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Stomatal conductance and xylem-sap abscisic acid (ABA) in adult oak trees during a gradually imposed drought

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Summary — Thirty- to 40-year-old oak trees (Quercus petraea Matt Liebl and Q robur L) growing in a forest stand near Champenoux (Nancy, France) were submitted to an imposed drought in a lysimeter during two successive summers. Xylem sap was extracted from leafy twigs of two trees per species at regular intervals during the onset of drought, and of two controls in parallel. Predawn leaf water potential, soil water potential at different depths and midday stomatal conductance of sun-exposed leaves were recorded at the same pace. Concentrations of abscisic acid (ABA) and of the glucose ester of ABA (ABA-GE) were measured with an ELISA technique, after purification of the sap samples using high performance liquid chromatography (HPLC). Mean concentrations of ABA recorded in the absence of drought constraint were around 30 μmol m−3, independently from year and species, and 45 μmol m−3 for ABA-GE. No significant drought-related increase in either of these concentrations could be recorded while predawn leaf water potential dropped below −1.5 MPa, and stomatal conductance down to 10% of the values of controls. A good correlation between stomatal conductance and predawn leaf water potential was detected, and even a better one between stomatal conductance and soil water potential at 25 cm. It may be concluded from these results that, even if a close correlation exists between stomatal aperture and soil moisture, the existence of a root signalling process based solely on increased ABA.

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Abbreviations: ABA: abscisic acid; ABA-GE: glucose ester of abscisic acid; MeABA: methyl ester of ABA; ABAxy and ABA-GExyl: concentrations of, respectively, ABA and ABA-GE in the xylem sap (μmol m−3); Qext: volume of xylem sap extracted from the twigs (μL); BSA: bovine serum albumin; ELISA: enzyme-linked immunosorbent assay; gs: stomatal conductance (mmol m−2 s−1); Ψwp: predawn leaf water potential (MPa).
or ABA-GE transport in the xylem sap may be questioned. Stomatal conductance under natural conditions is probably controlled by a rather complex chain of processes which has still to be elucidated.

ABA / ABA-GE / drought / stomatal conductance / water stress / xylem sap / Quercus robur / Quercus petraea

INTRODUCTION

Oak trees frequently suffer from drought-induced dieback (Landmann et al, 1993). Intensive studies on water relations of 30-year-old oak trees in a stand near Nancy, France, have shown that the two European species (Quercus robur L and Q. petraea Matt Liebl) were rather drought tolerant, due to a deep and efficient rooting system (Bréda et al, 1995b), associated with a low sensitivity to cavitation in the xylem conduits (Cochard et al, 1992), and the maintenance of substantial transpiration rates and open stomata till rather low soil moisture (Bréda et al, 1993a,b; Epron and Dreyer, 1993). The question therefore arose of the factors controlling stomatal opening in these trees, and whether root-issued abscisic acid (ABA) was involved in this control.

Much accumulated evidence suggests that stomatal closure during drought may be triggered by a nonhydraulic signal originating from the roots exposed to drying soils (see reviews by Davies and Zhang, 1991; and Davies et al, 1994). ABA is the most frequently suggested chemical effector thought to be released from drying roots, and transported via the transpiration stream in the xylem to the apoplastic spaces in the leaves where it interacts with the guard cells and induces stomatal closure (Davies et al, 1994). Actually, ABA concentrations in xylem and in apoplastic sap have been shown to increase with soil water depletion.
in many plant species including trees (Scuiller, 1990; Wartinger et al, 1990; Hartung and Slovik, 1991; Khalil and Grace, 1993), and to correlate rather well with stomatal conductance in many cases (Tardieu and Davies, 1992; Khalil and Grace, 1993; Correia and Pereira, 1994). Nevertheless, the relationship was very diverse, leading to the assumption that the stomatal sensitivity to xylem ABA could vary among species, and in a given species in relation to other physiological parameters, such as leaf water status (Tardieu and Davies, 1992) or chemical composition of the xylem sap (Schurr et al, 1992).

