

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 (ψ_1) 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 ψ_1 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 ($[ABA]_{xy1}$) was the same whether ABA had an endogenous origin (droughted plants) or was artificially fed. However stomatal response of maize and poplar to $[ABA]_{xy1}$ markedly changed with varying evaporative demand or ψ_1 , 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,

$[ABA]_{xy1}$ was related to soil water status with common relationships for different experimental conditions, but with markedly different responses among species. Diurnal variations of $[ABA]_{xy1}$ with evaporative demand were small in all studied species. Results are synthesized in a model which accounts for observed behaviours of g_s , ψ_1 and $[ABA]_{xy1}$ in fluctuating conditions and for several species. The validity of this model, in particular the physiological meaning of $[ABA]_{xy1}$, 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

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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 $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the field compared with $200\text{--}600 \mu\text{mol m}^{-2} \text{s}^{-1}$ 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 (ψ_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 ψ_l (see values of ψ_l at 4 a.m. in Figs 1c, 2c, 3d, e, f). So predawn ψ_l can be used as a reliable indicator of the maximum soil water

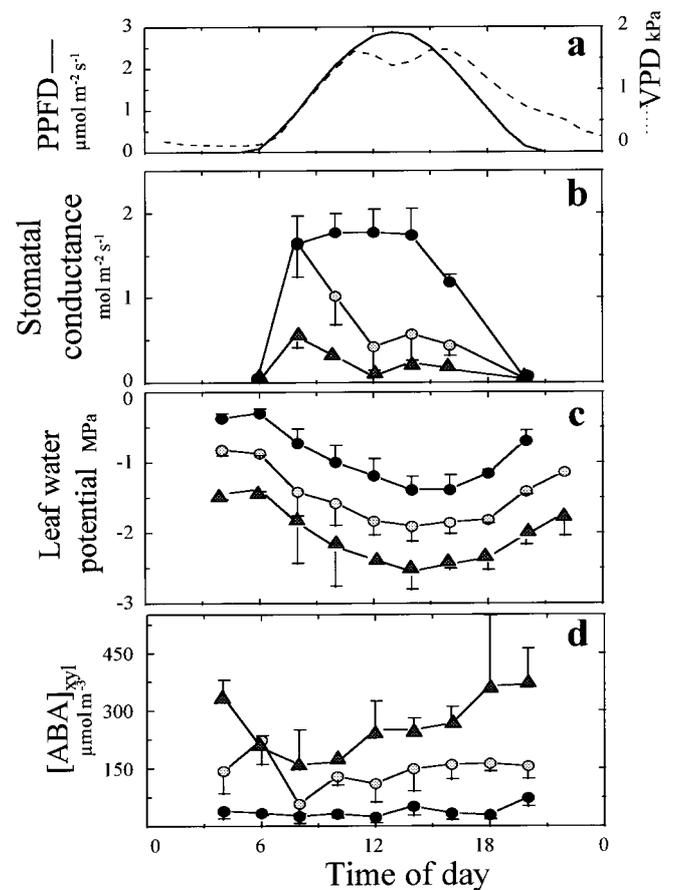


Fig. 1. Change with time in stomatal conductance of field-grown sunflower (*Helianthus annuus* L cv. Albena), presented together with *PPFD* (plain line), air *VPD* (dotted line), leaf water potential and concentration of ABA in xylem sap collected by pressurization (T Simonneau, unpublished data). Plants 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.

