_{xyl}$ with evaporative demand were small in all studied species. Results are synthesized in a model which accounts for observed behaviours of $g_s$, $\psi_l$ and [ABA]$_{xyl}$ in fluctuating conditions and for several species. The validity of this model, in particular the physiological meaning of [ABA]$_{xyl}$, is discussed.

Key words: Stomata, vapour pressure deficit, water deficit, ABA, maize, poplar, sunflower, water transport, field, genetic variability.

Introduction

Following a general trend described by Passioura (1979), analysis and modelling of stomatal control at the intermediate scales of a whole plant in natural conditions or of a square metre of canopy have tended to receive less attention lately than those either at a cellular level or at a regional level. The former can appear as more adapted to the analysis of mechanisms, the latter are presumably more useful in environmental modelling. However, three reasons led us to continue experiments and modelling at the scale of whole plants subjected to naturally fluctuating environmental conditions.

Integration of processes. Mechanisms involved in the stomatal response to environmental conditions are multiple and have different quantitative effects (even sometimes opposite effects) on stomatal conductance. Scaling up to the whole-plant level cannot be considered as a
sum of individual mechanisms whose weights would be independent of environmental conditions. It requires identification of the mechanisms which have the largest contribution to stomatal behaviour in a given range of environmental conditions. The whole plant or the square metre of canopy are the smallest possible integration level for that, but are also the largest integration levels at which environmental conditions and plant controls can be experimentally manipulated in order to test the bases for modelling and to avoid confusion of effects.

**Peculiarities and fluctuations of environmental conditions in the field.** Plant behaviour in the field frequently differs from that in the laboratory due to a variety of factors among which three probably have a determinant weight. (i) Evaporative demand is usually considerably higher in the field than in the laboratory, due to differences in irradiance (up to 2000 μmol m\(^{-2}\) s\(^{-1}\) in the field compared with 200–600 μmol m\(^{-2}\) s\(^{-1}\) at leaf level in a growth chamber). Maximum transpiration rate is therefore higher and minimum leaf water potential is lower in the field than in the laboratory. Potentially, this gives greater weight to hydraulic rather than chemical mechanisms of stomatal control in the field than in the laboratory (Kramer, 1988). (ii) Soil substrates in laboratory experiments have much lower unsaturated hydraulic conductivity than natural soils such as clays or loams (Jones and Tardieu, 1998). This increases the probability of short and local water deficits in the roots, even in wet soil and at relatively low evaporative demands. (iii) All environmental conditions are fluctuating in the field, with time-scales ranging from min to days. Plant characters such as water potential, turgor, concentrations of several hormones, and stomatal conductance have synchronous variations so a confusion of effects is likely if environmental conditions or plant control are not manipulated.

**Analysis of the genetic variability of stomatal control.** Detailed evaluation of different components of the genetic variability of ‘resistance to drought’ becomes essential, as genetic manipulation of individual characters is now feasible. A recent study (Borel et al., 1997) shows that this evaluation requires a framework of analysis that allows real genetic differences in stomatal control (e.g. differences among lines in ABA production or in stomatal conductance at a given soil water status) to be distinguished from developmental differences among lines (e.g. differences in cumulated transpiration due to differences in leaf areas among lines, so that plants with the lowest leaf area experienced the highest soil water potential after some days without irrigation).

The objective of this paper is to analyse jointly the stomatal controls of different species under naturally fluctuating evaporative demand and soil water status, and to contribute to a framework of analysis valid for several species and lines.

**Difference in stomatal control among species: isohydric and anisohydric behaviours**

**Role of stomatal conductance in the control of leaf water status**

Leaf water potential (\(\phi_l\)) of well-watered plants fluctuates during the day following both evaporative demand and stomatal opening with light (Figs 1c, 2c, 3d). Its maximum value, observed before dawn, results from an equilibrium between soil and plant water potentials in the absence of water flux. Plants growing in soil with decreasing water availabilities have decreasing predawn \(\phi_l\) (see values of \(\phi_l\) at 4 a.m. in Figs 1c, 2c, 3d, e, f). So predawn \(\phi_l\) can be used as a reliable indicator of the maximum soil water potential.

**Fig. 1.** Change with time in stomatal conductance of field-grown sunflower (Helianthus annuus L cv. Albena), presented together with PPFD (plain line), air VPD (dotted line), leaf water potential and concentration of ABA in xylem sap collected by pressurization (T Simonneau, unpublished data). Plants were grown in the field and subjected to full irrigation (◯), mild (○) or severe (▲) water deficit at flowering time. Stomatal conductance was measured with a ventilated closed chamber (LI-6200, Li-Cor). Water potential of the same leaves was measured with a pressure chamber, and xylem sap was collected by pressurizing leaves with 0.2–0.5 MPa over balancing pressure. Crude sap was analysed for ABA by RIA (Quarrie et al., 1988). Sap was free of immunocontaminant (Tardieu et al., 1996). Lines in panels b to d join average values corresponding to one treatment. Error bars, interval of confidence at the 0.95 probability level.
Vapour pressure deficit (VPD) equation relating stomatal conductance \( (g_x) \) to leaf-to-air vapour pressure deficit \( (VPD_{la}) \):

\[
\phi_l = \phi_{soil} - (R_{soil} + R_{plant}) \left( \frac{g_x}{g_s + g_a} \right) \frac{M_w}{R_g} VPD_{la}
\]