Despite this evidence, there is still some discussion about the quantitative implication of root-issued ABA in the control of stomatal conductance. In particular, data obtained on adult trees, where a long distance transport of root-issued ABA is required, are still scarce (Wartinger et al, 1990). Moreover, most ABA data have been obtained from unpurified sap samples with an ELISA test, which does not accurately detect combined ABA such as the glucose ester (ABA-GE) that might play an important role as a transport form. To test if drought promoted significant increases in the concentrations of both forms of ABA in relation to stomatal closure, we collected xylem sap during two successive summers (1992 and 1993) on oak trees in an experimental field plot submitted to a gradual soil water depletion, and quantitated ABA using high performance liquid chromatography (HPLC) purification and ELISA detection (Label et al, 1994). It has often been argued that root signalling could allow an adequate stomatal response to the desiccation of the upper soil levels where rooting is dense while deeper layers remain humid (Davies and Zhang, 1991). To verify this hypothesis, we tested for correlations between soil water potential at several depths in the soil and stomatal conductance of sun-exposed leaves.

**MATERIALS AND METHODS**

**Experimental design**

The experiment was conducted during the summers of 1992 and 1993 in an oak forest at Champenoux near Nancy, northeastern France, on 30-year-old and approximatively 18 m high trees. Four dominant trees (two Quercus robur and two Quercus petraea) were selected in a group of 17 trees included in a water-stressed plot. An artificial drought was applied: rain and throughfall were prevented by a plastic roof built under the canopy and lateral drainage was removed in 1 m deep trenches. The roof was installed before bud-break, at the end of February 1992, and trees depleted gradually the extractable soil water. During 1992, a heavy thunderstorm promoted an uncontrolled rewetting on d266. Four other trees were chosen as controls. They were watered manually during the measurement period in 1992, and experienced natural water balance during 1993. During 1993, sap collection was restricted to two stressed and two control trees of Q petraea. For additional details about the experimental design, see Bréda et al (1993a). Climate was recorded with a weather station installed above the canopy, yielding global radiation, air temperature, wind speed and vapour pressure deficits at a half-hourly pace. Stomatal conductance (gs) was recorded with a steady-state porometer (LI-1600, LI-Cor, Lincoln, NE, USA) during bright sunny days. Measurements were made between 12 and 14 PM UT on ten sun-exposed leaves of the upper canopy of each tree. Predawn leaf water potential (Ψwp, two replicates per tree) was measured on the same days with a Scholander pressure chamber. Soil water potential at 25, 50, 80, 110 and 140 cm depth was recorded with soil psychrometers (Wescor HR 33T). To avoid artefacts due to uncontrolled soil rehydration, we restricted the analysis of the relationships between soil water and stomatal conductance to trees grown in the lysimeter, and to the period of onset of drought (Q petraea and Q robur, 1992 and 1993).

**Extraction of xylem sap**

Immediately after the measurements of stomatal conductance, one extraction was made per tree (two per treatment). A 20 to 30 cm long twig with
four to ten leaves was cut off the upper crown and rapidly enclosed into a Scholander pressure chamber. The bark was removed from the distal end to avoid pollution of xylem sap by ABA exuding from phloem tissues (Else et al., 1994). Fifty to 400 μL (measured by differential weighing of the vials before and after collection) were sampled by pressurising the twigs at about 0.5 MPa above the balancing pressure. The sap was absorbed directly from exuding vessels with a micropipette; sap droplets were absorbed as soon as they appeared to minimize evaporation. The samples were immediately deep-frozen in liquid nitrogen and stored at -20 °C for later analysis.

**Purification of xylem sap**

Tritiated ABA (2.26 Bq mol⁻¹, Amersham) was added as an internal standard after sample thawing. The sap was filtered through a 0.22 mm filter and purified with reverse phase C18 SepPack cartridges before injection onto an HPLC column (Lichrospher 100 RP18, 250 x 5 mm, 5 mm, Merck during 1992, and a Lichrospher 100-5C18, 250 x 8 mm, 4.6 μm, during 1993). This solid phase was eluted with an H₂O-acetonitrile gradient, that allowed separation of ABA-GE from ABA. Fractions (0.15 min each) were collected between 8.75 and 10.25 min, which interval included retention times of ABA and ABA-GE. The ten fractions (160 μL each) were evaporated to dryness and methylated with diazomethane dissolved in diethylether. After the methylation step, fractions were evaporated to dryness a second time and redissolved in 1 mL of an aqueous phase with 200 mg/L NaN₃. The incubation before immunoassay was longer than 12 h.

