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Stomatal conductance, growth and root signaling in *Betula pendula* seedlings subjected to partial soil drying

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Summary Seedlings of *Betula pendula* Roth were grown with their root systems separated between two soil compartments. Four treatments were imposed: (i) adequate irrigation in both compartments (WW, controls); (ii) adequate irrigation in one compartment and drought in the other compartment (WD); (iii) drought in both compartments (DD); and (iv) half of the root system severed and the remainder kept well-watered (root excision, RE). Predawn leaf water potential, stomatal conductance, soil-to-leaf specific hydraulic conductance, and root and leaf growth decreased in DD-treated seedlings, which also displayed severe leaf shedding (30% loss in leaf area). The DD treatment also resulted in increased concentrations of abscisic acid (ABA) and its glucose ester in the xylem sap of roots and shoots compared to concentrations in control seedlings (about 200 versus 20 nM). Despite the difference in xylem sap concentrations, total ABA flux to the shoots was similar in the two treatments (1–2 pmol ABA m^{-2} leaf area s^{-1}) as a result of reduced transpiration in the DD-treated seedlings. Compared with root growth in control plants, root growth increased in the RE-treated plants and decreased in the drying compartment of the WD treatment; however, the RE and WD treatments only slightly reduced leaf expansion, and had no detectable effects on shoot water relations or ABA concentrations of the root and shoot xylem sap. We conclude that short-term soil water depletion affecting only 50% of the root system does not cause a measurable stress response in birch shoots, despite root growth cessation in the fraction of drying soil.

Keywords: abscisic acid, birch, water stress, xylem sap.

Introduction

Many studies have shown that, with increasing water stress, abscisic acid (ABA) is released from root tips into the transpiration stream and transported to the leaves where it triggers stomatal closure (Davies and Zhang 1991, Davies et al. 1994, Dodd et al. 1996). A logarithmic relationship between the ABA concentration ([ABA]) of xylem sap and stomatal conductance is frequently observed in herbaceous (e.g., Tardieu et al. 1996, Socias et al. 1997) and woody (e.g., Wartinger et al. 1990, Correia et al. 1995, Jackson et al. 1995, Sturm et al. 1998) species. It has been suggested that increased ABA delivery by the fraction of roots growing in the drying soil, rather than an increase in shoot xylem sap [ABA], is the signal for stomatal closure (Neales et al. 1989, Khalil and Grace 1993, Masia et al. 1994, Jokhan et al. 1996) or for expression of ABA-responsive genes (Griffiths and Bray 1996). However, Triboulot et al. (1996) reported no increase in xylem sap [ABA] of mature oak trees despite drought-induced stomatal closure, and Fort et al. (1997) found no increase in ABA delivery to the shoots of *Quercus robur* L. seedlings subjected to soil drying even though root xylem sap [ABA] was increased and stomatal conductance was decreased.

The importance of root-originated ABA in stomatal control, as well as the apparent sensitivity of stomatal conductance to root-originated ABA may vary with genotype, within genotypes as a function of phenotypic plasticity, or with short-term changes in environmental parameters. For example, because oaks are drought-tolerant species they may not rely on precocious and efficient stomatal closure (Dreyer et al. 1996) and so the role of root-originated ABA in stomatal control may be negligible (Fort et al. 1997). Dodd and Davies (1994, 1996) reported that, in wheat, the reduction in leaf growth induced by ABA supplied in the xylem sap is larger at high temperatures than at low temperatures. Honour et al. (1995) found that sensitivity of stomata to ABA is less at low temperatures than at high temperatures. Furthermore, apparent sensitivity of stomatal conductance to xylem sap ABA decreases with time in water-stressed plants (Correia and Pereira 1995).

Plants grown in split-root pots, where only a fraction of the root system is subjected to soil drying, frequently exhibit stomatal closure despite the maintenance of leaf water potentials close to optimum (Gowing et al. 1990). We carried out a split-root experiment with birch seedlings to analyze the effects of partial soil drying on water relations, stomatal conductance, xylem sap [ABA], and growth. We chose birch because it is a pioneer species characterized by high stomatal conductances and high transpiration rates (Ranney et al. 1991, Federer 1997). With increasing water stress, below a threshold value of predawn leaf water potential close to –1.1 MPa, birch seedlings exhibit a severe reduction in stomatal conductance and an increase in leaf shedding (Fort 1997). We tested two hypothe-

ses: (i) root-originated ABA is involved in stomatal closure and leaf abscission in drought-stressed birch; (ii) xylem sap [ABA] and delivery rates of ABA to the shoots increase in drought-stressed birch.