where \( R_{soil} \) and \( R_{plant} \) are the hydraulic resistances from bulk soil to roots and from roots to shoot, respectively, \( g_s \) and \( g_a \) are the boundary layer and stomatal conductances, \( M_w \) is the molar weight of water, and \( R_g \) is the gas constant. Equation 1 is an oversimplification of the reality (see later), but provides a broadly correct framework for the analysis of the change in \( \phi_l \) with time. In the absence of evaporative demand, \( \phi_l \) is only dependent on \( \phi_{soil} \) and declines as soil water is depleted. When plants transpire, \( \phi_l \) is further decreased according to the second term of Eq. (1) and could reach very low values in the absence of stomatal regulation. Such a situation is observed in wilt mutants and is generally lethal, unless plants are grown under very low VPDs (Tal and Imber, 1972; C Borel and T Simonneau, unpublished results). In other plants, stomatal conductance decreases with soil water status (Figs 1b, 2b, 3a, b, c) thereby reducing the effect of VPD\(_a\). However, the behaviour of \( \phi_l \) of droughted plants markedly differs among species, with a large difference in midday \( \phi_l \) among watering treatments in sunflower (Fig. 1), in opposition to the cases of maize and poplar (Figs 2, 3). It has been argued (Tardieu, 1993) that this difference in behaviours (called here ‘anisohydric’ and ‘isohydric’, respectively, following Stocker, 1956) is linked to stomatal control.

**Isohydric and anisohydric behaviours**

A typical anisohydric behaviour is presented in Fig. 1 for three groups of field-grown sunflower plants growing in soils with contrasting water availabilities (as characterized by predawn \( \phi_l \)) under high PPFD. Change with time in \( \phi_l \) with evaporative demand was similar in the three studied treatments, so differences in \( \phi_l \) among treatments remained approximately constant over the day (difference between midday \( \phi_l \) similar to difference between predawn \( \phi_l \) in Fig. 1c). The concentration of ABA in xylem sap \( ([ABA]_{xyl}) \) increased as predawn \( \phi_l \) decreased, but underwent slight fluctuations with time of the day (Fig. 1d). It is noteworthy, however, that there was a transient decrease in \( [ABA]_{xyl} \) from 6–10 h which was observed on all the days and treatments studied. Stomatal conductance decreased with predawn \( \phi_l \) and approximately followed changes in PPFD in all treatments, except for a transient stomatal opening in droughted plants during the morning \( h \) during which \( [ABA]_{xyl} \) had lower values (Fig. 1b). Stomatal closure therefore provided plants with a coarse control against dehydration, but did not completely buffer daytime \( \phi_l \) against changes in evaporative demand and \( \phi_{soil} \), as day–night alternations in \( \phi_l \) were similar in well-watered and in droughted plants.

A different, isohydric, behaviour was observed in maize and poplar plants (Figs 2, 3). \( \phi_l \) differed among watering treatments before dawn, but reached a plateau from 8–16 h which did not depend on soil water status (Figs 2c, 3d, e, f). This was the case in poplar plants, in particular, whose \( \phi_l \) was sequentially measured on the same plants. \( \phi_l \) of these plants was stable during the afternoon and did not differ among watering treatments. As in the case of sunflower, \( [ABA]_{xyl} \) remained nearly...
Fig. 3. Change with time in stomatal conductance of poplar plants (*Populus euramericana* cv. I-214) grown in the greenhouse during sunny days, presented with leaf water potential and concentration of ABA in xylem sap collected by pressurization (Tardieu and Trejo, unpublished data). Plants were grown until 6-month-old (plants were from 1.5–2 m tall at time of sampling). Irrigation rate was managed such that plants experiencing contrasting soil water status could be sampled on the same day. Measurements were carried out in the greenhouse, with PPFD greater than 800 μmol m⁻² s⁻¹ during the afternoon and with air VPDs ranging from 2–3 kPa. Measurements were carried out in the same way as in maize. Lines join points corresponding to one plant, as leaves belonging to the same plant could be sampled at different times of the day without appreciably changing total leaf area and water flux through the plant.

constant during the day, with a decline during the early hours in maize and an increase from 8–17 h in poplar (Fig. 4b). Midday stomatal conductance decreased with [ABA]ₓyl, but the typical asymmetric pattern with a lower gs in the afternoon than in the morning was not linked to a diurnal change in [ABA]ₓyl. In both species, stomatal control therefore allowed ψ₁ to remain constant during the afternoon by progressively closing stomata, thereby balancing the increase in evaporative demand (Eq. 1). It also allowed ψ₁ to be similar in droughted and well-watered plants, by a stomatal closure which balanced the decrease in soil water potential.