The radioactivity was determined in an aliquot of each sample with a liquid scintillation counter (Beckman LS 1801). Recovery was in all cases higher than 60%. For each sample the recovery efficiencies for ABA-GE and ABA were considered to be identical and used to correct the measured ABA and ABA-GE levels.

**Measurement of Me-ABA and ABA-GE**

Quantification of methylated ABA (Me-ABA) and of ABA-GE was established with an enzyme-linked immunosorbent assay (ELISA). Plate wells were coated with 200 μL of an Ovalbumin complex in each well, and plates were incubated overnight at room temperature. The emptied plates were washed four times with 400 μL of a washing solution (Photoflo 0.1%). Fifty μL of a saturated PBS-gelatine buffer were added to each well followed by 100 μL of either Me-ABA standard or sample. Four standard ranges were set on each plate. The first column was used to determine maximal colouration (absence of Me-ABA) and the last one to determine nonspecific colouration (no Me-ABA nor anti-ABA antibody). Two replicates of each sample were used. Then, 50 μL of the rabbit anti-ABA IgG were added to each well except those of the last column. The dilution of the antibody corresponded to 50% of maximum binding to wells. The plates were stirred for 1 min, capped and stored at +4 °C for 2 h. After the competition step, plates were washed again and each well was filled with 200 μL of the biotinylated goat antirabbit IgG. After 1 min gentle stirring, the plates were incubated 1 h at +40 °C. Wells were washed another time and 200 μL of the streptavidine-alkaline-phosphatase complex added. The plates were stirred, and incubation carried out at +40 °C for 1 h. They were washed again and 200 mL of p-nitrophenyl phosphate added. The reaction proceeded at +40 °C during 1–2 h until maximal absorbance reached 1 AU. The reading of plates was taken at 405 and 610 nm with a double wavelength spectrophotometer (Dynatech MR 5000). The relative absorbance (B₮ₐ₀/B₮ₓₓₓₓ) was plotted against the log of Me-ABA concentration. The quantity of Me-ABA in samples was determined on the basis of calibration curves for each plate. This technique had been validated by Label et al. (1994) with poplars.

Purification of xylem sap separated ABA from ABA-GE. The rabbit anti-ABA IgG shows cross-reactivity with ABA-GE, which concentration was therefore determined with the same procedure, except that calibration curves used ABA-GE. ABA-GE measurements were made only for *Q. petraea*.

**RESULTS**

**Leaf water potential and stomatal conductance**

Drought induced an important decline of predawn leaf water potential (Ψₚₚ) during
Fig 1. Seasonal time course of (a, b) predawn leaf water potential \( (\psi_{wp}) \), (c and d) stomatal conductance \( (g_s) \), (e and f) ABA content in the xylem sap \( (\text{ABA}_{xy}) \), and (g) ABA-GE content in the xylem sap \( (\text{ABA-GE}_{xy}) \) as recorded on adult oak trees submitted to an imposed drought during summer 1992. Closed symbols: water-stressed trees; open symbols controls. Two trees were used in each species (Quercus robur and Quercus petraea) and treatment. Mean ± standard deviation.
1992. $\Psi_{wp}$ was lower in stressed trees than in controls during the period between d210 and d270 (fig 1a, b). It dropped below $-1.5$ and $-2$ MPa in Q robur and Q petraea, respectively, and remained above $-0.8$ MPa in controls. At the end of the summer, rewatering due to heavy thunderstorms occurred in the dry plot (d270) and $\Psi_{wp}$ of stressed trees recovered levels of controls. During the second year, a wet spring followed by a dry summer resulted in parallel decreases of $\Psi_{wp}$ in both treated and controls down to $-1.5$ MPa (fig 2a). Stomatal conductance ($g_s$) of control trees stabilised at 250 mmol m$^{-2}$ s$^{-1}$ in both species during 1992, after a spring peak value which was related to leaf maturation (fig 1c, d). During autumn, it decreased to 100 mmol m$^{-2}$ s$^{-1}$. Drought induced stomatal closure, with low conductances (values below 50 mmol m$^{-2}$ s$^{-1}$).

