Canopy stomatal conductance and xylem sap abscisic acid (ABA) in mature Scots pine during a gradually imposed drought

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Summary We investigated the effect of drought on canopy stomatal conductance ($g_c$), and examined the hypothesis that $g_c$ is controlled by the chemical messenger abscisic acid (ABA) produced in roots. Beginning in November 1994, we subjected a mature stand of Scots pine (*Pinus sylvestris* L.) to an imposed 11-month drought. Control plots were maintained at average-season soil water content. Xylem sap was extracted from shoots at regular intervals from April to November 1995. Soil water, sap flow and leaf water potentials (predawn to dusk) were recorded at the same time. Canopy stomatal conductance was calculated from sap flow data and xylem sap ABA concentrations ([ABA$_{xyl}$]) were measured by radioimmunoassay.

Mean [ABA$_{xyl}$] in control trees was 250 µmol m$^{-3}$. No diurnal variation in [ABA$_{xyl}$] was detected. With soil drying, [ABA$_{xyl}$] increased to a maximum in summer (600 µmol m$^{-3}$), but decreased again toward autumn; however, no significant increase in ABA flux to the leaves occurred. A decline in $g_c$ was detected when volumetric soil water content declined below 0.12. The decline in $g_c$ could not have been mediated by increasing [ABA$_{xyl}$] because stomatal closure appeared to precede any increase in [ABA$_{xyl}$].

Peak sap flow velocity data were used to estimate delivery times for root-to-shoot signals in 15-m tall trees. Under normal field conditions, a signal would take 12 days to travel from the site of production (roots) to the presumed site of action (shoots). However, under drought conditions it may take a chemical signal in excess of 6 weeks. We conclude that a feed-forward model of short-term stomatal response to soil drying, based solely on the action of a chemical messenger from the roots, is not applicable in mature conifer trees because signal transmission is too slow.

Keywords: canopy conductance, *Pinus sylvestris*, sap flow velocity, soil water content, root-to-shoot signal.

Introduction

The avoidance of water deficits in plant tissues was originally thought to be achieved by stomatal closure caused by a decline in leaf turgor or leaf water potential or both (e.g., Turner 1974). In the 1980s, studies on field-grown plants provided evidence that stomatal responses were more closely linked to soil water content than to leaf water status (Bates and Hall 1981, Jones 1985). These findings were supported by experiments on plants whose stomata were induced to close in response to soil water deficit, even when leaf turgor was maintained close to zero by experimental manipulation (Schurr et al. 1992). Further evidence was obtained from split root experiments, in which stomatal conductance declined in response to drying of soil associated with one part of the root system only, whereas no significant perturbation in shoot water status was evident (Gowing et al. 1990, Khalil and Grace 1993). The observation that stomatal closure occurs independently of changes in bulk leaf water status prompted the hypothesis that stomatal conductance is controlled by a chemical signal moving from root to shoot in the xylem stream (Davies 1995). Abscisic acid (ABA) was implicated as the most likely signal (Schulze 1986, Zhang and Davies 1987).

Most ABA signalling investigations have involved controlled experiments on small plants grown in containers (e.g., Jackson et al. 1995). There have been few studies of ABA signalling in forest trees (Blake and Ferrell 1977, Roberts and Dumbroff 1986, Davies 1995, Auge et al. 2000) and, in particular, ABA fluxes in conifers have been given only scant attention (Jackson et al. 1995, Sturm et al. 1998). In tall trees growing in their natural environment, long-distance transport would be required to convey the signal from root to shoot (Wartinger et al. 1990, Triboulet et al. 1996, Loewenstein and Pallardy 1998) and thus, studies on small trees grown in containers may be misleading (Hartung and Davies 1991). In this report we explore the hypothesis that stomatal conductance is controlled by an ABA signal conveyed from the root to the shoot in the xylem stream of mature *Pinus sylvestris* L.

