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Summary Leaf conductance, water relations, growth, and abscisic acid (ABA) concentrations in xylem sap, root apices and leaves were assessed in oak seedlings (*Quercus robur* L.) grown with a root system divided between two compartments and subjected to one of four treatments: (a) well watered, WW; (b) half of root system exposed to soil drying and half kept well watered, WD; (c) whole root system exposed to drought, DD; and (d) half of root system severed, RE. Sharp decreases in plant stomatal conductance, leaf water potential, hydraulic conductance and leaf growth were observed during DD treatment. No significant differences in plant leaf water potential and stomatal conductance were detected between the WW and WD treatments. Nevertheless, the WD treatment resulted in inhibition of leaf expansion and stimulation of root elongation only in the well-watered compartment. Abscisic acid concentrations did not change in leaves, root tips, or xylem sap of WD- compared to WW-treated plants. Increased concentrations of ABA were observed in xylem sap from DD-treated plant roots, but the total flux of ABA to shoots was reduced compared to that in WW-treated plants, because of decreases in transpiration flux. Similar plant responses to the WD and RE treatments indicate that the responses observed in the WD-treated plants were probably not triggered by a positive signal originating from drying roots.

Keywords: abscisic acid, Quercus robur, root growth, shoot growth, water stress, xylem sap.

Introduction

Soil drying generally induces changes in plant growth and stomatal closure, independently of actual shoot water status. Many studies suggest that these responses to soil drying are controlled by a nonhydraulic signal that originates in the roots, is released to xylem vessels and transported through the shoot to apoplastic spaces in the leaves (Davies and Zhang 1991, Davies et al. 1994). Although the action of diverse compounds such as cytokinins cannot be excluded (Blackman and Davies 1985), abscisic acid (ABA) is considered to be the chemical most likely to be involved in this root-to-shoot signaling, because drying roots often synthesize ABA (Cornish and Zeevaart 1985, Zhang et al. 1987). The role of apoplastic ABA in leaves as a signal for stomatal closure is now widely accepted (Hartung and Slovik 1991, Davies et al. 1994). Moreover, convincing relationships between increases in ABA concentration in xylem sap and stomatal closure have sometimes been found in response to drought (Tardieu et al. 1992, Khalil and Grace 1993). In addition, many experiments have shown that ABA may also play an important role in regulating shoot and root growth in response to water stress (Watts et al. 1981, Creelman et al. 1990).

Despite wide acceptance of ABA as the signal inducing stomatal closure, there are still arguments concerning the role of ABA originating in the root in mediating a response to drought. Munns and King (1988) showed that changes in xylem sap ABA concentration $[ABA]_{xyl}$, were not sufficient to explain the reduction in transpiration rate observed during drought. Trejo and Davies (1991) and Schurr et al. (1992) observed only a weak coupling between $[ABA]_{xyl}$ and stomatal conductance. Drought-induced increases in $[ABA]_{xyl}$ have been observed in almond trees (Wartinger et al. 1990) and oak seedlings (Scuiller 1990); however, Triboulot et al. (1996) detected no significant increase in $[ABA]_{xyl}$ despite stomatal closure induced by a severe drought in adult oaks.

In addition, there is still some debate about the real nature of the ABA signal (Trejo et al. 1995). Despite large increases in $[ABA]_{xyl}$, total flux of ABA from roots to leaves sometimes decreases during drought as a result of reduced transpiration flux (Jackson et al. 1995). The relative importance of $[ABA]_{xyl}$ versus total mass flux of ABA to the leaf apoplast and guard cells may be related to ABA half-life. For example, ABA in the apoplast of *Prunus avium* L. leaves has a rapid turnover and a half-life of around 30–40 min (Gowing et al. 1993). Under such conditions of rapid turnover, total flux of ABA to leaves would be more important than increases in $[ABA]_{xyl}$. Furthermore, relative changes in pH among different leaf tissues may induce a redistribution of ABA pools, which increase ABA content in the apoplast, and thus result in stomatal closure (Hartung and Slovik 1991).