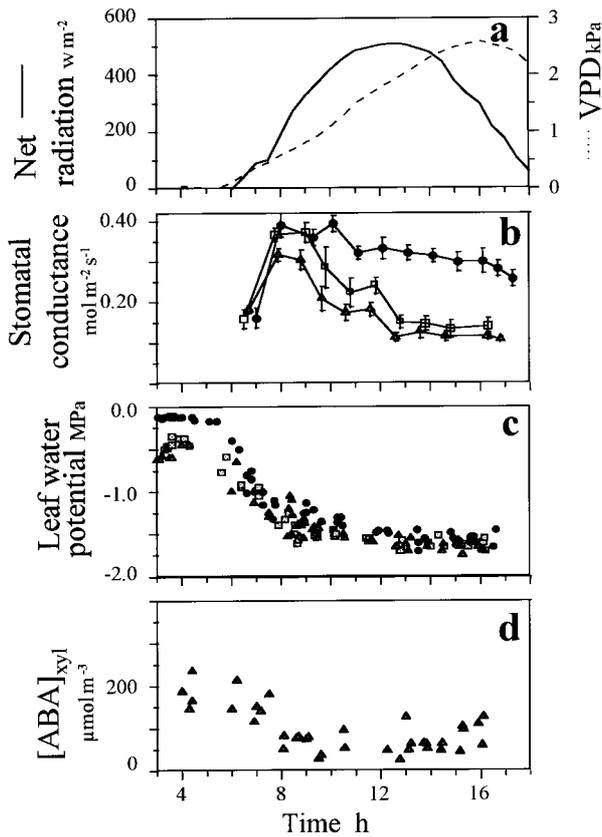


Fig. 2. Change with time in stomatal conductance of field-grown maize (*Zea mays* L, F₁ hybrid LG1) presented together with *PPFD* (plain line), air *VPD* (dotted line), leaf water potential and concentration of ABA in xylem sap collected by pressurization. Redrawn from Tardieu and Davies (1992). Plants were grown in the field and subjected to either full irrigation (●), mild water deficit (□) or mild deficit with soil compaction (▲) at flowering time. Stomatal conductance was measured with a porometer (Delta T). Water potential of the same leaves was measured and sap was collected in the same way as in the case of sunflower. Data in panel (d) correspond to the treatment with water deficit. Lines in panel (b) join average values corresponding to one treatment; each point in panels (c) and (d) corresponds to one plant.

potential to which the roots of the plant are exposed. Leaf water potential at a given time of the day is, therefore, the result of both soil water status, which determines the baseline ψ_1 in the absence of evaporative demand, and of the transpiration rate linked to evaporative demand. These changes in ψ_1 can be synthesized (Eq. 1) by combining classical equations of water flux (Eqs 4, 6; Fig. 4) and the equation relating stomatal conductance (g_s) to leaf-to-air vapour pressure deficit, VPD_{la} .

$$\psi_1 = \psi_{soil} - (R_{soil} + R_{plant}) \left(\frac{g_a \times g_s}{g_a + g_s} \right) \frac{M_w}{\mathcal{R}T_a} VPD_{la} \quad (1)$$

where R_{soil} and R_{plant} are the hydraulic resistances from bulk soil to roots and from roots to shoot, respectively, g_a and g_s are the boundary layer and stomatal conductances, M_w is the molar weight of water, and \mathcal{R} 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 ψ_1 with time. In the absence of evaporative demand, ψ_1 is only dependent on ψ_{soil} and declines as soil water is depleted. When plants transpire, ψ_1 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 wilted 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_{la} . However, the behaviour of ψ_1 of droughted plants markedly differs among species, with a large difference in midday ψ_1 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 ‘aniso-hydric’ and ‘isohydric’, respectively, following Stocker, 1956) is linked to stomatal control.

Isohydric and aniso-hydric behaviours

A typical aniso-hydric 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 ψ_1) under high *PPFD*. Change with time in ψ_1 with evaporative demand was similar in the three studied treatments, so differences in ψ_1 among treatments remained approximately constant over the day (difference between midday ψ_1 similar to difference between predawn ψ_1 in Fig. 1c). The concentration of ABA in xylem sap ($[ABA]_{xyl}$) increased as predawn ψ_1 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 ψ_1 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 ψ_1 against changes in evaporative demand and ψ_{soil} , as day–night alternations in ψ_1 were similar in well-watered and in droughted plants.

A different, isohydric, behaviour was observed in maize and poplar plants (Figs 2, 3). ψ_1 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 ψ_1 was sequentially measured on the same plants. ψ_1 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