Materials and methods

Study site

The site was located in Devilla Forest, near Kincardine Bridge, Fife, Scotland. The study was carried out in a 41-year-old Scots pine stand within Compartment 474 of this commercial plantation, owned by the Forestry Commission (Table 1).
The soil is a homogeneous sandy loam with occasional pockets of less free-draining soil. Within the forest, eight square plots of 100 m² were selected, each contained eight to 15 trees. Around the perimeter of each plot a 1.5-m deep trench was excavated, an impermeable plastic septum was inserted vertically into the trench and the trench was then refilled with soil. The septum minimized lateral movement of water from the surrounding soil, allowing the soil water contents of the plots to be manipulated independently. Precipitation and stem flow were excluded from the four drought-treated plots by construction of clear polythene covers that intercepted rain-water and diverted it outside the vertical soil septum. The covers permitted access to the tree boles and allowed natural aeration of the soil surface. The control treatments were without covers and received some irrigation to maintain average-season soil water content. The year of study (1995) was exceptionally dry, with March to August rainfall 128 mm lower than the 20-year mean, a reduction of 40%. Over this period 125 mm of water was added as irrigation to the control plots.

The general conditions of the experiment are further described by Irvine et al. (1998). Site construction was complete by November 1994, after which time water was withheld from the drought-treated plots. Measurements commenced in April 1995. At the beginning of November 1995, after removal of the covers, the drought-treated plots were irrigated to return soil water content to pretreatment values.

Microclimate

Sensors for air temperature and relative humidity (HMP35AC, Campbell Scientific, Loughborough, U.K.), net radiation (Q7, Campbell Scientific), photosynthetically active radiation (PAR) (Skye 215, Skye Instruments, Llandrindod, Powys, Wales) and wind speed (A100R, Vector Instruments, Powys, Wales) were permanently installed on the tower, 3 m above the canopy. Hourly means of climatological variables were recorded with a data logger (CR21X, Campbell Scientific). Vapor pressure deficit (VPD) was calculated from air temperature and relative humidity.

Table 1. Climate data were obtained from a Meteorological Office station at Grangemouth, about 4 km to the south of the study site. Dimensions at breast height and numbers of trees ha⁻¹ were based on measurements of all trees within 0.25 ha. Standard deviation is given in brackets where appropriate.

<table>
<thead>
<tr>
<th>Latitude</th>
<th>56°2’</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longitude</td>
<td>3°43’</td>
</tr>
<tr>
<td>Elevation (m)</td>
<td>75</td>
</tr>
<tr>
<td>Mean January temperature (°C)</td>
<td>2.5</td>
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<tr>
<td>Mean July temperature (°C)</td>
<td>14.8</td>
</tr>
<tr>
<td>Mean annual precipitation (mm)</td>
<td>820</td>
</tr>
<tr>
<td>Planting year</td>
<td>1954</td>
</tr>
<tr>
<td>Mean height (m)</td>
<td>15</td>
</tr>
<tr>
<td>Mean diameter at breast height (cm)</td>
<td>20.1 (4.9)</td>
</tr>
<tr>
<td>Overbark basal area (m² ha⁻¹)</td>
<td>28</td>
</tr>
<tr>
<td>Sapwood basal area (excluding heartwood) (m² ha⁻¹)</td>
<td>21</td>
</tr>
<tr>
<td>Trees (ha⁻¹)</td>
<td>836</td>
</tr>
</tbody>
</table>

Sap flow

Sap flux density was measured by the heat pulse method (Custom system, Soil Conservation Centre, Palmerston North, New Zealand) on one tree per plot (four per treatment) with estimates checked for accuracy. Sap flow velocity was calculated by averaging values per treatment (cm h⁻¹), after standard corrections, on a sapwood area basis. The nomenclature adopted for sap flow measurements follows that suggested by Edwards et al. (1997). The sap flux measurement protocol is reported in Irvine et al. (1998).