During 1993, both stressed and control trees displayed a similar time course of $g_s$ with a low and gradual decrease to 100 mmol m$^{-2}$ s$^{-1}$ (fig 2b). The relationship between drought intensity (expressed by $\Psi_{wp}$) and $g_s$ is shown in figure 5 (a, b). It was not linear, as has been frequently observed in oaks (Bréda et al, 1993a; Epron and Dreyer, 1993): a steep decline of $g_s$ at high $\Psi_{wp}$ was followed by a much slower one at lower $\Psi_{wp}$ (below $-1.0$ MPa). The 1993 data differed in this respect, as they displayed much higher values of $g_s$ at $\Psi_{wp}$, around $-1$ to $-1.5$ MPa. This was probably an effect of the lower vapour pressure deficits during these measurements (around 14.5 versus 21.5 Pa kPa$^{-1}$ during 1992), and was also perceptible on whole tree xylem sap flux densities which displayed a 25% decrease in 1993 compared to 1992 on the same trees (Bréda et al, 1995a).

The relationship between $g_s$ and soil water potential at increasing depths was much tighter. A second order polynomial could be fit with soil water potential as exemplified in figure 3. Such a significant regression could be established at all depths, but

![Fig 2. Seasonal time course of (a) predawn leaf water potential ($\Psi_{wp}$), (b) stomatal conductance ($g_s$), (c) ABA content in the xylem sap (ABA$_{xy}$), and (d) ABA-GE content in the xylem sap (ABA-GE$_{xy}$) as recorded on adult trees of Quercus petraea submitted to an imposed drought during summer 1993. Closed symbols: water-stressed trees; open symbols: controls. Two trees were used per treatment. Mean ± standard deviation.](image-url)
the best fit with the highest regression coefficient was obtained with the soil water potential at 25 cm (table I).

**ABA concentration of xylem sap**

The amount of sap extracted from the twigs and its ABA content (ABA$_{xyl}$) displayed a high variability. In order to search for potential artefacts due to the extraction procedure, we checked for correlations between these two variables and the drought intensity (expressed as the predawn leaf water potential, Ψ$_{wp}$). In fact, we found no relation between Ψ$_{wp}$ and the volume of extracted sap (Q$_{ext}$; table II), but a statistically significant one between Q$_{ext}$ and ABA$_{xyl}$ (table II; fig 4), with a decrease of ABA$_{xyl}$ at increasing Q$_{ext}$.

ABA$_{xyl}$ displayed no self-evident seasonal time course in Q petraea or in Q robur regardless of drought treatment or year (fig 1e, f, and fig 2c). No species related differences were detected: ABA$_{xyl}$ was 65.4 ± 14.4 and 53 ± 29.4 μmol m$^{-3}$ in well-watered Q petraea and Q robur during 1992 (n = 5), and 33.2 ± 7.8 in well-watered Q petraea during 1993 (n = 22). The differences between well-watered trees (Ψ$_{wp}$ above -0.6 MPa) and those submitted to the most severe stress (Ψ$_{wp}$ below -1.5 MPa) were not significant (+60%; table III).

The concentration of the conjugate ABA-glucose ester in the xylem sap (ABA-GE$_{xyl}$) were of the same order of magnitude and significantly correlated to ABA$_{xyl}$ (ABA-GE$_{xyl}$ = 0.856 +12.9, r$^2$ = 0.206, n = 41). As for ABA$_{xyl}$, no seasonal trend (figs 1 and 2) and no drought-related increase (table III) could be detected. A 100-fold difference occurred nevertheless between 1992 (152 ± 66 μmol

**Table I.** Regression coefficients between midday stomatal conductance (g$_s$, mean of ten values per tree) recorded in the crown of two Q robur (during 1992) and two Q petraea (during 1992 and 1993), and the soil water potential (Ψ$_s$) recorded in the direct vicinity of each tree with psychrometers at different depths in the soil (g$_s$ = a + bΨ$_s$ + cΨ$_s^2$, correlation coefficient r$^2$).