Material and methods

Experimental design and treatments

Seedlings of *Betula pendula* Roth were grown from seed in moist sand, in a greenhouse near Nancy, northeastern France. Temperature did not fall below 10 °C and rarely exceeded 25 °C during the day and irradiance was about 70% of outdoor values. After four weeks, the seedlings were removed from the sand and each root system was divided into two parts and repotted in a 14-l container that was divided vertically into two water-tight compartments. The soil comprised a 2:1 (v/v) sand:peat mix and was covered with a paraffined cardboard disk to minimize direct evaporation. Plants were fertilized (5 kg m^{-3} of slow-release fertilizer; N,P,K 13,13,13 plus trace elements) and were kept well-watered for 3 months. At the end of June, four treatments were imposed: (i) both compartments were watered to run-off daily (WW, controls, $n = 8$); (ii) one compartment was watered to run-off daily and the other compartment was not watered from July 10 to July 25 (Days 191 to 206) (WD, $n = 9$); (iii) water was withheld from both compartments from July 10 to July 25 (Days 191 to 206) (DD, $n = 8$); and (iv) half the root system was excised and the compartment containing the remainder of the root system was watered to run-off daily (RE, $n = 8$).

Growth and leaf abscission during drought

Height and root growth were measured twice a week. The progress of root tip extension was marked on the transparent sides of the containers. Between measurements, the containers were covered with black plastic to avoid light penetration and algal development. Leaf expansion in all treatments was followed from Days 193 to 203 by manually drawing the outline of each leaf every second day. Two of the youngest leaves were chosen per plant, the first leaf on the main axis and the other on a lateral branch. Shed leaves were harvested at regular intervals and their dry weights and areas measured. At the end of the experiment, plants were harvested for biomass and final leaf area measurements (determined with an area meter, Delta-T Devices, Cambridge, U.K.). Total leaf area at any given date was calculated from final leaf area corrected by the area of leaves shed in the interval. In the WD treatment, roots in the wet and dry compartments were harvested separately.

Soil water status

Volumetric soil water content was measured by Time Domain Reflectometry (TDR) (Soil Moisture Equipment Corp., Santa Barbara, CA). One 20-cm long wave guide was installed in each compartment one month before the onset of the experiment. Measurements were made at the end of the afternoon, just before watering the wet compartments and one hour after watering.

Leaf water relations and stomatal conductance

Leaf water potential was measured twice a week, at predawn (Ψ_{pd}) and mid-afternoon (Ψ_{pm}), on one mature leaf per seedling, with a pressure chamber. Stomatal conductance to water vapor (g_s, mmol m^{-2} s^{-1}) was measured at midday (1200 to 1300 h solar time) with a steady-state porometer (LI-1600, Li-Cor, Lincoln, NE) on one expanding, well-exposed leaf per plant. Throughout the experiment, whole-plant transpiration (E_p, mmol s^{-1}) was assessed gravimetrically, by weighing the containers at 2-h intervals, before and after Ψ_{pm} measurements. Values of whole-plant transpiration flux density (E, mmol m^{-2} s^{-1}) were calculated by dividing E_P by the corresponding leaf area. Soil-to-leaf specific hydraulic conductance (g_L, mmol s^{-1} MPa^{-1}) was calculated by dividing E by the difference between Ψ_{pd} and Ψ_{pm} (Reich and Hinckley 1989).

Extraction of xylem sap from shoots and roots

Xylem sap was extracted on Days 205 (DD treatment), 206 (WD), 207 (RE) and 208 (WW). Three days before each extraction, seedlings were transferred to a growth chamber providing a 12-h photoperiod, day/night temperatures of 21/10 °C, and a day/night relative humidity of 60/90%. Light (300 μmol m^{-2} s^{-1}) was provided by fluorescent tubes (Sylvania VHO). The day before extraction of xylem sap, stomatal conductance and instantaneous values of transpiration flux density were measured 5 h after the beginning of the photoperiod.