Canopy stomatal conductance

Canopy stomatal conductance (gₛ) had been estimated previously from hourly measurements of sap flux and atmospheric humidity (Irvine et al. 1998). Data were normalized as the quotient: midday water-stressed canopy conductance/midday control canopy conductance, hereafter the canopy conductance fraction. This derived variable expresses conductance relative to controls, and thus produces a data set that is uninfluenced by hour to hour variations in the weather.

Delivery rate of ABA

An estimate of the time taken for ABA to be transported was calculated from sap flow data, restricted to the 12-h period of maximal sap flow, obtained from diurnal profiles. This 12-h period of maximal flow accounted for about 90% of total daily sap flow. The effect of stem sapwood taper was accounted for by integration among three heights: (a) the height of measurement (1 m); (b) an arbitrary crown base height of 9 m; and (c) at an assumed tree height of 15 m. We then estimated the minimum time required for ABA to travel from the roots to shoots, based on data averaged from four trees per treatment.

Soil water

Soil water deficit (SMD) was measured by time domain reflectometry (TDR) every 2 weeks. A two-pin balanced design was used, and measurements were taken with a cable testing oscilloscope (1502B, Tektronics Corporation, Redmond, OR) fitted with an inline SDM1502 interface connected to a CR21X data logger (Campbell Scientific). Probes were permanently installed in each plot: 3 × 20 cm and 2 × 50 cm depth. Calibration and measurements were as in Irvine et al. (1998).

Leaf water potential

On selected days throughout the season, leaf water potential (Ψₛ) was assessed at 3-h intervals from dawn to dusk. Shoots were obtained from the mid-canopy with pole pruners, one random sample being taken from each plot (four per treatment). To minimize water loss, samples were immediately placed in polythene bags containing moist towels. From each sample, Ψₛ was measured on two, fully expanded 1-year-old needles with a Scholander pressure chamber (Model 1400, Skye Instruments). Measurement was completed within 20 min of sample collection.
Abscisic acid

On the same days that \( \Psi_L \) was measured, xylem sap for ABA analysis was collected during the early afternoon. On two dates, diurnal variation in ABA concentration in xylem sap ([ABA\(_{xyl}\)]) was measured by collection of sap over the course of the day. Large shoots, 20 to 30 cm long (4–5 years old), were collected randomly from one tree per plot with pole pruners and immediately placed in polythene bags containing moist towels to minimize water loss. Bark was removed from the distal end of the shoot to avoid possible contamination of xylem sap by ABA exuding from the phloem tissues (Else et al. 1995), and the shoots placed in a Scholander pressure chamber (Model 1400, Skye Instruments). About 50 mm\(^3\) of sap was collected from each shoot by gradually (< 0.3 MPa min\(^{-1}\)) raising the pressure 0.5–1.5 MPa above the balancing pressure. It has previously been reported that there is no correlation between the applied over-pressure and [ABA\(_{xyl}\)] (Wartinger et al. 1990). The expressed sap was collected directly from the exuding xylem vessels, via a micro-capillary tube, in a 1.5-cm\(^3\) polypropylene Eppendorf vial. The vial was protected from bright light by encapsulation with aluminium foil and wrapped in Nescofilm\(^{\text{TM}}\) (Nescofilm, Nippon Shoji, Osaka, Japan). After the collection of sap, the vials were immediately frozen in liquid nitrogen and stored at –80 °C pending analysis.

Measurement of ABA concentration in xylem sap

We quantified [ABA\(_{xyl}\)] by the radioimmunoassay (RIA) protocol (Quarrie et al. 1988). The monoclonal antibody used (AFRC MAC 62) is specific for (+)-cis,trans-ABA. Standard ABA samples were included in each assay for construction of the standard curve. The validation of the RIA for use with unpurified sample extracts was confirmed by a dilution/spike recovery test for nonspecific interference (Jones 1987). Values of [ABA\(_{xyl}\)] are presented as means for four trees (i.e., one tree from each plot).