Data in the literature allow classification of several species in one of these two categories (Table 1), which do not follow other classical classifications of species (monocots versus dicots, C₃ versus C₄ etc.). The difference in behaviour was probably not linked to the respective roles of stomatal and boundary layer resistances in the control of transpiration (McNaughton and Jarvis, 1983; Jones, 1998). In ‘coupled’ canopies, with high ratio gs/gₛ, transpiration largely depends on stomatal conductance while the opposite occurs in ‘uncoupled’ canopies with low gs/gₛ. As ψ₁ depends on transpiration rate and not solely on gs (Eq. 4; Fig. 4) one could expect that ψ₁ could be largely independent of gs in uncoupled canopies. This was not sufficient to explain the differences in behaviour among species. Isolated plants of poplar or maize grown in a ventilated greenhouse or in the field with wind (coupled canopy) still displayed an isohydric behaviour, similar to that shown for field-grown plants. Conversely, sunflower plants grown either in the field or isolated all displayed anisohydric behaviour (Tardieu et al., 1996). Other experimental conditions, such as the rate of soil water depletion, temperature or growth stage did not affect this behaviour either.

Which framework of analysis to study differences in stomatal control among species?

Stomatal control differs between isohydric and anisohydric plants, and for the cases described here between sunflower on one hand and maize and poplar on the other hand. However, Eq. 1 does not allow an analysis of this difference in control because of several weak points. Leaf-to-air VPD is not an independent driving variable, as it is the result of the leaf energy budget which itself depends on gs. When stomata close, leaf temperature can increase by several degrees, thereby increasing VPDₛ by up to 1 or 2 kPa (Ben Haj Salah and Tardieu, 1997). VPDₛ is therefore both a cause and a consequence of the water movement through the plant. In the framework of analysis presented in Fig. 4, Eq. 1 was then replaced by...
Modelling stomatal control in whole plants

Equation 3 applies when PPFD is higher than 800 \( \text{mol m}^{-2} \text{s}^{-1} \). Otherwise, \( g_s \) is set at the minimum between that predicted by the model and that predicted by the response to PPFD. Arrows symbolize transfers of water and/or ABA. Input variables: \( W_n \) (net radiation), \( VPD \) (air pressure vapour deficit) and \( \psi_{soil} \) (soil water potential). State variables are \( g_s \) (stomatal conductance), \( \psi_r \) and \( \psi_l \) (root and leaf water potentials), \( J_w \) (water flux) and \([\text{ABA}]_{\text{xyl}}\) (concentration of ABA in the xylem sap). For explanation of the other symbols in equations, see text.

### Table 1. Classification of several species in isohydric or anisohydric behaviours

<table>
<thead>
<tr>
<th>Species</th>
<th>Growing conditions</th>
<th>Behaviour</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lupin</td>
<td>Pot</td>
<td>Isohydric</td>
<td>Henson et al., 1989</td>
</tr>
<tr>
<td>Maize</td>
<td>Field, pot</td>
<td>Isohydric</td>
<td>Tardieu et al., 1993</td>
</tr>
<tr>
<td>Pea</td>
<td>Field</td>
<td>Isohydric</td>
<td>Bates and Hall, 1981</td>
</tr>
<tr>
<td>Poplar</td>
<td>Pot</td>
<td>Isohydric</td>
<td>This study</td>
</tr>
<tr>
<td>Sugarcane</td>
<td>Pot</td>
<td>Isohydric</td>
<td>Salindra and Meinzer, 1989</td>
</tr>
<tr>
<td>Almond tree</td>
<td>Field</td>
<td>Anisohydric</td>
<td>Wartinger et al., 1990</td>
</tr>
<tr>
<td>Barley</td>
<td>Pot</td>
<td>Anisohydric</td>
<td>Borel et al., 1997</td>
</tr>
<tr>
<td>Peach tree</td>
<td>Field</td>
<td>Anisohydric</td>
<td>Xiloyannis et al., 1980</td>
</tr>
<tr>
<td>Soybean</td>
<td>Pot</td>
<td>Anisohydric</td>
<td>Allen et al., 1994</td>
</tr>
<tr>
<td>Subterranean clover</td>
<td>Pot</td>
<td>Anisohydric</td>
<td>Socia et al., 1997</td>
</tr>
<tr>
<td>Sunflower</td>
<td>Field, pot</td>
<td>Anisohydric</td>
<td>Tardieu et al., 1996</td>
</tr>
<tr>
<td>Wheat</td>
<td>Pot</td>
<td>Anisohydric</td>
<td>Henson et al., 1989</td>
</tr>
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three equations, the Penman–Monteith equation (Eq. 2; Fig. 4) and the Van den Honert (1948) water flux equations (Eqs 4, 6).

\[
R_{\text{soil}} = \left[ \ln \left( \frac{d^2}{r^2} \right) \right] / 4\pi K(\theta) \tag{7}
\]

if \( d \) is half the mean distance between neighbouring roots and \( r \) is mean root diameter. This equation provides acceptable estimates of \( R_{\text{soil}} \) when root spatial arrangement is close to randomness but underestimates it if roots tend to be clumped (Tardieu et al., 1992c). Equation 6 in Fig. 4 results from the combination of Eq. 7 and the Van den Honert equation applied to the soil–root transport.

Controls of stomatal conductance as a function of environmental conditions must be inserted in the model. This was achieved (Fig. 4) by two equations describing ABA concentration in the xylem sap (Eq. 5), and stomatal control by hydraulic and chemical messages (Eq. 3).
The resulting model (Fig. 4) has $\phi_{\text{soil}}$, net radiation and air vapour pressure deficit ($VPD_{\text{air}}$) as input variables, and consists of five equations with five unknowns ($\psi_i$, $\phi_{\text{root}}$, [ABA]$_{\text{xyl}}$, $g_s$, $J_a$). It applies to periods with high $PPFD$ corresponding to maximum $g_s$. At lower $PPFD$s, $g_s$ is set to the minimum between that predicted by the model and that predicted by the response to $PPFD$. The set of equations was solved numerically (Tardieu, 1993). The following paragraphs aim to discuss the formalisms which have been used for describing plant controls (Eqs 3, 5) in several species.