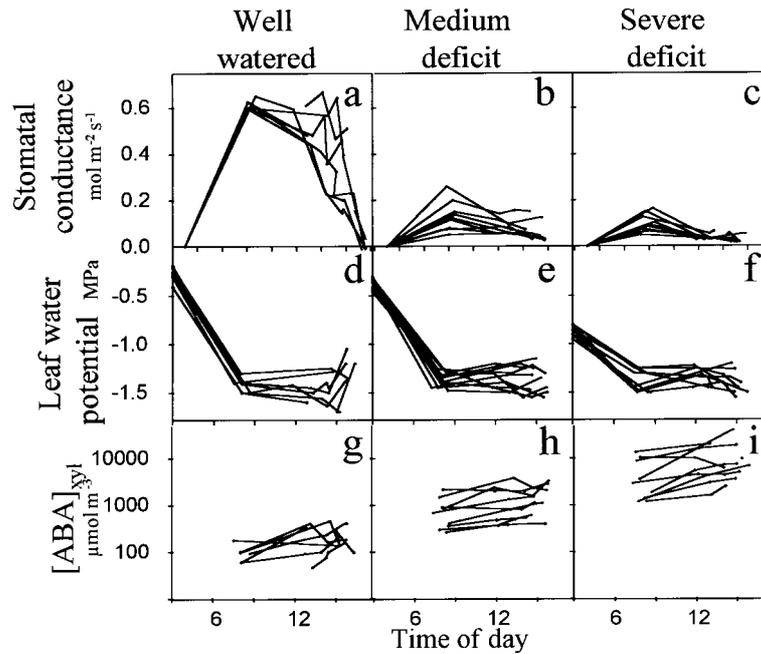


Fig. 3. Change with time in stomatal conductance of poplar plants (*Populus euramericana* cv. I-214) grown in the greenhouse during sunny days, presented with leaf water potential and concentration of ABA in xylem sap collected by pressurization (Tardieu and Trejo, unpublished data). Plants were grown 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 $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ 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 $[\text{ABA}]_{\text{xyl}}$, but the typical asymmetric pattern with a lower g_s in the afternoon than in the morning was not linked to a diurnal change in $[\text{ABA}]_{\text{xyl}}$. In both species, stomatal control therefore allowed ψ_1 to remain constant during the afternoon by progressively closing stomata, thereby balancing the increase in evaporative demand (Eq. 1). It also allowed ψ_1 to be similar in droughted and in 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, C_3 versus C_4 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 g_a/g_s , transpiration largely depends on stomatal conductance while the opposite occurs in ‘uncoupled’ canopies with low g_a/g_s . As ψ_1 depends on transpiration rate and not solely on g_s (Eq. 4; Fig. 4) one could expect that ψ_1 could be largely independent of g_s 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 g_s . When stomata close, leaf temperature can increase by several degrees, thereby increasing VPD_{la} by up to 1 or 2 kPa (Ben Haj Salah and Tardieu, 1997). VPD_{la} 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

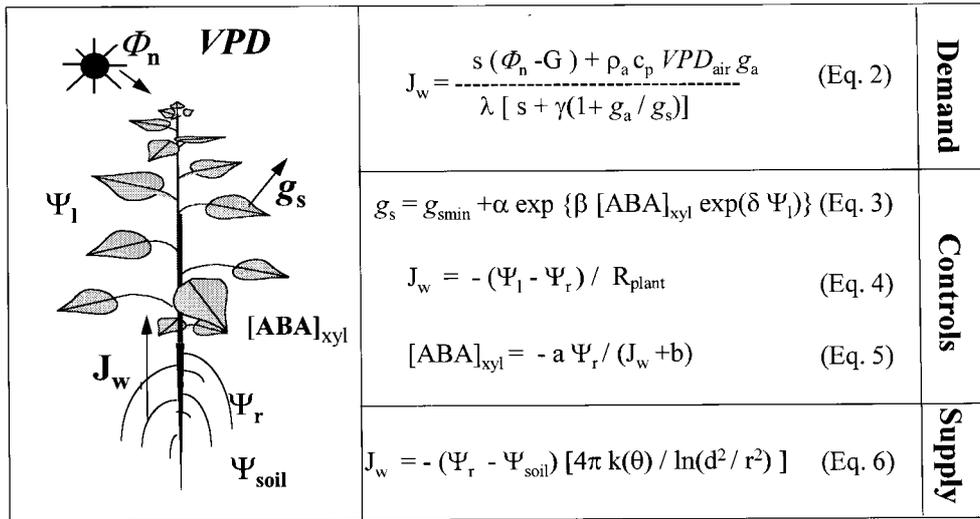


Fig. 4. Diagrammatic illustration of a model of stomatal control by ABA, environmental conditions and water potential (from Tardieu, 1993). Equation 3 applies when $PPFD$ is higher than $800 \mu\text{mol m}^{-2} \text{s}^{-1}$. Otherwise, g_s is set at the minimum between that predicted by the model and that predicted by the response to $PPFD$. Arrows symbolize transfers of water and/or ABA. Input variables: Φ_n (net radiation), VPD (air pressure vapour deficit) and ψ_{soil} (soil water potential). State variables are g_s (stomatal conductance), ψ_l and ψ_r (root and leaf water potentials), J_w (water flux) and $[ABA]_{xyl}$ (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