Xylem sap pH

After [ABA\(_{xyl}\)] was determined, xylem sap pH was measured with a Mettler-Toledo Electrode (Mettler-Toledo, Leicester, U.K.) that allowed determination of pH in sample volumes of > 50 \( \mu \)l.

Statistical analysis

Where a particular measured variable showed a treatment effect, a series of \( t \)-tests was performed to quantify the significance of treatment at each date or time. Where no clear interaction was observed, a two-way ANOVA with one “repeat measures” factor was performed. Lag-day data was analyzed by covariance with varying lag. All data were analyzed using SAS software (SAS Institute, Cary, NC).

Results

Seasonal variation in [ABA\(_{xyl}\)] between treatments, grouped over all dates, was statistically significant (\( P < 0.01 \)) (Figure 1a). A gradual upward trend in [ABA\(_{xyl}\)] was evident in the control treatment over the summer, followed by a decline in autumn (Figure 1a). Water-stressed trees displayed much higher [ABA\(_{xyl}\)] than control trees between July 20 and August 31 (Figure 1a, shaded area: \( P < 0.001 \)), reaching a peak of 600 \( \mu \)mol m\(^{-3}\) on August 9. Abscisic acid flux was not significantly different between treatments because of a reduction in sap flow in the water-stressed trees (Figure 1b). From September, when \( \Psi_{LPD} \) had recovered, [ABA\(_{xyl}\)] of control and water-stressed trees returned to within the same range. No significant differences in soil water fraction were detected in late summer (Figure 1d).
stressed trees converged (cf. Figures 1a and 1d). Canopy conductance ($g_c$) declined in a near linear manner from mid-July until the end of August (Figure 1c).

In the water-stressed treatment, soil water content integrated over the top 20- and 50-cm depths declined to a minimum of 0.5 and 0.11, respectively, at the end of August 1995. The soil water content in the control treatment never fell below 0.2 (50-cm depth). There was a statistically significant difference in soil water content between treatments from April 1995 (Figure 1d). Predawn needle water potential ($\Psi_{LMP}$) of water-stressed trees was lower than that of control trees throughout the season (Figure 1e). From July 27 until the beginning of November, the difference in $\Psi_{LMP}$ between treatments was statistically significant ($P < 0.05$).

Near-constant concentrations of [ABA xyl] were generally observed over the course of each day (Figure 2e). During the driest period, water-stressed trees had higher [ABA xyl] than control trees (Figure 2e) ($P < 0.05$). Diurnal fluctuations in leaf water potential ($\Psi_L$) were evident in both control and water-stressed trees (Figure 2c). On August 9, $\Psi_L$ was about 0.3 MPa lower in water-stressed trees than in control trees (Figure 2c, $P < 0.01$). Differences in diurnal flux of ABA were not significant because of reduced sap flow rate in water-stressed trees (Figure 2f).

There was no clear relationship between [ABA xyl] and $\Psi_{LMP}$. There were, however, relationships between minimum daily leaf water potential ($\Psi_{LMM}$) and [ABA xyl] (Figure 3a) and midday canopy conductance ($g_c$) (Figure 3b). A negative linear reduction in $g_c$ was evident with decreasing $\Psi_{LMM}$ ($r^2 = 0.55$) and the relationship between $\Psi_{LMM}$ and [ABA xyl] could be described by a polynomial expression ($R^2 = 0.64$). There was also a roughly linear negative relationship between [ABA xyl] and $g_c$ in the water-stressed trees (Figure 3c).