<table>
<thead>
<tr>
<th>Depth in the soil (cm)</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>r$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>-25</td>
<td>255</td>
<td>164</td>
<td>32.4</td>
<td>0.842</td>
</tr>
<tr>
<td>-50</td>
<td>259</td>
<td>124</td>
<td>14.8</td>
<td>0.800</td>
</tr>
<tr>
<td>-60</td>
<td>240</td>
<td>120</td>
<td>14.4</td>
<td>0.785</td>
</tr>
<tr>
<td>-110</td>
<td>219</td>
<td>81.6</td>
<td>-4.8</td>
<td>0.703</td>
</tr>
<tr>
<td>-140</td>
<td>242</td>
<td>202</td>
<td>53.6</td>
<td>0.660</td>
</tr>
</tbody>
</table>
m⁻³) and 1993 (9.9 ± 4.1 μmol m⁻³) in well-watered trees, for which no convincing explanation could be found.

In order to test for the relationship between drought intensity and both ABAₓyl and ABA-GEₓyl, we related them to Ψ/wp (fig 5 for ABAₓyl). No correlation was found between Ψ/wp and ABAₓyl or ABA-GEₓyl. Table III shows the correlation coefficients obtained: none was significant. As a consequence, the observed decreases in stomatal conductance could not be related to increases in ABAₓyl nor in total (ABA + ABA-GEₓyl); all correlation coefficients were not significant (table III).

DISCUSSION

Many published results indicate a direct relationship between soil moisture and stomatal conductance, independently from leaf water status (Gollan et al, 1986, and review by Davies and Zhang, 1991). This evidence has been obtained with potted plants, either using root pressurisation (Gollan et al, 1992;...
Schurr et al., 1992), or split-root devices (Khalil and Grace, 1993), and was used as an evidence for the occurrence of chemical root-shoot signalling as a control mechanism for stomatal conductance.

It is less easy to demonstrate a direct control of stomata by soil moisture in field grown plants. The relative independence between midday stomatal conductance and midday leaf water potential has been well established in oaks, and a better relationship was demonstrated with predawn leaf water potential ($\Psi_{wp}$, Bréda et al., 1993a, and our results). Nevertheless, this relationship always exhibited a nonlinear shape (Bréda et al., 1993a,b; Epron et al., 1993), with steep decreases of stomatal conductance at high $\Psi_{wp}$, and more gradual ones for lower values. Moreover, the relationship among $\Psi_{wp}$ and $g_s$ exhibited climate-related shifts: during 1993, where vapor pressure deficits in the air were lower than in 1992, $g_s$ was much higher for any given $\Psi_{wp}$.

Bréda et al. (1995b) demonstrated that $\Psi_{wp}$ in the oak trees studied here was close to the soil water potential of the deepest rooted soil layers, and that substantial decreases in water potential could already occur in the upper layers without any noticeable effect on $\Psi_{wp}$, but with already significant stomatal closure. Much closer relationships, with less curvilinearity, could also be established here between $g_s$ and soil water potentials at different depths. The best fit was obtained with values measured at -25 cm, where the rooting density was highest and the water depletion fastest: this may be used as an argument for a control of stomatal conductance by the water availability in the upper soil layers, and not by bulk water reserves. However, care should be taken that in a soil during drought, all layers dry out gradually even if not at the same pace, and that therefore all parameters describing soil water in the different horizons are more or less correlated. Furthermore, diurnal variations in stomatal conductance still occur at any given soil moisture content, with midday closure depending on the actual vapor pressure deficit and other factors: this was demonstrated on the

![Fig 5](image_url). Relationship between predawn leaf water potential and (a,b) stomatal conductance ($g_s$), and (c,d) ABA content in the xylem sap ($ABA_{xy}$) of adult oak trees as recorded during 1992 on Q. petraea and Q. robur, and during 1993 on Q. petraea submitted or not to soil water depletion.
oak trees used here (Epron et al, 1992) as well as for many other species (Tenhunen et al, 1984). Stomatal aperture of trees in the field is far from being exclusively controlled by soil moisture.