Species	Growing conditions	Behaviour	References
Lupin	Pot	Isohydric	Henson <i>et al.</i> , 1989
Maize	Field, pot	Isohydric	Tardieu <i>et al.</i> , 1993 Ben Haj Salah and Tardieu, 1997
Pea	Field	Isohydric	Bates and Hall, 1981
Poplar	Pot	Isohydric	This study
Sugarcane	Pot	Isohydric	Saliendra and Meinzer, 1989
Almond tree	Field	Anisohydric	Wartinger <i>et al.</i> , 1990
Barley	Pot	Anisohydric	Borel <i>et al.</i> , 1997
Peach tree	Field	Anisohydric	Xiloyannis <i>et al.</i> , 1980 Steinberg <i>et al.</i> , 1989
Soybean	Pot	Anisohydric	Allen <i>et al.</i> , 1994
Subterranean clover	Pot	Anisohydric	Socias <i>et al.</i> , 1997
Sunflower	Field, pot	Anisohydric	Tardieu <i>et al.</i> , 1996
Wheat	Pot	Anisohydric	Henson <i>et al.</i> , 1989

three equations, the Penman–Monteith equation (Eq. 2; Fig. 4) and the Van den Honert (1948) water flux equations (Eqs 4, 6).

R_{soil} increases by several orders of magnitude as soil dries, depending on the unsaturated hydraulic conductivity of the studied soil ($K(\theta)$ if θ is soil water content). As a consequence, soil near the roots becomes less and less permeable as water flux increases and as ψ_{soil} decreases, thereby tending to increase the gradient of ψ_{soil} around roots (Dunham and Nye, 1973, Lafolie *et al.*, 1991). ψ_l therefore becomes more sensitive to VPD_{la} than predicted by Eq. 1 as evaporative demand increases. This effect is greatest in soils with low unsaturated hydraulic conductivity, such as potting composts, sand or vermiculite (Jones and Tardieu, 1998). R_{soil} also depends on the distance that water must travel in the soil in order to reach the nearest root. It is then a function of both

hydraulic conductivity of the considered soil at current water potential and root density (Gardner, 1960).

$$R_{soil} = [\ln(d^2/r^2)]/4\pi K(\theta) \quad (7)$$

if d is half the mean distance between neighbouring roots and r is mean root diameter. This equation provides acceptable estimates of R_{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 ψ_{soil} , net radiation and air vapour pressure deficit (VPD_{air}) as input variables, and consists of five equations with five unknowns (ψ_1 , ψ_{root} , $[ABA]_{\text{xyl}}$, g_s , J_w). It applies to periods with high $PPFD$ corresponding to maximum g_s . At lower $PPFD$ s, g_s is set at 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_{air} (Bunce, 1985; Grantz *et al.*, 1987; Aphalo and Jarvis, 1991; Dai *et al.*, 1992; Franks *et al.*, 1997). In these conditions, a reduction in g_s is observed at each step. Recently, Monteith (1995) showed that much of the effects of changing VPD_{air} could be accounted for by changes in transpiration rate (and not directly by changes in VPD), and that the feedforward effect with the decrease in transpiration rate was exceptional. This was consistent with Mott and Parkhurst's (1991) experiments comparing stomatal responses to VPD in air and helox (mixture of oxygen and helium, with higher gas diffusivity than air). In this view, stomatal response to evaporative demand should be considered as a feedback process. However, Bunce (1996) showed that stomatal response of soybean to VPD_{air} was suppressed at low CO_2 concentrations, although this treatment increased transpiration. Response to VPD could be attributed neither to whole plant transpiration nor to ψ_1 , as the same response to VPD was obtained when ψ_1 of the studied leaf was varied by changing the transpiration rate of the other leaves of the plant.

A lack of response to evaporative demand in well-watered plants grown in the field?

An additional difficulty arises from a comparison of the behaviours of plants subjected either to steps in VPD_{air} in laboratory conditions or to natural variations of VPD_{air} in the field. A 'midday depression' of g_s is traditionally described in the field and attributed to high VPD_{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_{air} (Figs 5, 6, 7). Sequences with nearly constant g_s during the afternoon in spite of high VPD_{air} have also been described in kiwifruit (Gucci *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_{air} nor to decreases in ψ_1 . In contrast, Grantz *et al.* (1987) concluded that a stomatal response to VPD_{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 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The difference in g_s between the abnormally high value expected at 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and the observed value was attributed to the effect of VPD .