All parameters measured showed a response to the soil water deficit treatment (Figure 4), except xylem sap pH (Figure 4c). Below a soil water content of 0.12, approximate exponential declines in $g_c$ (Figure 4b) and $\Psi_{LMM}$ (Figure 4d) were observed. However, an approximate exponential increase in [ABA xyl] occurred only when soil water content fell below 0.8, indicating that the increase in [ABA xyl] was in response to a much lower threshold soil water potential than the decreases in $g_c$ (Figure 4b) and $\Psi_{LMM}$ (Figure 4a). The textural properties of the soil at the study site are such that a steep decline in soil water potential ($\Psi_{soil}$) occurs (determined as described by Saxton et al. 1986) below a soil water content of 0.15. Hence the observed results may be interpreted as a response to $\Psi_{soil}$.

To explore the relationship between seasonal variation in [ABA xyl] and $g_c$, we fit a smooth curve to the seasonal pattern of [ABA xyl] to enable an estimate of [ABA xyl] to be made by interpolation for any specified day. An empirical model was constructed with Sigmaplot (SPSS, Chicago, IL). Seasonal trends of [ABA xyl] were described by empirical equations, enabling daily values to be estimated. These daily estimates were then correlated with midday $g_c$, using a form of time-series analysis to ascertain the lag-time between the two variables with SAS/ETS software (SAS Institute, Cary, NC), which varied linearly with respect to one another (Figure 5a). The analysis with increasing lag times confirmed the trend indicated in Figure 1 that declines in $g_c$ occurred before increases in [ABA xyl] in water-stressed trees, with significant correlations ($P < 0.01$, $r = 0.56$, $n = 20$) across a range of 7 to 48 days (Figure 5b).

**Estimation of delivery times of ABA**

Typical sap velocities in the water-stressed trees during the pe-
period of severe drought were 3 cm h⁻¹, implying that delivery from root to shoot would take about 500 h. Examples of estimated delivery times are given in Table 2, and indicate that water-stressed trees may be expected to receive a chemical signal from the root later than the control trees. Under well-watered conditions, we estimated a delivery time of 12–17 days. However, when the imposed drought was most severe, a steep increase in delivery time was noted because of the decline in sap flux in the water-stressed treatment. Delivery times in August were between 6 and 8 weeks (Table 2).

Discussion

It has been shown previously that the water stress treatment was sufficient to cause a 14, 34 and 20% reduction in basal area, shoot and needle growth, respectively, compared with the corresponding control values by the end of the 1995 growing season (Figure 8, Irvine et al. 1998). Such water stress is severe compared with the severity of drought that P. sylvestris normally experiences in its natural environment, even so, some recovery of plant water status occurred when the weather deteriorated during autumn.

Xylem ABA concentration ([ABA xyl]) showed no pronounced fluctuation throughout the day. This finding is in agreement with the results of Wartinger et al. (1990), but contrasts with results obtained with container-grown saplings (Jackson et al. 1995). The peak [ABA xyl] value during the period of severe drought, 600 µmol m⁻³, was of the same order of magnitude as previously reported in mature trees of other conifer species (Wartinger et al. 1990, Triboulot et al. 1996, Sturm et al. 1998). Nevertheless, despite the observed twofold drought-induced increase in [ABA xyl], no significant increase in ABA flux to the leaves occurred, even though ABA flux in the control trees was lower than that previously reported for field-grown oak (Triboulot et al. 1996).

There was a threshold relationship between soil water content and $g_c$, [ABA xyl] and bulk $\Psi_{LMIN}$. This does not necessarily demonstrate direct control of stomata by soil water deficit, mediated by a root-sourced hormone, because it could simply be the result of a sharp decline in soil water content below a threshold value of 0.12 (Figure 6). It is evident, however, that the ABA response did not occur until soil water content declined below 0.08. Canopy stomatal conductance of the water-stressed trees began to decline after May 23; however, a
significant increase in [ABA_{xyl}] was not noted until after June 27, suggesting that the reduction in g_c observed from May 23 was not the direct result of an ABA signal carried in the xylem. More importantly, declines in sap flow caused ABA flux to remain relatively constant.