To extract xylem sap from roots and shoots, seedlings were severed at the root collar and 20 cm of bark was removed from the stem to avoid contaminating xylem sap with ABA exuding from phloem tissues (Else et al. 1994). The twig was rapidly enclosed in a large Scholander pressure chamber. After the balancing pressure was reached, an additional over-pressure of 0.5 MPa was applied and xylem sap exuding from the cut end was collected with a micropipette. Samples were immediately frozen in liquid nitrogen. Thereafter, roots were washed free of sand and harvested and white root apices were excised and immediately frozen. Root xylem sap was collected by the same technique used for stems. It was not always possible to collect enough sap from roots of drought-stressed plants for determination of ABA. Xylem sap was stored at –70 °C until analyzed. Abscisic acid was extracted from freshly ground root tips in 80% methanol for 60 h at 4 °C in darkness.

Measurements of ABA and ABA-GE and estimation of ABA mass flow

Aqueous root tissue extracts and stem and root xylem sap samples were purified by HPLC. Ten fractions were collected, centered on the retention time of ABA and ABA-GE. The concentrations of ABA and ABA-GE in the fractions were measured by ELISA (Enzyme Linked Immunosorbent Assay) as described by Label et al. (1994). Details of the procedure have been described by Fort et al. (1997).

Abscisic acid mass flow from roots to leaves was estimated from the xylem sap ABA concentration (ABA_x) measured in the shoot multiplied by the instantaneous transpiration flux density measured the day before sap collection with a steady-

state porometer. Values calculated on a leaf area basis took into account the reduction in leaf area resulting from increasing water stress.

Results

Water relations

In well-watered plants, volumetric soil water content (H_v) fluctuated diurnally between 12 (before watering at the end of the afternoon) and 17% (after watering, Figure 1i). Predawn leaf water potential (Ψ_{pd}) remained close to –0.25 MPa throughout the experiment (Figure 1ii). The large differences in stomatal conductance (g_s) and whole-plant transpiration (E_p) observed from one day to another were the result of changing weather conditions (Figure 2i). Well-watered birch seedlings were characterized by high values of g_s, ranging from 200 in the early morning and late evening to 900 mmol m^{-2} s^{-1} (Figure 2ii). As a result, daily plant water consumption was close to 600 g day^{-1} $plant^{-1}$. The rooting medium contained about 2–2.5 kg water, corresponding to about four days of water consumption by the seedlings in the absence of watering.

Severing half of the root system had no significant effect on Ψ_{pd} (Figure 1ii), g_s, E_p (Figure 2), or on whole-plant transpiration flux density (E) and soil-to-leaf specific hydraulic conductance (g_L) (Table 1). In the dry compartments of the WD and DD treatments, H_v rapidly decreased and reached values close to 5 (WD) and 3% (DD) after six days of drought and values close to 3 (WD) and 1.5% (DD) by the end of the drought period (Figure 1i). In the wet compartment of the WD treatment, H_v fluctuated diurnally from a minimum of 12 (before watering) to a maximum of 17% (after watering). Withholding water from one compartment had no detectable

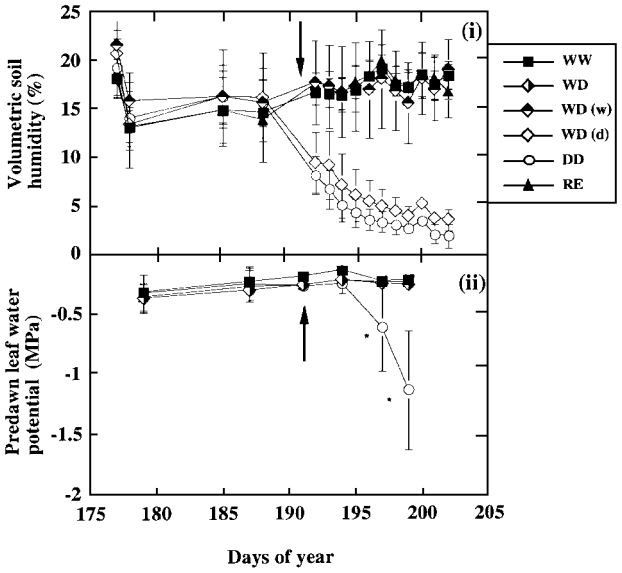

Figure 1. Time course of (i) volumetric soil humidity (H_v); and (ii) predawn leaf water potential (Ψ_{pd}) of *B. pendula* seedlings grown in split-root containers. Treatments were: WW = controls; WD = drought in one compartment; WD (w) and WD (d) represent wet and dry compartments of the WD treatment, respectively; DD = drought in both compartments; and RE = roots severed in one compartment. Arrows indicate the beginning of drought. Means ± SD (n = 5 for H_v and 8 or 9 for Ψ_{pd}). In (i), dry compartments differed from well-watered compartments from the beginning of drought on; in (ii), an asterisk (*) indicates significant differences from controls (P < 0.05, ANOVA followed by Bonferoni test).