**Stomatal control by evaporative demand in stable and fluctuating conditions**

*Which variables account for the effect of evaporative demand on stomatal control?*

It is well accepted that $PPFD$ has a typical ‘feedforward’ effect, with an increase in $g_s$ with increasing $PPFD$ until a plateau value (Jones, 1998; Zeiger and Zhu, 1998). The effect of $VPD$ on stomatal control is more controversial in the recent literature (Aphalo and Jarvis, 1991; Monteith, 1995; Bunce, 1996). Stomatal response to $VPD$ has typically been analysed by subjecting plants to a series of steps of 1–2 h duration with increasing $VPD$, but has typically been analysed by subjecting plants to a series of steps of 1–2 h duration with increasing $VPD$. At lower $VPD$s, $g_s$ is set to the minimum between that predicted by the model and that predicted by the response to $PPFD$. The set of equations was solved numerically (Tardieu, 1993). The following paragraphs aim to discuss the formalisms which have been used for describing plant controls (Eqs 3, 5) in several species.

An additional difficulty arises from a comparison of the behaviours of plants subjected either to steps in $VPD_{\text{air}}$ in laboratory conditions or to natural variations of $VPD_{\text{air}}$ in the field. A ‘midday depression’ of $g_s$ is traditionally described in the field and attributed to high $VPD_{\text{air}}$s in the afternoon. However, such decreases in $g_s$ during the afternoon were generally not observed in well-irrigated plants (Figs 1, 2, 3) unless plants were subjected to a mild water deficit and [ABA]$_{\text{xyl}}$ differed from 0. Furthermore, maximum values of $g_s$ at near-zero [ABA]$_{\text{xyl}}$ were independent of $VPD_{\text{air}}$ (Figs 5, 6, 7). Sequences with nearly constant $g_s$ during the afternoon in spite of high $VPD_{\text{air}}$ have also been described in kiwifruit (Guacci et al., 1996), clover (Vadell et al., 1995) and apple trees (Jones, 1992). Correia et al. (1995) described a decline in $g_s$ of vines during the afternoon, but could relate it neither to changes in $VPD_{\text{air}}$ nor to decreases in $\psi_i$. In contrast, Grantz et al. (1987) concluded that a stomatal response to $VPD_{\text{air}}$ was observed in sugarcane, both in the field and in step experiments with varying $VPD$. This conclusion was based on somewhat misleading data processing in which a multiple regression analysis of $g_s$ was carried out with $PPFD$ and $VPD$. The resulting equation implied that $g_s$ nearly doubled when $PPFD$ increased from 1000 to 2000 $\mu$mol m$^{-2}$ s$^{-1}$. The difference in $g_s$ between the abnormally high value expected at 2000 $\mu$mol m$^{-2}$ s$^{-1}$ and the observed value was attributed to the effect of $VPD$.

**Fig. 5.** Relationship between stomatal conductance and concentration of ABA in the xylem sap of sunflower plants subjected to contrasting soil water status and evaporative demand (a–c) or fed with artificial ABA (d) as explained in Tardieu et al. (1996). (e) Detached leaves were fed with artificial sap with contrasting concentrations of ABA. Redrawn from Tardieu et al. (1996).
Fig. 6. Relationship between stomatal conductance and concentration of ABA in the xylem sap of maize plants subjected to contrasting soil water status and evaporative demand (a–c) or fed with artificial ABA (d–f). Experimental procedures and method for supplying ABA are described in Tardieu et al. (1995). Data from Tardieu et al. (1992, 1993) and Ben Haj Salah and Tardieu (1997). (a, d) Experiments in which \( \psi_r \) was higher than \(-1.3\) kPa as a result of low VPD: (a) morning in the field in 1990 (▲) or 1991 (▲); (d) ABA feeding of well-irrigated plants (▲). (b, e) Experiments in which \( \psi_r \) ranged between \(-1.3\) and \(-1.6\) kPa in the field in 1990 (▲) or 1991 (▲). (e) ABA feeding of plants subjected to mild soil water deficit (▲). (c, f) Experiments in which \( \psi_r \) was lower than \(-1.6\) kPa in the field in 1990 (▲) or 1991 (▲). (f) ABA feeding of plants subjected to water deficit (▲).

The possibility is therefore raised that an effect of VPD on stomatal control in droughted plants is isohydric of well-watered plants was considered in the model presented in Fig. 4. However, indirect effects were computed by the model in two cases.

1. Evaporative demand had a marked effect on the response curve of \( g_s \) to [ABA]\text{xyl} in isohydric plants subjected to a mild soil water deficit, consistent with the observation of Saliendra et al. (1995) that the effect of VPD\text{air} on \( g_s \) was prevented if roots were pressurized. At near-zero [ABA]\text{xyl}, maximum \( g_s \) in maize, sunflower or poplar were similar in the morning and in afternoons regardless of VPD in the range from 0.5–3.5 kPa (Figs 5, 6, 7). As a consequence, no direct effect of VPD on stomatal control of well-watered plants was considered in the model presented in Fig. 4. However, indirect effects were computed by the model in two cases.