Our sap flow velocities are similar to those reported for mature conifer trees of other species: data from Milburn (1979) for _Pinus contorta_ (Dougl.) equate to a rate of 86 cm day^{-1} and data from Köstner et al. (1996) for _P. sylvestris_ (L.) equate to a rate of 204 cm day^{-1}. We note that these sap flow velocities are means and therefore do not directly measure the maximum potential; however, peak flow in 40-year-old Scots pine trees has been estimated at 175 cm over a 4-day period (44 cm day^{-1}) using radioisotopes that allow the leading edge of the pulse, the “fastest stream,” to be defined (see Table 2, Waring and Roberts 1979). During the period of severe drought, we estimated that the time taken for a signal to travel to the crown increased to more than 6 weeks. Even if sap velocities were expressed per unit conducting area instead of per sapwood area (i.e., including non-transporting conduit walls) delivery time would still have been more than 3 weeks. These data support the conclusion of Schulze (1991) that “in conifers a root signal transported in the xylem is too slow to be effective.”

Previous studies have highlighted the important effect of sap flow rate on [ABA_{xyl}] (the ABA flux into the leaves) (Gowing et al. 1993, Else et al. 1995, Jackson et al. 1995). We found no discernible relationship between sap flow and [ABA_{xyl}] or the reciprocal of [ABA_{xyl}] (cf. Jarvis and Davies 1997).

Loading of ABA at the guard cell may increase as a result of variation in xylem sap pH (Wilkinson and Davies 1997). We found no significant variation in bulk xylem pH in response to water stress except on one date (August 31), but we have little confidence in this result because n = 1. However, a role for ABA in the control of stomatal aperture mediated through changing apoplastic pH (Thompson et al. 1997) cannot be discounted.

Threshold relationships were evident between soil water content and both Ψ_{LMIN} and [ABA_{xyl}]. The correlation between soil water content and [ABA_{xyl}] does not, however, preclude the presence of a rapid hydraulic signaling system from root to shoot in which stomata respond to the resulting complex gradients of Ψ in the leaf (c.f. Saliendra et al. 1995, Fuchs and Livingston 1996, Comstock and Mencuccini 1998). We noted a correlation between midday Ψ_L and g_c.

We confirmed the presence of ABA in the xylem of mature conifers, and demonstrated an increase in [ABA_{xyl}] in response to an increase in soil water deficit. However, we found that stomatal closure preceded increases in [ABA_{xyl}] and ABA flux remained constant in response to the drought treatment, so we reject the hypothesis that stomatal closure is caused by increased [ABA_{xyl}]. Moreover, in tall conifers, sap velocities are generally too low for root to shoot ABA signalling to be effective in causing stomatal closure in time to avoid tissue water deficits. Therefore, although it is difficult to determine whether the correlation between variables might explain a threshold phenomenon for Ψ_L, it appears unlikely because of the long signal delivery times.

Acknowledgments

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Table 2. Peak sap flow and estimated delivery times of xylem sap ABA, on four dates over the experimental period. Treatments are (a) water-stressed and (b) control. Delivery times are presented to the nearest half-day.

<table>
<thead>
<tr>
<th>Date</th>
<th>Treatment</th>
<th>May 4</th>
<th>June 27</th>
<th>August 9</th>
<th>August 31</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Sap flow at 1 m (m s^{-1} × 10^{-5})</td>
<td>1.34</td>
<td>1.21</td>
<td>1.20</td>
<td>1.66</td>
<td>0.68</td>
</tr>
<tr>
<td>Sap flow at 9 m (m s^{-1} × 10^{-5})</td>
<td>2.68</td>
<td>2.42</td>
<td>2.40</td>
<td>3.32</td>
<td>1.36</td>
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<tr>
<td>Delivery time (h)</td>
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<td>207</td>
<td>209</td>
<td>151</td>
<td>512</td>
</tr>
<tr>
<td>Delivery time (days)</td>
<td>15.5</td>
<td>17.5</td>
<td>17.5</td>
<td>12.5</td>
<td>42.5</td>
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References