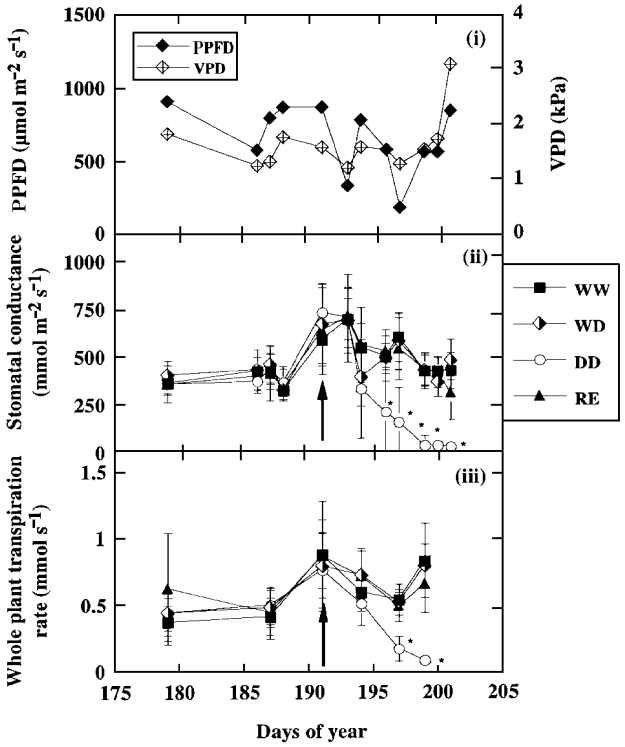

Figure 2. Time course of (i) photosynthetic photon flux density (PPFD) and vapor pressure deficit (VPD); (ii) stomatal conductance; and (iii) whole-plant transpiration (E_p) of *B. pendula* seedlings grown in split-root containers. Treatments were: WW = controls; WD = drought in one compartment; DD = drought in both compartments; and RE = roots severed in one compartment. Arrows indicate the beginning of drought. Mean ± SD, n = 8, except for WD where n = 9. An asterisk (*) indicates significant differences from controls (P < 0.05, ANOVA followed by Bonferoni test).

Table 1. Whole-plant transpiration flux density (E) and soil-to-leaf specific hydraulic conductance (g_L) of *B. pendula* seedlings grown with roots split between two containers. Treatments were: WW = controls; WD = drought in one compartment; DD = drought in both compartments; and RE = half the root system severed. Measurements were made at the end of the experiment. Means ± SD (n = 8 for WW, DD, RE and n = 9 for WD). For a given variable, mean values not sharing common letters are significantly different (P < 0.05, ANOVA followed by Bonferoni test).

Treatment	E (mmol m^{-2} s^{-1})	g_L (mmol m^{-2} s^{-1} MPa^{-1})
WW	1.41 ± 0.48 a	2.13 ± 0.68 a
WD	1.66 ± 0.39 a	2.48 ± 0.85 a
DD	0.26 ± 0.15 b	0.32 ± 0.27 b
RE	1.43 ± 0.65 a	1.74 ± 1.05 ab