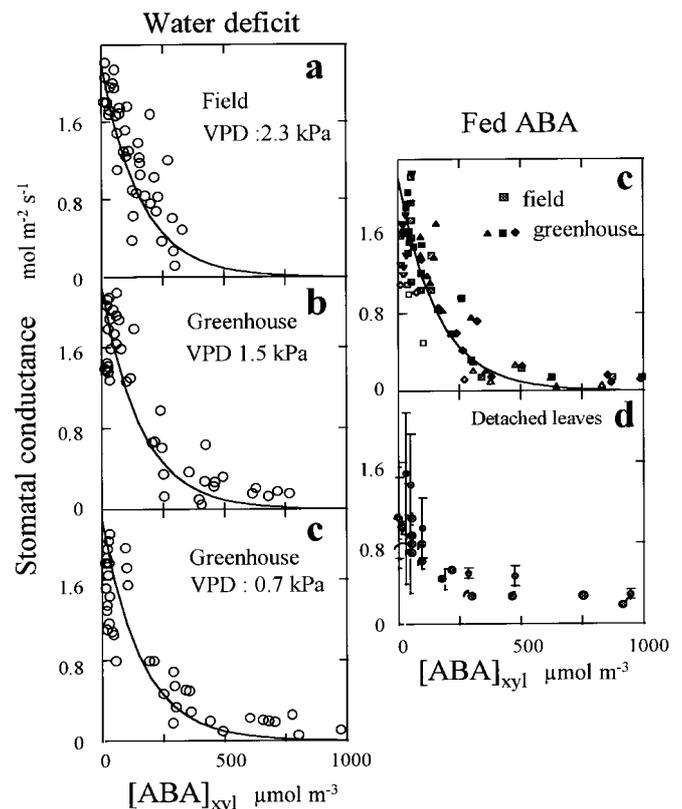


Fig. 5. Relationship between stomatal conductance and concentration of ABA in the xylem sap of sunflower plants subjected to contrasting soil water status and evaporative demand (a–c) or fed with artificial ABA (d) as explained in Tardieu *et al.* (1996). (e) Detached leaves were fed with artificial sap with contrasting concentrations of ABA. Redrawn from Tardieu *et al.* (1996).

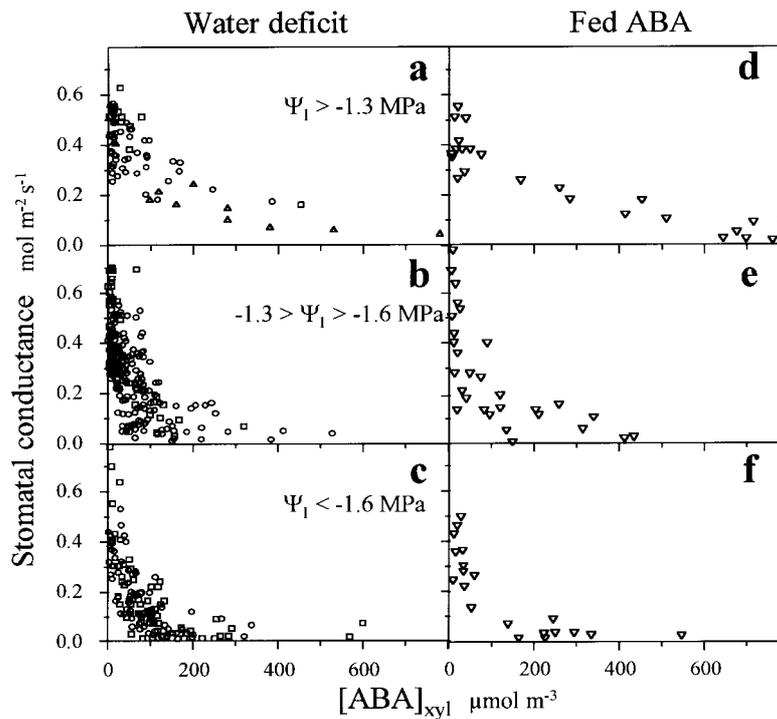


Fig. 6. Relationship between stomatal conductance and concentration of ABA in the xylem sap of maize plants subjected to contrasting soil water status and evaporative demand (a–c) or fed with artificial ABA (d–f). Experimental procedures and method for supplying ABA are described in in Tardieu *et al.* (1993). Data from Tardieu *et al.* (1992, 1993) and Ben Haj Salah and Tardieu (1997). (a, d) Experiments in which ψ_1 was higher than -1.3 kPa as a result of low VPD : (a) morning in the field in 1990 (\circ) or 1991 (\square) or afternoon in a greenhouse with low VPD (\blacktriangle); (d) ABA feeding of well-irrigated plants (\blacktriangledown). (b, e) Experiments in which ψ_1 ranged between -1.3 and -1.6 kPa in the field in 1990 (\circ) or 1991 (\square). (e) ABA feeding of plants subjected to mild soil water deficit (\blacktriangledown). (c, f) Experiments in which ψ_1 was lower than -1.6 kPa in the field in 1990 (\circ) or 1991 (\square). (f) ABA feeding of plants subjected to water deficit (\blacktriangledown).