Several experimental designs have been applied to analyze the contribution to drought responses of nonhydraulic signals from the root. For example, plants grown in pressurized pots were subjected to fine control of turgor in the shoots during soil drying (Gollan et al. 1986, Passioura 1988, Gollan et al. 1992).

Others applied the technique of splitting roots into several (generally two) soil compartments (Zhang et al. 1987, Gowing et al. 1990, Khalil and Grace 1993). In these designs, the fraction of the root system in the compartment subjected to soil drying is expected to produce the signal inducing drought responses, whereas the well-watered fraction still supplies sufficient water to maintain optimal turgor in the leaves. Possible stomatal closure may then be ascribed to a nonhydraulic signal originating from roots in the dry soil compartment. This technique was applied in our study by growing oak plants (*Quercus robur* L.) with root systems divided between two separate compartments of one container. Water was withheld from one compartment while the other was kept well-irrigated. Root growth was observed through the transparent Perspex (Plexiglas) walls of the containers. This split-root system was designed: (i) to determine if growth and stomatal drought responses can result from nonhydraulic root signals; and (ii) to investigate the potential role of ABA from the root in mediating the observed responses to drought.

Material and methods

Plant material and growth conditions

In mid-March 1994, acorns of pedunculate oak were germinated on sand in a greenhouse near Nancy (northeastern France) (70% outdoor irradiance and an air temperature of 10–25 °C). After two weeks, seedlings were gently removed from the soil and the tap root was severed with a razor blade. Seedlings were kept in sand for two weeks to allow lateral roots to develop. Each root system was then split into two parts and repotted in containers that were divided vertically into two watertight compartments (each with a capacity of 7 l), with a 2/1 (v/v) sand/peat mix. Transparent sides to each compartment allowed direct observation of root growth. Plants were fertilized (5 kg m^{-3} of slow-release fertilizer Nutricote; N,P,K 13,13,13 + trace elements) and kept well watered for 4 months. Soil was covered with paraffin cardboard disks to minimize direct evaporation.

Experimental design

The following root treatments were applied: (1) control (WW, n = 11), in which all compartments were watered manually once daily; (2) half-droughted (WD, n = 9), in which one compartment was watered twice daily to drip point and the other compartment was not watered at all; (3) unwatered (DD, n = 9), in which water was withheld from both compartments; (4) half root system severed (RE, n = 6), in which all roots in one compartment were removed on Day 201, and the other compartment was watered twice daily to drip point.

In the WD and DD treatments, drought was imposed from Julian Day 203 (July 22) to Day 228 (August 16) by withholding irrigation. Plant water status, growth, stomatal conductance to water vapor and total transpiration were measured at regular intervals for four weeks until Day 228. Soil water content was recorded in the dry compartments of the WD and DD treatment containers, three times a week. Leaf, root and xylem sap ABA concentration were measured on Day 216 (RE treatment) and at the end of the drought treatment on Day 228 (WD and DD treatments only).

Growth analysis

Stem height and root-collar diameter were measured weekly from April to August. Root elongation was monitored once or twice a week by marking length increases of the visible roots on the transparent sides. Between measurements, pots were kept wrapped with black plastic to minimize the development of algae in the rhizosphere. Plants were harvested on Day 228, and separated into leaves, stems and roots for biomass measurement. All samples were dried at 80 °C for 48 h and weighed. Dry biomass of roots remaining after excision on Day 201, was determined in the three RE-treated plants. In the other treatments, roots in each compartment (dry or wet) were harvested separately (n = 5 (WW, DD, WD) or 3 (RE)).

Water relations

Volumetric soil water content in dry compartments was measured by TDR (Time Domain Reflectometry, Soil Moisture Equipment Corp., Santa Barbara, CA) with 20-cm long buried wave guides in five replicates of WD- and DD-treated plants (one wave guide per dry compartment, installed at the onset of the experiment).