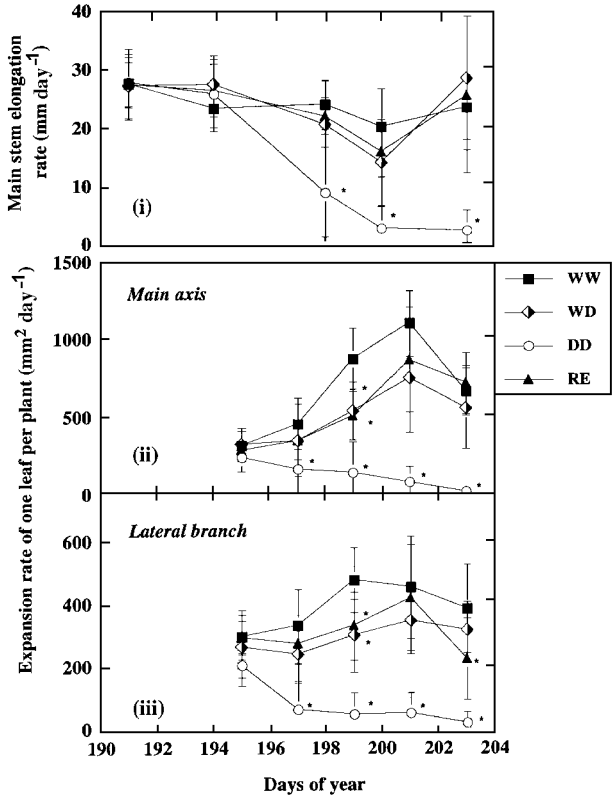

Figure 3. Time course of main stem elongation rate (cm day^{-1}) (i) and leaf expansion rate (mm^2 day^{-1}) of one leaf located on the main axis (ii) and one leaf located on a lateral branch (iii) of young *B. pendula* seedlings grown in split-root containers. Treatments were: WW = controls; WD = drought in one compartment; DD = drought in both compartments; and RE = roots severed in one compartment. Mean ± SD of eight replicates per treatment, except for WD where $n = 9$. An asterisk (*) indicates significant differences from controls ($P < 0.05$, ANOVA followed by Bonferoni test).

effect on Ψ_{wp} and values of g_s, E_p, E and g_L remained close to control values (Figure 2 and Table 1). In contrast, withholding water from both compartments resulted in marked decreases in Ψ_{pd}, g_s and E_p within a few days of the onset of drought (Figure 2ii and 2iii). At the end of the drought, when Ψ_{pd} reached values below –1 MPa, stomatal closure was almost complete, E_p and E were close to zero and g_L was severely reduced (Figure 2 and Table 1).

Shoot and root growth

Stem elongation rate remained almost constant at 20 to 30 mm day^{-1} in seedlings in the WW, RE and WD treatments, whereas it rapidly dropped to almost zero in seedlings in the DD treatment (Figure 3i). Despite this severe effect, final shoot length did not differ significantly between WW- and DD-treated plants because of the short duration of the drought period and the large variability among seedlings (Table 2). Although leaf expansion rates tended to be lower in the RE and WD treatments compared with control values (Figure 3ii and 3iii), final plant leaf area and dry weight did not differ significantly from control values (Table 2). In contrast, leaf expansion rates of DD-treated seedlings were severely reduced (Figures 3ii and 3iii) and accompanied by leaf shedding. Total leaf dry weight (attached + shed leaves) was lower in DD-treated seedlings than in seedlings in the other treatments, indicating that leaf initiation was also inhibited by drought stress. As a result, at harvest, seedlings in the DD treatments had much lower leaf areas and biomass than seedlings in the other treatments (Table 2).

Final root biomass was similar among seedlings in the WW, RE and WD treatments but it was significantly reduced in DD-treated seedlings (Table 2). There was no difference in root biomass between the wet and dry compartments of the WD treatment. A slight increase in root growth rate was detected in the remaining half of the root system of RE-treated plants compared with control seedlings (Figure 4), whereas a severe reduction in root growth rate was observed in DD-treated seedlings, with values close to zero by the end of the drought period. A decrease in root growth rate was also recorded in the dry compartment of the WD-treated seedlings, although the reduction was less than in the DD treatment (Table 3).

Concentrations of ABA and ABA-GE in xylem sap and root tips

Concentrations of ABA in xylem sap extracted from shoots and roots of WW-treated plants were around 20 nM (Table 4), and ABA concentrations of root apices were 14 pmol g_{fw}^{-1}. The total leaf-specific flux of ABA to the shoots was about 1.8 pmol m^{-2} s^{-1} (Table 4).

The DD treatment increased xylem sap [ABA] and [ABA-GE] of roots and shoots more than tenfold. Concentrations of ABA in xylem sap reached above 300 nM in the shoots and

Table 2. Final shoot length, biomass and leaf area of *B. pendula* seedlings grown with roots split between two containers. Treatments were: WW = controls; WD = drought in one compartment; DD = drought in both compartments; and RE = half of the root system severed. Means ± SD ($n = 8$ for WW, DD, RE, and $n = 9$ for WD). For a given variable, mean values not sharing common letters are significantly different ($P < 0.05$, ANOVA followed by Bonferoni test).