The possibility is therefore raised that an effect of VPD_{air} is only observed if plants are subjected to a mild soil water deficit, consistent with the observation of Saliendra *et al.* (1995) that the effect of VPD_{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.

(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 water deficit, thereby reproducing the classical ‘midday depression’ when $[ABA]_{\text{xyl}}$ differed from 0. This would be consistent with the conclusion of Bunce (1996) who attributed to ABA the stomatal response to VPD_{air} .

(2) A depression of g_s by VPD_{air} even at high ψ_{soil} was predicted in soils with low unsaturated hydraulic conductivity. An increase in evaporative demand greatly reduces ψ_{soil} in the vicinity of roots (Eq. 7) even if bulk ψ_{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_{air} observed in laboratory experiments where plants are grown in soils (potting compost or vermiculite) with low unsaturated conductivity. The depressing effect of VPD_{air} was predicted by the model in such soils, but not in field soils. This point is currently under investigation in our laboratory (T. Simonneau, unpublished data).

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 ψ_{root} in numerous species and that root-sourced ABA contributes to this control (Henson *et al.* (1989) in lupin and wheat; Wartinger *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

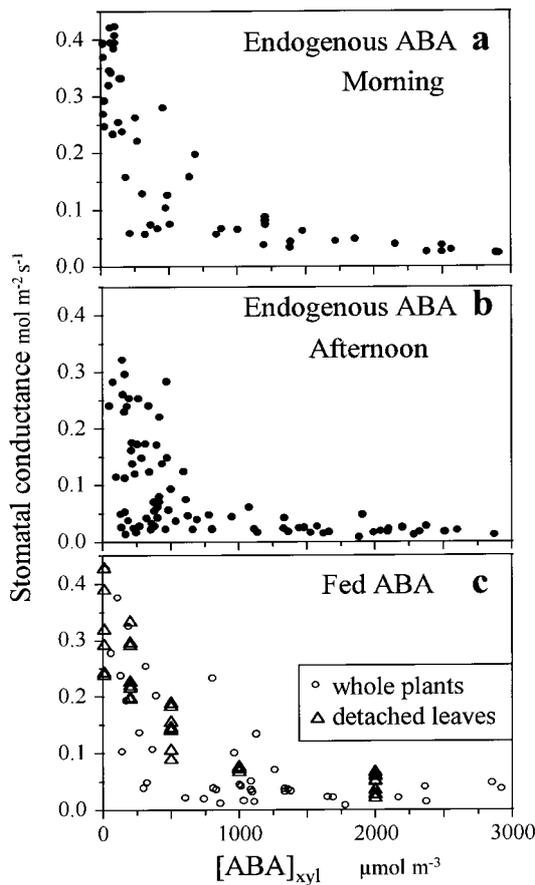


Fig. 7. Relationship between stomatal conductance and concentration of ABA in the xylem sap of poplar plants subjected to contrasting soil water status and evaporative demand (a, b) or fed with artificial ABA (c, ○). (c) Detached leaves were fed with artificial sap with contrasting concentrations of ABA (▲) (Tardieu and Trejo, unpublished data).

out on sunflower, maize and poplar are presented in Figs 5, 6 and 7. The response of g_s to $[ABA]_{xy1}$ was analysed in a series of experiments varying evaporative demand, soil water status and ABA origin (endogenous or artificial). Plants were subjected to a range of soil water potentials under contrasting air vapour pressure deficits (VPD_{air} , from 0.5 to 2.5 kPa) in the field, in the greenhouse or in a growth chamber. They were also fed through the xylem with varying concentrations of artificial ABA, in the greenhouse and in the field. Finally, detached leaves were fed directly with varying concentrations of ABA under three contrasting $VPDs_{air}$.

Anisohydric behaviour (sunflower, wheat and barley): a single relationship between $[ABA]_{xy1}$ and g_s regardless of evaporative demand

In sunflower (Fig. 5), a unique relationship between $[ABA]_{xy1}$ and g_s was observed at evaporative demands ranging from 0.5 to 3.2 kPa. The same relationship applied to plants subjected either to soil water deficit or to ABA feeding. It was slightly (but non-significantly)

different in experiments with detached leaves, in which stomatal conductance was calculated from transpiration rate and VPD_{la} 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 $[ABA]_{xy1}$ 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_{air} . This leads to two conclusions. (i) As endogenous and fed ABA had quantitatively similar consequences on g_s , $[ABA]_{xy1}$ 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 $[ABA]_{xy1}$ were similar at markedly different transpiration rates. It was also independent of ψ_1 .