Measurements were made on five seedlings per treatment, except in the case of RE-treated plants as only three individuals were kept after Day 216. Predawn (Ψ_b) and midday (Ψ_{min}) leaf water potentials were measured weekly with a pressure chamber on a mature leaf located in the middle of the shoot. Stomatal conductance to water vapor (g_s, mmol m^{-2} s^{-1}) was measured at midday (1200 to 1300 h) with a steady-state porometer (LI-1600, Li-Cor, Inc., Lincoln, NE) on three leaves per plant. Soil to leaf specific hydraulic conductance (g_L, mmol m^{-2} s^{-1} MPa^{-1}) was calculated from the relation:

$$g_L = \frac{E}{(\Psi_{min} - \Psi_b)},$$

where E = transpiration flux density (mmol m^{-2} s^{-1}), measured gravimetrically by loss of weight over 2 h, and expressed on a leaf area basis. Leaf area was measured with a DeltaT area meter (Delta-T-Devices, Burwell, England).

Extraction of xylem sap, and harvest of roots and leaves

Concentrations of ABA were measured in leaf tissues, root apices, and xylem exudate from roots and shoots of seedlings. A first series of extractions was conducted on Day 216 (3 RE-treated and 4 WW-treated plants selected at random in each treatment), and a second series on Day 228 (4 WD-, 4 DD- and 2 WW-treated plants). In the DW treatment, root samples of wet and dry compartments were harvested separately. Before extraction, seedlings were transferred to the laboratory and kept overnight in a 16 h day/night photoperiod. Extractions were made after at least 3 hours irradiance following darkness, to limit potential variability due to diurnal cycles in $[ABA]_{xyl}$ (Tardieu et al. 1992).

On each harvest date, one leaf per plant was sampled, immediately wrapped in aluminum foil and frozen in liquid nitrogen. After severing the shoot at the root collar, bark was removed to a height of 20 cm to avoid xylem sap contamination by ABA exuded from phloem tissue (Else et al. 1994). The twig was then enclosed in a Scholander pressure chamber and the pressure slightly increased above the balance pressure, while sap droplets exuding from the vessels were collected with a micropipette. About 200 µl of sap was obtained by increasing the pressure by 0.5 MPa, but significant differences in this amount were observed among treatments (Table 1). Samples were immediately frozen in liquid nitrogen. After shoot sap extraction, roots were gently washed to remove soil, and white apices were cut and frozen. Root xylem sap was collected by the same procedure as for stems. All samples were stored at –70 °C.

Extraction of ABA from leaf and root tissues, purification of xylem sap and measurements of ABA and ABA-GE

Extraction of ABA from freshly ground leaves and roots was performed in 80% methanol at 4 °C in darkness for 60 h. Tritiated ABA was added to the different samples (xylem sap and root or leaf extracts) as an internal standard to allow estimation of recovery rates. Samples were purified on a Sep-Pak C18 cartridge (Waters, USA), and injected into a reverse phase HPLC column (High Pressure Liquid Chromatography) (Lichrospher 5 mm, ODS 100 RP18, end capped 250 × 4 mm, Merck, Germany) thermoregulated at 4 °C. Ten fractions were collected, centered on the retention time of ABA (9.13 min) and ABA-GE (glucose ester of ABA) (8.32 min), and determined with a separate injection of pure ± ABA and ABA-GE standards. The ABA fractions were methylated with an ethereal solution of diazomethane, as in Label et al. (1994), to improve the cross reactivity of anti-ABA antibodies.