Growth parameter	WW	WD	DD	RE
Final shoot length (cm)	101 ± 17.7 a	104 ± 17.7 a	88.6 ± 10.5 a	103 ± 17.3 a
Total root dry weight (g)	20.9 ± 4.84 a	15.9 ± 3.72 a	9.22 ± 1.54 b	14.3 ± 3.84 a
Leaf dry weight (g)	23.0 ± 5.83 a	18.0 ± 4.92 a	10.0 ± 1.92 b	20.9 ± 6.37 a
Leaf area (dm^2)	60.4 ± 15.9 a	49.6 ± 12.8 a	25.8 ± 4.40 b	48.8 ± 13.1 a
Abscised leaf area (dm^2)	0.00 a	0.48 ± 0.77 a	8.79 ± 5.70 b	0.75 ± 0.94 a
Abscised leaf dry weight (g)	0.00 a	0.25 ± 0.34 a	3.27 ± 1.42 b	0.31 ± 0.42 a

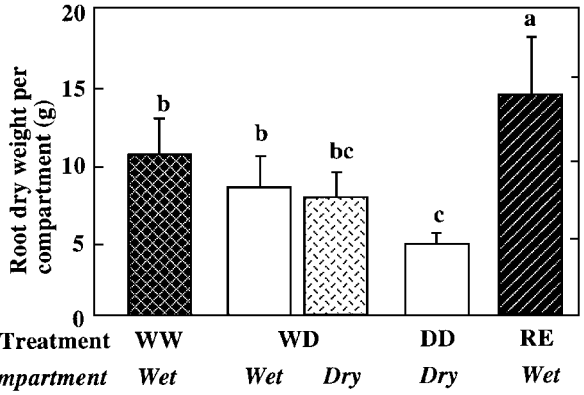

Figure 4. Root dry weight per individual compartment of young *B. pendula* seedlings grown in split-root containers. Treatments were: WW = controls; WD = drought in one compartment; DD = drought in both compartments; and RE = roots severed in one compartment. Measurements were made at the end of the experiment. The two compartments of WD plant were measured separately. Data represent the mean ± SD of eight replicates per treatment, except for WD where $n = 9$. Mean values not sharing common letters are significantly different ($P < 0.05$, ANOVA followed by Bonferoni test).

800 nM in the roots. In root apices, drought significantly increased [ABA-GE] but had no significant effect on [ABA], because of high variability among plants. There was a linear relationship between xylem sap [ABA_x] of shoots and stomatal conductance in DD-treated plants (Figure 5). Despite the large drought-induced changes in xylem sap [ABA], total flux of ABA to the shoots, computed from transpiration data, was similar in water-stressed and well-watered plants (Table 4) because of severe reductions in transpiration flux in the water-stressed seedlings.

The WD and RE treatments did not result in detectable increases in [ABA] in xylem sap or root apices. Furthermore, [ABA] in xylem sap and root apices did not increase in roots in the drying compartment of the WD treatment despite the severe decline in soil water content in this compartment.

Discussion

Birch seedlings maintained high transpiration fluxes on a leaf area basis, high stomatal conductances and high predawn leaf water potentials at low soil water contents. These features are thought to characterize pioneer woody species (Bazzaz 1979) and contrast with the relatively low transpiration rates and the more gradual stomatal closure recorded in drought-tolerant oak seedlings in response to soil drying (Fort 1997). A comparison with oak seedlings grown under similar conditions revealed that birch seedlings maintained higher predawn leaf water potentials at significantly lower soil water contents than oak (Fort 1997). However, birch seedlings displayed a lower root dry weight to leaf area ratio, as a result of both a lower root biomass (15–20 versus 40–50 g) and higher leaf area (50–60 versus 30 dm^2), than oak seedlings, suggesting that the high water extraction capacity of birch seedlings is associated with differences in the architecture (relatively more fine roots in birch than in oak) or the hydraulic conductivities of the roots. Roots of woody species have lower conductivities than herbaceous species, and large variations occur among species (Steudle and Heydt 1997). Soil drying also resulted in cessation of leaf expansion, stem and root elongation and ultimately leaf shedding.