An alternative hypothesis to consider is the one underlining the fine tuning of stomatal conductance to leaf water potential in a feedforward regulation aimed at maintaining it close to, but slightly above, the cavitation threshold (Jones and Sutherland, 1991). Bréda et al (1993a) and Cochard et al (1996) showed that stomata of the tested oak trees were almost completely closed when embolism began to develop, and that a good relationship could be found between stomatal control of transpiration and the total, soil-to-leaf hydraulic conductance of oak trees. The physiological mechanisms relating hydraulic conductance and stomatal conductance are still a matter of discussion, and it may also be stated that stomatal control and vulnerability to cavitation could be co-evolved traits.

The ability of ABA to induce stomatal closure is generally accepted (eg, Harris and Outlaw, 1991, and reviews by Hartung and Slovik, 1991 and Davies et al, 1994). ABA receptors are thought to be localized at the outer membrane of guard cells, but they remain to be precisely identified (Anderson et al, 1994). It is also clear that drought induces a remobilisation of existing ABA pools transported to guard cells, rather than a de novo synthesis, which occurs only during strong stress when stomata are already closed (Hartung and Slovik, 1991). The origin of this ABA is still a matter of debate. A model based on the known physicochemical properties of ABA (pKa, permeability, turnover rate, etc) developed by Hartung and Slovik (1991) showed that a pH drift in the apoplast could induce remobilisation of chloroplastic pools of ABA, and an efflux into the leaf apoplast resulting in stomatal closure. Increases in apoplastic ABA (ie, in sap extracted with a pressure chamber directly from leaves) have been evidenced.

<table>
<thead>
<tr>
<th></th>
<th>Well watered</th>
<th>n</th>
<th>Stressed</th>
<th>n</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABA$_{xyl}$ (μmol m$^{-3}$)</td>
<td>33.2 ± 7.8 22</td>
<td></td>
<td>51.8 ± 14.7 9</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>ABA-GE (1992) (μmol m$^{-3}$)</td>
<td>152 ± 32.9 4</td>
<td></td>
<td>132 ± 43.9 5</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>ABA-GE (1993) (μmol m$^{-3}$)</td>
<td>9.92 ± 4.05 13</td>
<td></td>
<td>16.7 ± 1.85 2</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>$g_s$ (nmol m$^{-2}$ s$^{-1}$)</td>
<td>289.3 ± 20.7 23</td>
<td></td>
<td>77.3 ± 13.4 9</td>
<td></td>
<td>***</td>
</tr>
</tbody>
</table>

Values from trees with predawn leaf water potential above −0.6 MPa (well-watered) and below −1.5 MPa (stressed) were used. Values of ABA$_{xyl}$ for Q robur and Q petraea and for 1992 and 1993 were pooled together because of lack of differences. Values for ABA-GE were from Q petraea and both years were distinguished. NS: not significant.
by Harris and Outlaw (1991) and Correia and Pereira (1994) among many others.

Nevertheless, the question whether root-issued ABA is involved in this control of stomatal conductance remains open. The presence of ABA (and ABA-GE) in the xylem sap of plants, including trees, has been firmly established. Values around 60 (Prunus armeniaca, Loveys et al, 1987), 100 (Prunus dulcis, Wartinger et al, 1990; Acer saccharum, Bertrand et al, 1994), 100–250 (seedlings of Quercus petraea, Q robur, Q pubescens, Scuiller, 1990) and up to 500 μmol m⁻³ (Quercus agrifolia, Hartung and Slovik, 1991) have been reported, and are of the same order of magnitude as the 30 and 50 μmol m⁻³, respectively, for ABA and ABA-GE we detected in Quercus robur and Q petraea. Unfortunately, this consensus does not hold for drought-induced increases: the just cited authors frequently observed a three- to ten-fold increase during drought. This was not the case in our trees; the slight increase detected for the most severe stress intensities (+50%) was statistically not significant and, in any case, remained much below the previous range.