2. A depression of \( g_s \) by VPD\text{air} even at high \( \psi_{\text{soil}} \) was predicted in soils with low unsaturated hydraulic conductivity. An increase in evaporative demand greatly reduces \( \psi_{\text{soil}} \) in the vicinity of roots (Eq. 7) even if bulk \( \psi_{\text{soil}} \) remains high. This generates an increase in [ABA]\text{xyl} (Eq. 5) and a stomatal closure. This mechanism might explain the effect of VPD\text{air} observed in laboratory experiments where plants are grown in soils (potting compost or vermiculite) with low unsaturated conductivity. The depressing effect of VPD\text{air} was predicted by the model in two cases.

Stomatal control in droughted plants. Is isohydric behaviour linked to an interaction between the effects of [ABA]\text{xyl} and leaf water status?

There is a consensus that stomatal control depends on \( \psi_{\text{root}} \) in numerous species and that root-sourced ABA contributes to this control (Henson et al. (1989) in lupin and wheat; Waringlegter et al. (1990) in almond trees; Davies and Zhang (1991) and Tardieu et al. (1992b) in maize; Khalil and Grace (1993) in sycamore seedlings; Correia and Pereira (1994) in white lupin; Correia et al. (1995) in grapevine; Tardieu et al. (1996) in sunflower; Liang et al. (1996) in two tropical trees; Borel et al. (1997) in barley). In several of these studies, the effects of endogenous and of exogenous ABA were compared, suggesting that ABA plays a crucial role in the chemical signalling involved in stomatal control. Analyses carried out...
different in experiments with detached leaves, in which stomatal conductance was calculated from transpiration rate and \( VPD_{\text{air}} \) instead of being measured with a porometer as in other experiments. In opposition to a suggestion of Jarvis and Davies (1997), the response curve of \( g_s \) to \([ABA]_{\text{xyl}}\) cannot be interpreted in this experiment as the consequence of an increased dilution of the ABA flux by the water flux when \( g_s \) increased. Stomata closed with the same response to exogenous and endogenous ABA, independently of water flux which largely differed among experiments with contrasting \( VPD_{\text{air}} \). This leads to two conclusions. (i) As endogenous and fed ABA had quantitatively similar consequences on \( g_s \), \([ABA]_{\text{xyl}}\) as it was measured can be considered as a relevant controlling variable. (ii) Stomatal control was largely independent of evaporative demand, transpiration rate and ABA flux through plants as coupled values of \( g_s \) and \([ABA]_{\text{xyl}}\) were similar at markedly different transpiration rates. It was also independent of \( \psi_l \).

The conclusions drawn here for sunflower were essentially conserved in two other anisohydric species, barley (Borel et al., 1997) and wheat (Guichard, Tardieu and Brisson, unpublished data). The response of \( g_s \) to \([ABA]_{\text{xyl}}\) was common for several experiments carried out at contrasting evaporative demands, resulting in large differences in leaf water potentials which had no apparent consequences on stomatal control.

**Isohydric behaviour (maize, poplar): a response of \( g_s \) to \([ABA]_{\text{xyl}}\) which depends on leaf water status or on transpiration rate**

In maize (Fig. 6), the response curve of \( g_s \) to \([ABA]_{\text{xyl}}\) markedly differed in the morning and in the afternoon, except in afternoons with low \( VPD_{\text{air}} \). Plants fed with ABA had usually a higher \( \psi_l \) than droughted plants, due to partial equilibration of \( \psi_l \) with \( \psi_{\text{osm}} \) when stomata closed. In each range of \( \psi_l \), the relationship between \([ABA]_{\text{xyl}}\) and \( g_s \) did not differ whether changes in \([ABA]_{\text{xyl}}\) were due to soil dehydration or to ABA feeding. Detached leaves fed with ABA solutions had a \( \psi_l \) close to 0, and consistently had a response of \( g_s \) to [ABA] still less sensitive than that in Fig. 6d (not shown; Tardieu et al., 1993). A similar behaviour was observed in poplar, but with greater scatter of points (Fig. 7).

A variable linked either to transpiration rate or to \( \psi_l \) is likely to affect stomatal control in the cases of maize and poplar, with an increasing apparent stomatal sensitivity to \([ABA]_{\text{xyl}}\) as transpiration rate increased or as \( \psi_l \) decreased (Tardieu and Davies, 1992). Experiments involving ABA feeding show that \( VPD_{\text{air}} \) could not act per se on stomatal sensitivity to \([ABA]_{\text{xyl}}\). Feeding ABA closed stomata, thereby increasing leaf temperature and \( VPD_{\text{air}} \), but also caused a drop of transpiration rate and a large increase in \( \psi_l \). The resulting apparent stomatal sensitivity...
was low, as in plants with low transpiration rate due to low \( VPD_h \). Comparison of \( g_s \) of ABA-fed plants growing in wet and dry soils (low transpiration rate in both cases, but with contrasting \( \phi_l \)) suggests that \( \phi_l \) could account for changes in stomatal sensitivity to \([ABA]_{xyl}\). \( \phi_l \) was consequently chosen as a controlling variable in Eq. 3 (Fig. 4) instead of transpiration rate.