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 $[ABA]_{xy1}$ 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 $[ABA]_{xy1}$ which depends on leaf water status or on transpiration rate

In maize (Fig. 6), the response curve of g_s to $[ABA]_{xy1}$ markedly differed in the morning and in the afternoon, except in afternoons with low VPD_{air} . Plants fed with ABA had usually a higher ψ_1 than droughted plants, due to partial equilibration of ψ_1 with ψ_{soil} when stomata closed. In each range of ψ_1 , the relationship between $[ABA]_{xy1}$ and g_s did not differ whether changes in $[ABA]_{xy1}$ were due to soil dehydration or to ABA feeding. Detached leaves fed with ABA solutions had a ψ_1 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 ψ_1 is likely to affect stomatal control in the cases of maize and poplar, with an increasing apparent stomatal sensitivity to $[ABA]_{xy1}$ as transpiration rate increased or as ψ_1 decreased (Tardieu and Davies, 1992). Experiments involving ABA feeding show that VPD_{la} could not act *per se* on stomatal sensitivity to $[ABA]_{xy1}$. Feeding ABA closed stomata, thereby increasing leaf temperature and VPD_{la} , but also caused a drop of transpiration rate and a large increase in ψ_1 . The resulting apparent stomatal sensitivity

was low, as in plants with low transpiration rate due to low VPD_{la} . Comparison of g_s of ABA-fed plants growing in wet and dry soils (low transpiration rate in both cases, but with contrasting ψ_1) suggests that ψ_1 could account for changes in stomatal sensitivity to $[ABA]_{\text{xyl}}$. ψ_1 was consequently chosen as a controlling variable in Eq. 3 (Fig. 4) instead of transpiration rate.

Differences in behaviours and interaction between $[ABA]_{\text{xyl}}$ and leaf water status

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

$$g_s = g_{\text{smin}} + a \exp(\beta[ABA]_{\text{xyl}}) \quad (8)$$

where g_{smin} and $g_{\text{smin}} + a$ are the minimum and maximum stomatal conductances of the considered cultivar, respectively, and β (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 $[ABA]_{\text{xyl}}$ which depended either on leaf water status or on transpiration rate. This was translated in an equation in which g_s depended on $[ABA]_{\text{xyl}}$, but with a sensitivity which depended on ψ_1 with a second negative exponential function with parameter δ (negative value):

$$g_s = g_{\text{smin}} + a \exp\{\beta[ABA]_{\text{xyl}} \exp(\delta\psi_1)\} \quad (3)$$

Changing apparent sensitivity of g_s to $[ABA]_{\text{xyl}}$ with ψ_1 provides the plant with a way of controlling ψ_1 much more tightly than in the anisohydric case. Whenever ψ_1 undergoes a small decrease, increased sensitivity to $[ABA]_{\text{xyl}}$ causes a slight stomatal closure for an unchanged $[ABA]_{\text{xyl}}$. This reduces the water flux through the plant and, therefore, causes an increase in ψ_1 . Isohydric behaviour could be simulated with the model presented in Fig. 4 when the interactive term (δ in Eq. 3) was set to a non-zero value (Tardieu, 1993). When δ was set to 0, the model simulated that ψ_1 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 ψ_1 and g_s without assuming any controlling effect of ψ_1 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 $[ABA]_{\text{xyl}}$ 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 $[ABA]_{\text{xyl}}$ 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_{air} 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 $[ABA]_{\text{xyl}}$ 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 $[ABA]_{\text{xyl}}$ 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 $[ABA]_{xy1}$ and ψ_1 (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 ψ_1 or on evaporative demand.

Progress in knowledge since the model of Fig. 4 was published therefore questions the meanings of $[ABA]_{xy1}$ and of the interactive term of Eq. 3. However, $[ABA]_{xy1}$ 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 (Tardieu, 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 ψ_1) and $[ABA]_{xy1}$ in maize, sunflower and poplar (Fig. 8) as well