Other differences between oaks and birches included a lower xylem sap [ABA] in well-watered birch than in well-watered oak, and a larger (more than tenfold) increase in xylem sap [ABA] and [ABA-GE] in response to soil drying in birch than in oak (cf. Fort et al. 1997). Thus, birch closely resembles many herbaceous species (cf. Davies et al. 1994, Schurr and Schulze 1995, 1996, Tardieu et al. 1996) and some woody species including maple (Khalil and Grace 1993), pine, spruce (Jackson et al. 1995), acacia, and litchi (Liang et al. 1996). Drought-induced increases in ABA concentrations were smaller in root apices than in xylem sap; however, root apices, despite their ability to synthesize large amounts of ABA, only contribute a small percentage of total root ABA (Zhang and Tardieu 1996).

Well-watered seedlings exhibited ABA fluxes from roots to shoots (i.e., total fluxes divided by leaf area) of around 1.8 pmol m^{-2} s^{-1}, which were only slightly lower than in oak seedlings (above 2 pmol m^{-2} s^{-1}, Fort et al. 1997) despite the much higher xylem sap [ABA] in oak seedlings compared with birch seedlings. *Cedrella odorata* L. exhibited ABA fluxes of between 5 and 15 pmol m^{-2} s^{-1} (Jarvis and Davies 1997), and ABA fluxes were around 10 pmol m^{-2} s^{-1} in *Ricinus communis* L. (Jokhan et al. 1996) and around 5 pmol m^{-2} s^{-1} during

Table 3. Root growth rate (mm day^{-1}) of *B. pendula* seedlings grown with roots split between two containers. Treatments were: WW = controls; WD = drought in one compartment; DD = drought in both compartments; and RE = half of the root system severed. Means ± SD ($n = 8$ for WW, DD, RE, and $n = 9$ for WD). For a given date, values not sharing common letters are significantly different ($P < 0.05$, ANOVA followed by Bonferoni test).

Date	WW	WD		DD	RE
		Dry compartment	Wet compartment		
Day 191	54.1 ± 31.0 a	89.9 ± 52.8 a	88.7 ± 40.9 a	69.8 ± 29.8 a	87.8 ± 56.7 a
Day 193	106 ± 78.4 a	71.1 ± 58.1 a	129 ± 51.8 a	93.7 ± 80.6 a	142 ± 88.3 a
Day 198	39.8 ± 23.5 abc	32.1 ± 53.3 bc	55.4 ± 27.0 ab	15.0 ± 20.2 c	74.1 ± 38.1 a
Day 201	65.1 ± 38.0 ab	17.11 ± 14.8 bc	105 ± 97.6 a	2.27 ± 5.52 c	105 ± 57 a
Day 203	103 ± 88.5 ab	44.3 ± 36.8 bc	91.0 ± 47.1 ab	4.56 ± 10.2 c	168 ± 85.4 a
Day 208	36.3 ± 22.0 b	3.20 ± 4.64 c	22.2 ± 12.1 b	0 c	71.6 ± 15.8 a

Table 4. Concentrations of ABA and ABA-GE in xylem sap extracted from shoots or roots, and in root apex tissues of *B. pendula* seedlings grown in split-root containers. Treatments were: WW = controls; WD = drought in one compartment; DD = drought in both compartments; and RE = roots severed in one compartment. Measurements were made at the end of the experiment. Means ± SD. For a given variable, values not sharing common letters are significantly different ($P < 0.05$, ANOVA followed by Bonferoni test).

	Compartment	WW (n = 8)	WD (n = 9)	DD (n = 7)	RE (n = 8)
Shoot $[ABA]_x$ (nM)	–	23.2 ± 10.3 a	16.4 ± 7.20 a	309 ± 199 b	18.7 ± 11.0 a
Shoot $[ABA\text{-}GE]_x$ (nM)	–	7.50 ± 3.07 a	7.88 ± 5.40 a	151 ± 101 b	6.40 ± 4.20 a
Root $[ABA]_x$ (nM)	Dry	–	76.5 ± 32.6 a	829 ± 267 b	–
	Wet	22.4 ± 11.0 a	90.1 ± 108 a	–	31.3 ± 16.6 a
Root $[ABA\text{-}GE]_x$ (nM)	Dry	–	47.3 ± 23.4 a	263 ± 62.9 b	–
	Wet	10.4 ± 4.44 a	30.7 ± 30.1 a	–	15.4 ± 16.2 a
ABA in root apices	Dry	–	77.7 ± 124 a	93.2 ± 61.3 a	–
(pmol g_{fw}^{-1})	Wet	14.2 ± 6.63 a	25.7 ± 35.3 a	–	48.1 ± 18.7 a
ABA-GE in root apices	Dry	–	15.7 ± 7.34 a	31.8 ± 11.1 b	–
(pmol g_{fw}^{-1})	Wet	6.77 ± 3.10 a	10.3 ± 6.52 a	–	15.0 ± 5.88 a
Total ABA flux from roots to leaves (pmol m^{-2} s^{-1})	– –	1.76 ± 0.73 a	1.19 ± 0.71 a	1.02 ± 1.12 a	1.25 ± 0.79 a