**Differences in behaviours and interaction between \([ABA]_{xyl}\) and leaf water status**

Analysis of three species with anisohydric behaviour (sunflower, wheat, barley) revealed that stomatal response to \([ABA]_{xyl}\) was independent of evaporative demand and of leaf water status. It is possible, therefore, to write a single equation between \( g_s \) and \([ABA]_{xyl}\) with a negative exponential function fitted on experimental relationships.

\[
g_s = g_{\text{min}} + a \exp(\beta[ABA]_{xyl})
\]

where \( g_{\text{min}} \) and \( g_{\text{min}} + a \) are the minimum and maximum stomatal conductances of the considered cultivar, respectively, and \( \beta \) (negative value) is the parameter of the common negative exponential function fitted from experimental data (e.g. Fig. 5 for sunflower). In contrast, analysis for maize and poplar yielded a response curve of \( g_s \) to \([ABA]_{xyl}\) which depended either on leaf water status or on transpiration rate. This was translated in an equation in which \( g_s \) depended on \([ABA]_{xyl}\), but with a sensitivity which depended on \( \phi_l \) with a second negative exponential function with parameter \( \delta \) (negative value):

\[
g_s = g_{\text{min}} + a \exp(\beta[ABA]_{xyl} \exp(\delta \phi_l))
\]

Changing apparent sensitivity of \( g_s \) to \([ABA]_{xyl}\) with \( \phi_l \) provides the plant with a way of controlling \( \phi_l \) much more tightly than in the anisohydric case. Whenever \( \phi_l \) undergoes a small decrease, increased sensitivity to \([ABA]_{xyl}\) causes a slight stomatal closure for an unchanged \([ABA]_{xyl}\). This reduces the water flux through the plant and, therefore, causes an increase in \( \phi_l \). Isohydric behaviour could be simulated with the model presented in Fig. 4 when the interactive term (\( \delta \) in Eq. 3) was set to a non-zero value (Tardieu, 1993). When \( \delta \) was set to 0, the model simulated that \( \phi_l \) was not maintained when evaporative demand or soil water potential varied, consistent with the experimentally observed behaviour of anisohydric species. It simulated the frequently reported correlation between \( \phi_l \) and \( g_s \) without assuming any controlling effect of \( \phi_l \) on \( g_s \) (Tardieu et al., 1996).

**Which variables can account for stomatal control by ABA and evaporative demand?**

**ABA and stomatal control**

In spite of the consistent relationships between \([ABA]_{xyl}\) and \( g_s \) reported here and in other above-mentioned papers, the choice of appropriate variable(s) governing stomatal conductance in droughted plants is still a matter of debate. Concentration of ABA in the apoplast near guard cells could be the best candidate, since perception of ABA has been located at the external surface of the plasmalemma of the guard cell (Anderson et al., 1994; Wilkinson and Davies, 1997; Hartung et al., 1998). However, this concentration cannot be measured by using current techniques. It has been long considered as close to the concentration of ABA in the xylem sap, but this view is now unlikely as ABA is trapped in the cytoplasm of epidermis or mesophyll cells (Hartung and Slovik, 1991) and then degraded rapidly (Gowing et al., 1993; Daeter and Hartung, 1995). The concentration of ABA around guard cells is therefore a balance between the rate of sequestration in the symplast of mesophyll or epidermis cells and the delivery rate of ABA by the xylem sap. It may appreciably differ from that in the xylem, as ABA solutions with concentrations of ABA similar to that observed in the xylem sap of well-watered plants can close stomata of epidermal strips (Trejo et al., 1993).

The use of ABA flux delivered to the leaf (\( J_{ABA} \)) instead of \([ABA]_{xyl}\) as a controlling variable for \( g_s \) may seem adequate for avoiding the above-mentioned problems. However, \( J_{ABA} \) seldom correlates with \( g_s \) (Gowing et al., 1993; Tardieu et al., 1993; Trejo et al., 1995). In the sunflower experiment reported in Fig. 6, as in those reported by Trejo et al. (1995) and by Jia et al. (1996), changing \( VPD_{sat} \) influenced the relationship between \( g_s \) and \( J_{ABA} \) with an accumulation of ABA in leaves (in Trejo et al.’s experiment) and unchanged relationship between \([ABA]_{xyl}\) and \( g_s \).

It therefore seems paradoxical that \( g_s \) still correlates closely with the concentration of ABA in the sap collected by pressurizing leaves (called \([ABA]_{xyl}\) in Figs 5, 6, and 7), in spite of large changes in concentration within the leaf from the xylem sap to the vicinity of guard cell plasmalemma. Two non-exclusive possibilities may explain this result. (i) The concentration of ABA at its site of action may be controlled by feedback processes, leading to the lack of stomatal response to \( J_{ABA} \) described in the previous paragraph. (ii) The concentration of ABA in the sap expressed by pressurization may relate to a ‘mean’ concentration in the apoplast rather than to the concentration of ABA in the xylem sap which was entering into the leaf before excision. When a leaf is excised, a large proportion of apoplastic water is taken up by the symplast and is then released to the apoplast when the leaf is pressurized (Passioura, 1991). One can therefore expect that saps in the xylem and in the apoplast are thoroughly mixed, resulting in an intermediate concentration of ABA in pressurized sap. This is consistent with the results of Borel et al.’s (1997) experiments in which the concentrations of ABA in the saps of roots and shoots of the same barley plants were collected by pressurization.
The resulting concentration in the sap of roots, probably close to the concentration in the leaf xylem sap before excision, was consistently higher than the concentration in the sap collected in leaves.