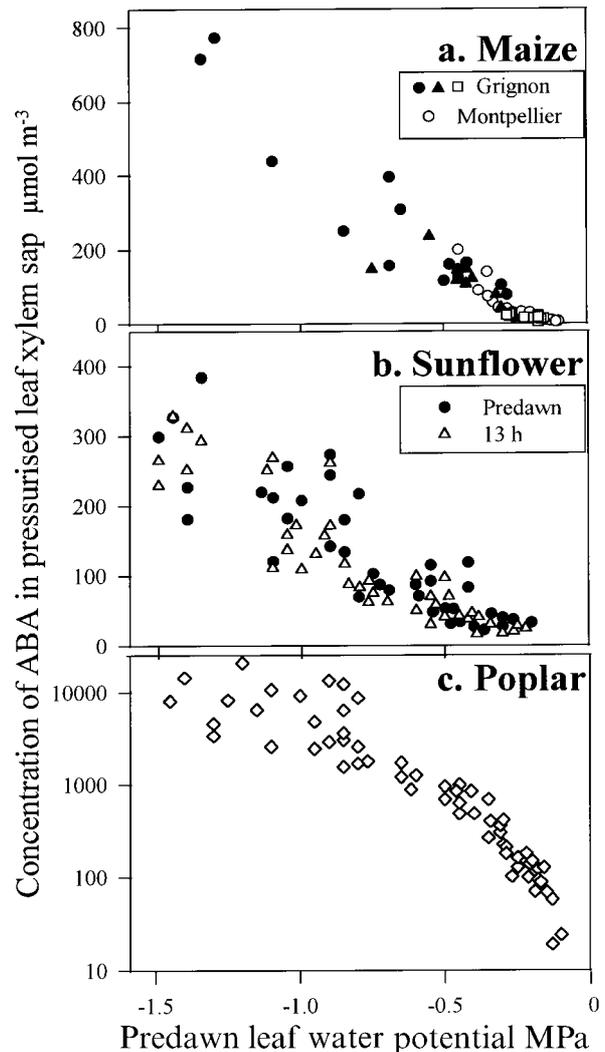


Fig. 8. Concentration of abscisic acid in xylem sap collected by leaf pressurization, as a function of predawn leaf water potential in sunflower, maize and poplar. Note that ranges of y -axes differ among panels and that the scale in (c) is logarithmic. (a) Field-grown maize, predawn values. (●,▲,□) Samples collected in northern France in 1990 and 1992. Change with time of $[ABA]_{xy1}$ in treatments with water deficit, water deficit plus soil compaction and irrigation, respectively (Tardieu *et al.* (1992) and Tardieu, unpublished data). (○) Samples collected in southern France with mild water deficit from 1993 to 1995 (Ben Haj Salah and Tardieu, unpublished data). (b) Sunflower, samples collected either before dawn or at 13 (solar) time. Redrawn from Tardieu *et al.*, 1996. (c) Poplar grown in pots in the greenhouse (see Fig. 3), morning values (Tardieu and Trejo, unpublished data).

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 ψ_1 corresponded to markedly different $[ABA]_{xy1}$ in the three species presented in Fig. 8, but species such as poplar with steepest response of $[ABA]_{xy1}$ to predawn ψ_1 had a less sensitive response of g_s to $[ABA]_{xy1}$. This resulted in relatively similar values of g_s at a given predawn ψ_1 among species.

In most of the above-mentioned studies, $[ABA]_{xy1}$ 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 $[ABA]_{xy1}$ measured at two times of the day had a common relationship with predawn ψ_1 . However, near constancy of $[ABA]_{xy1}$ 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 $[ABA]_{xy1}$ measured at the end of the day corresponded to predawn ψ_1 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 $[ABA]_{xy1}$ with root water status and time of day. Stability of $[ABA]_{xy1}$ 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 $[ABA]_{xy1}$ was viewed as reflecting root water status. During the day, ψ_{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 $[ABA]_{xy1}$, 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. $[ABA]_{xy1}$ 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 $[ABA]_{xy1}$ is largely independent of rapid changes in evaporative demand during the day and between days, consistent with experimental data. $[ABA]_{xy1}$ 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 $[ABA]_{xy1}$ during the day when R_{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 in $[ABA]_{xy1}$, when ψ_{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 $[ABA]_{xy1}$ 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 $[ABA]_{xy1}$. This framework could be considered as sound as the effect on g_s of endogenous change in $[ABA]_{xy1}$ 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 $[ABA]_{xy1}$ to soil water status or of g_s to $[ABA]_{xy1}$, but essentially on the combination of these two response curves. For instance, poplar plants exhibit much higher $[ABA]_{xy1}$ than maize in response to a decrease in soil water potential, but stomatal response to $[ABA]_{xy1}$ is less sensitive, resulting in similar stomatal behaviours during a sequence of soil water depletion. One of the most determinant parameters is the term δ which holds for the interaction between $[ABA]_{xy1}$ and leaf water status. If it is set to a non-zero value, it generates an isohydric behaviour with maintenance of day-time ψ_1 when soil

water status varies, and an absence of relationship between g_s and ψ_1 . In contrast, it generates an anisohydric behaviour with correlation between g_s and ψ_1 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_s 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 VPD_{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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