peak transpiration in maize (Ben Haj Salah and Tardieu 1997). Despite the large drought-induced increase in xylem sap [ABA], the total flux of ABA to shoots was similar in water-stressed and control birch seedlings as a result of severe reductions in transpiration flux in the water-stressed seedlings. A similar lack of increase in ABA delivery rates despite increased [ABA] was observed in pine and spruce seedlings (Jackson et al. 1995, Jarvis and Davies 1997). In contrast, herbaceous species generally exhibit increased ABA delivery rates during drought stress (Jorkhan et al. 1996, Ben Haj Salah and Tardieu 1997). Our data provide circumstantial evidence that xylem sap [ABA] rather than total ABA mass flux to shoots is the main signal issued by roots. Based on the half-life residence time of ABA in the mesophyll, which is as low as 30 min in cherry (Gowing et al. 1993) and 40 min in maize (Jia et al. 1996), it has been suggested that ABA is rapidly metabolized during transport from xylem vessels to guard cells (Gowing et al. 1993). Furthermore, the fraction of xylem sap ABA arriving at guard cells is small and it is likely that the concentration of ABA in the vicinity of guard cells is tightly regulated and independent of the mass flow of ABA entering the leaf (Tardieu and Davies 1993, Jia et al. 1996). However, Heckenberger et al. (1996) showed that the mass flow of ABA entering cut leaves was important for the regulation of stomatal conductance.

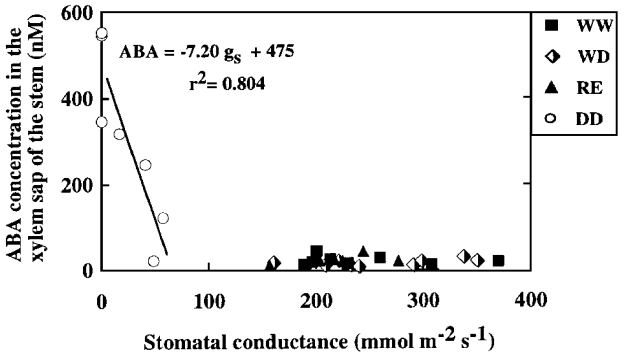

Figure 5. Relationship between stomatal conductance and ABA concentration in the xylem sap extracted from shoots of *B. pendula* seedlings grown in split root containers. Treatments were: WW = controls; WD = drought in one compartment; RE = roots severed in one compartment; and DD = drought in both compartments. Each point represents an individual measurement.

Leaf expansion was only slightly reduced in the WD treatment and root growth in the wet compartment remained close to that of the controls, although it was severely reduced in the dry compartment. The WD treatment had no effect on stomatal conductance, whole-plant transpiration, or on ABA and ABA-GE concentrations (whatever the compartment considered). This lack of response was observed even though the soil water content of the dry compartment of the WD treatment declined to values very close to those reached in the DD treatment. Our data differ from earlier reports based on split root designs that showed significant decreases in stomatal conductance when water was depleted from one root compartment (Neales et al. 1989, Gowing et al. 1990, Masia et al. 1994). We conclude that more than half of the root-explored soil needs to be depleted of water to induce drought-stress responses in birch seedlings (cf. Tan and Buttery 1982, Ebel et al. 1994). Similarly, no increase in root [ABA] was recorded in transpiring field grown maize until the whole soil profile was almost completely depleted of water (Tardieu et al. 1992).

In conclusion, birch seedlings were able to take up water from soils with very low soil water contents. However, at a threshold soil water content, predawn leaf water potential and stomatal conductance decreased markedly and leaf shedding occurred. These responses were correlated with an increase in xylem sap [ABA] of roots and shoots. When only half the root system was exposed to soil water depletion and the other half was well-watered, no stress responses were observed, and xylem sap [ABA] remained at control values.

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