The possibility that the concentration of ABA in the expressed sap is intermediate between those in the xylem sap and in the apoplast complicates the interpretation of response curves reported in Figs 5, 6 and 7, but it would also explain why tight relationships can be obtained in spite of changes in ABA content within the leaf. This possibility could also give a possible mechanism for the apparent interaction in stomatal control between $[\text{ABA}]_{\text{xyl}}$ and $\psi_l$ (Fig. 3b, c), as the trapping rate of ABA in the symplast probably differs between droughted and well-watered plants. Wilkinson and Davies (1997) recently showed in Commelina that xylem-delivered ABA is sequestered away from the apoplast by the mesophyll cells at low pH as observed in the xylem sap of well-watered plants. A stress-induced increase in sap pH lowers the sequestration of ABA, thereby allowing more ABA to reach the guard cells apoplast in droughted plants. This suggests that the xylem sap pH may act as an additional signal of drought which may explain the interaction described earlier if sap pH depended on $\psi_l$ or on evaporative demand.

Progress in knowledge since the model of Fig. 4 was published therefore questions the meanings of $[\text{ABA}]_{\text{xyl}}$ and of the interactive term of Eq. 3. However, $[\text{ABA}]_{\text{xyl}}$ as it was measured remains a variable which can easily be measured in field and laboratory studies. As it is, it relates closely to the stomatal conductance of all the species studied. The largest effort should now be aimed at relating it to the delivery of ABA in the xylem sap and to ABA trapping in the leaf.

**Delivery rate of root-sourced ABA as a function of root water status**

ABA can be synthesized by dehydrating detached roots of Phaseolus vulgaris (Walton et al., 1976), Xanthium (Cornish and Zeevaart, 1985), sunflower (Robertson et al., 1985) and maize (Ribaut and Pilet, 1991; Zhang and Tardieu, 1996). ABA content in roots is related to soil water status (Zhang and Davies, 1989; Tardieu et al., 1992a). It is probably acceptable to express ABA synthesis at the root system level, as different classes of maize roots (apices of seminal and of nodal roots, older parts of branches and of primary roots) synthesized ABA at the same rate when subjected to similar water potentials (T Simonneau, unpublished data). This was not the case if they were compared at similar water contents. At whole plant level, linear relationships were observed between soil water status (as evaluated by predawn $\psi_l$) and $[\text{ABA}]_{\text{xyl}}$ in maize, sunflower and poplar (Fig. 8) as well as in other species such as almond trees (Wartinger et al., 1990), or several cultivars of barley (Borel et al., 1997).

It is noteworthy that a common predawn $\psi_l$ corresponded to markedly different $[\text{ABA}]_{\text{xyl}}$ in the three species presented in Fig. 8, but species such as poplar with steepest response of $[\text{ABA}]_{\text{xyl}}$ to predawn $\psi_l$ had a less sensitive response of $g_s$ to $[\text{ABA}]_{\text{xyl}}$. This resulted in relatively similar values of $g_s$ at a given predawn $\psi_l$ among species.

In most of the above-mentioned studies, $[\text{ABA}]_{\text{xyl}}$ remained nearly constant with time of the day (Figs 1d,
2d, 3g, h, i; Ben Haj Salah and Tardieu, 1997). This near constancy is also illustrated in Fig. 8b, which shows that \([\text{ABA}]_{\text{xyt}}\) measured at two times of the day had a common relationship with predawn \(\psi_r\). However, near constancy of \([\text{ABA}]_{\text{xyt}}\) with time of the day does not apply to all conditions. It tended to decrease in the morning in droughted maize (Fig. 2d), but rapidly increased with time of the day when the root system had a low efficiency to take up water because of clumping (Tardieu et al., 1992). It also tended to increase with time of the day in poplar plants grown in pots with lower water reserve than in the field. In this case, water reserve in pots decreased during the day, so \([\text{ABA}]_{\text{xyt}}\) measured at the end of the day corresponded to predawn \(\psi_r\) of the following day. In a study where root water potential was manipulated by pressurizing the root system, Schurr and Schulze (1996) observed rapid variations in \([\text{ABA}]_{\text{xyt}}\) with root water status and time of day. Stability of \([\text{ABA}]_{\text{xyt}}\) with time of day is, therefore, the result of an equilibrium which is observed in many cases but cannot be considered as a base for modelling. Furthermore, this equilibrium could not be expected if \([\text{ABA}]_{\text{xyt}}\) was viewed as reflecting root water status. During the day, \(\psi_{\text{root}}\) is depressed as the transpiration rate increases, so it is expected that more ABA should be delivered by the roots.