Many hypotheses may be invoked to explain the lack of increase in ABAₓyl during our experiments as compared to other reports. Numerous artefacts may arise during xylem sap extraction, and induce either decreases or increases in ABAₓyl. A dilution by symplasmic water may occur during overpressurisation of the leafy shoot, and could possibly explain the negative correlation we found between the volume of extracted sap and ABAₓyl. Nevertheless, this correlation explained only a weak fraction of the variability, and furthermore, the amount of extracted sap was independent of the stress intensity (as estimated by Ψwp). Its incidence on the lack of difference between stressed and nonstressed trees was probably minor. A pollution of xylem sap by phloem ABA may frequently promote increases in the apparent value of ABAₓyl (Else et al, 1994), but removal of the bark before extraction minimized the risk. Finally, a mixture of xylem sap with apoplastic sap originating from the leaves (the latter being potentially enriched with ABA mobilised from the intracellular pools) may occur when sap is directly extracted from leaf petioles. The impact of this artefact was probably only minor in our case, due to the length of the stem of the twigs used. Despite the potential importance of such artefacts, they cannot alone explain the lack of drought-induced increase of ABAₓyl in our measurements.

The lack of increase in ABAₓyl in response to drought cannot either be analysed as a specific feature of oaks. Several works evidenced increased ABAₓyl in oak seedlings (Scuiller, 1990; Stiller, 1995; Pallardy, personal communication). Two other factors could help explain it: the size of the trees and the slow installation of the stress. Besides the study of Wartinger et al (1990), all other reports used seedlings as plant material, and did impose drought rather rapidly (several days). In the case of tall trees submitted to a gradual soil water depletion, the question of relative contributions of root-issued and leaf-issued ABA to the control of stomatal conductance arises.

Several facts show that the link between ABAₓyl may not be as strong as sometimes argued. Stomatal closure preceded the increase in ABAₓyl in Phaseolus (Trejo and Davies, 1991). Moreover, important changes in the relationships between ABAₓyl and stomatal conductance have been detected and ascribed to changes in the apparent sensitivity of stomata to ABAₓyl (Schurr et al, 1992; Tardieu and Davies, 1992) and related to reductions in leaf water potential (Tardieu and Davies, 1993). They might be of importance during gradually increasing and long-lasting drought stress, together with more subtle changes in xylem sap properties (Gollan et al, 1992), and could induce
changes in the balance between ABA issued from the roots or from intracellular leaf compartments.

Moreover, a high rate of sequestration followed by catabolisation of apoplastic ABA has been evidenced (Hartung and Slovik 1991); values to half-life time as low as 30 min have sometimes been reported (Prunus, Gowing et al., 1993). As a consequence, it may be argued that the factor controlling stomatal aperture may be closer to the total flux of ABA into leaves rather than to the concentration in xylem sap alone (Gowing et al., 1993a). We estimated the total amount of ABA transported to the crowns from ABAxyl and from measured total sap fluxes (Bréda et al., 1993a). As a consequence of the limited differences in ABAxyl between controls and stressed trees, and of the severe reduction in total sap fluxes (Bréda et al., 1993b), the total flux of ABA, varying between 20 and 600 nmol tree⁻¹ d⁻¹, was highest in the well-watered and actively transpiring individuals. Similar results were reported by Jackson et al. (1995) with saplings of Picea sitchensis and Pinus sylvestris. Despite an 11-fold increase in ABAxyl in response to drought, the total flux of ABA to the needles was of the same order of magnitude in stressed and in well-watered plants. The active concentration in the vicinity of stomatal guard cells would therefore have been lower in stressed oak trees if root-issued ABA was the unique source of ABA acting on stomata.

Finally, we may state, according to the work of Hartung and Slovik (1991) and to recent data published by Trejo et al. (1993), that the analysis of drought-induced and ABA-mediated stomatal closure has to take into account changes in the compartmentation of ABA both in leaves and in roots, transport from roots to leaves in the xylem sap, and from leaves to roots in the phloem, and finally the turnover rate of ABA in the apoplastic solution in the vicinity of the guard cells.

CONCLUSION

The drought imposed on oak trees did not result in detectable increases of concentration of ABA in the xylem sap. This may not necessarily be an argument for the lack of root control of stomatal aperture in tall trees, nor for the absence of root-issued ABA and its importance as drought signal, but stresses the need for additional research integrating all aspects of ABA transport in water-stressed plants.

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