A possibility that may account for the stability of \([\text{ABA}]_{\text{xyt}}\), and also for the lack of stability in the above-mentioned cases, is that the ABA delivery into the xylem sap of root system is a function of root water potential, but is diluted by the transpiration stream. \([\text{ABA}]_{\text{xyt}}\) would therefore depend on the ratio of the ABA delivery rate to the water flux (Eq. 5; Fig. 4). Simulations carried out using this formalism suggest that \([\text{ABA}]_{\text{xyt}}\) is largely independent of rapid changes in evaporative demand during the day and between days, consistent with experimental data. \([\text{ABA}]_{\text{xyt}}\) would then represent a relatively long-term factor, linked to soil water status and buffered against rapid changes in evaporative demand. Simulations also predict an increase in \([\text{ABA}]_{\text{xyt}}\) during the day when \(R_{\text{soil}}\) appreciably changes with evaporative demand as a consequence of root clumping (compacted soil) or of low unsaturated soil hydraulic conductivity (Tardieu, 1993). They also predict an increase with time \([\text{ABA}]_{\text{xyt}}\), when \(\psi_{\text{soil}}\) appreciably decreases during the day. This usually does not happen in the field where soil water reserve is much larger than the transpiration of one day, but is frequent in pot-grown plants. The ‘buffering capacity’ experimentally observed and predicted by our algorithm seems necessary from an environmental point of view if the message serves to inform the plant of soil water status. The sap takes several hours (possibly even days in tall trees) to travel from roots to shoots. If the information conveyed by the message depended on transient events such as clouds, windspeed or air temperature, the information would be obsolete by the time the sap reaches the leaf.

However, this formalism is an oversimplification of processes of ABA synthesis, delivery, recirculation, and degradation in the plant. It also has the inconvenience of calculating continuous changes in ABA and water fluxes as a sequence of steady states. This is probably acceptable at a time step of several hours during the day, but cannot account precisely for changes in \([\text{ABA}]_{\text{xyt}}\) during the night or when plants are subjected to a rapid sequence of events such as that described by Schurr and Schulze (1996). A more detailed model such as those of Wolf et al. (1990) or Slovik and Hartung (1992) would be more appropriate from a theoretical point of view, but would require a large number of parameters difficult to measure routinely, thereby making it difficult to be used in a large range of environmental conditions and in a multi-species analysis. An adequate approach would probably lie somewhere between the oversimplification presented here and the detailed model of Slovik and Hartung.

**Concluding remarks: toward a common modelling for several species and cultivars?**

In spite of its reported weak points, the framework of analysis proposed in 1993 (Fig. 4) has since proved useful for the analysis of the apparent variability of stomatal control under contrasting environmental conditions and for different species or lines.

A unique framework of analysis applied to several species with quite different behaviours, and to a large range of environmental conditions. The latter included both synchronous changes with time of several environmental conditions observed in natural conditions and asynchronous changes linked to manipulations of soil water status, evaporative demand and \([\text{ABA}]_{\text{xyt}}\). This framework could be considered as sound as the effect on \(g_s\) of endogenous change in \([\text{ABA}]_{\text{xyt}}\) with soil water status could be interpreted with the same framework as the effect of ABA feeding. It is noteworthy that a common set of parameters could apply to all these conditions in a given species and line (Tardieu and Davies, 1993; Tardieu et al., 1996).

This framework can help in the analysis of differences in stomatal control among several species. Observed differences in behaviour do not depend on individual response curves of \([\text{ABA}]_{\text{xyt}}\) to soil water status or of \(g_s\) to \([\text{ABA}]_{\text{xyt}}\), but essentially on the combination of these two response curves. For instance, poplar plants exhibit much higher \([\text{ABA}]_{\text{xyt}}\) than maize in response to a decrease in soil water potential, but stomatal response to \([\text{ABA}]_{\text{xyt}}\) is less sensitive, resulting in similar stomatal behaviours during a sequence of soil water depletion. One of the most determinant parameters is the term \(\delta\) which holds for the interaction between \([\text{ABA}]_{\text{xyt}}\) and leaf water status. If it is set to a non-zero value, it generates an isohydric behaviour with maintenance of day-time \(\psi_l\) when soil
water status varies, and an absence of relationship between $g_*$ and $\psi_*$. In contrast, it generates an anisohydric behaviour with correlation between $g_*$ and $\psi_*$ if it is set to 0. This analysis applied to all studied species with either anisohydric (sunflower, barley, wheat) or isohydric (maize, poplar) behaviours.

Finally, this framework also helped to analyse the stomatal behaviour of lines or cultivars differing in genetic background, geographical origin and ‘resistance to drought’. The result of this analysis in barley (Borel et al., 1997), consistent with that of a similar work in sunflower (Wery, unpublished results), suggested that genetic variability of stomatal control was low. This was in spite of the fact that stomatal behaviours apparently differed among lines. The lines which had faster leaf growth caused faster soil depletion rate, and therefore exhibited an earlier stomatal closure than the lines with slower leaf growth. However, both groups of lines had similar responses of $[ABA]_{xyl}$ to soil water status and similar responses of $g_*$ to $[ABA]_{xyl}$. ‘Resistance to drought’ was not due to intrinsic differences in stomatal control, but to differences in plant development rate.

The basis of this model has been questioned by recent progress in the understanding of stomatal control. In particular, the physiological meaning of the concentration of ABA in xylem sap obtained by pressurization and the modelling of ABA transfer inside the plant are clear weak points. The results reported here on a possible absence of effect of $V_{PD}^{air}$ on stomatal control in well-irrigated field-grown plants, and on the role of unsaturated hydraulic conductivity in the apparent discrepancy between behaviours observed in the field and in the laboratory also merit a new experimental approach.

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