After purification, ABA and ABA-GE concentrations in bulk-leaf and root apice tissues and in xylem sap ($[ABA]_{xyl}$) were measured by an ELISA method (Enzyme Linked Immunosorbent Assay). Polystyrene microtitration plates (NUNC, Denmark) were coated over night at room temperature with an ABA-ovalbumine conjugate prepared from ovalbumine (Sigma, St. Louis, MO) (Label et al. 1994). After washing the plates with H_2O-Photoflo 0.1% (Kodak), either methylated ABA standard, ABA-GE standard or methylated sample was added for competition to anti-ABA rabbit polyclonal antibodies (Laboratory of Immunology, University of Nancy I, France). Plates were incubated for 2 h at 40 °C in darkness and washed, and then incubated for 1 h at 40 °C with an excess of biotinylated anti-rabbit goat antibodies (Calbiochem, France). After washing, an excess of streptavidin alkaline phosphatase conjugate (prepared as described by Label et al. 1994) was added and incubated for 1 h at 40 °C. Phosphatase activity was determined with *p*-nitrophenylphosphate (Sigma), and measured with a spectrophotometer (MR 5000, Dynatech, USA) at 405 nm after 1–2 h at 40 °C. Phosphatase activity was inversely proportional to the amount of methylated ABA in the sample.

Mass flow of ABA from roots to shoots and from roots to leaves was estimated from $[ABA]_{xyl}$ in roots and shoot, and multiplied by the transpiration rate that was measured gravimetrically for 2 h at midday the day before sap collection.

Table 1. Amount (µl) of xylem sap extracted with a pressure chamber from the shoots, and from the roots in the wet or the dry compartment of seedlings from *Quercus robur* grown in a split-root device. Treatments: WW, controls; WD, drought in a single compartment; DD, drought in both compartments; RE, roots severed in one compartment. Different letters in a column indicate significant differences at $P < 0.05$ (nonparametric Kruskall and Wallis test).

Treatment	Shoots	Roots (wet)	Roots (dry)
WW (n = 6)	158 ± 70 a	238 ± 70 ab	
WD (n = 4)	266 ± 40 b	145 ± 80 a	119 ± 80 a
DD (n = 4)	121 ± 60 a		82 ± 60 a
RE (n = 3)	346 ± 80 b	286 ± 70 b	

Results

Water relations

Control plants (WW treatment) exhibited very stable and near-optimum values of predawn leaf water potential (Ψ_b) throughout the experiment (Figure 1). Changes in midday values of incident photon flux densities (PFD) and vapor pressure deficit (VPD) were recorded during the experiment with generally low values of PFD (Figures 2i and 2ii). As a consequence, stomatal conductance (g_s) of controls (WW) varied between measurement dates. Initial g_s values were relatively low (100 mmol m^{-2} s^{-1}), but increased from Day 207 on, and decreased after Day 212 with lowest values around 200 mmol m^{-2} s^{-1} (Figure 2iii). The observed soil to leaf specific hydraulic conductance (g_L) values of 1–2 mmol m^{-2} s^{-1} MPa^{-1}, were

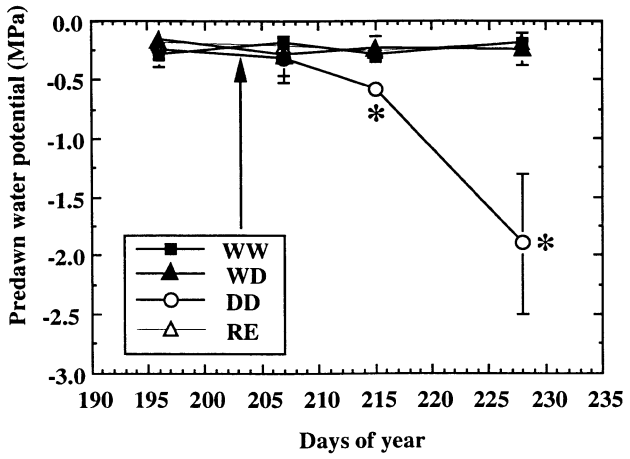

Figure 1. Time course of predawn leaf water potential of young *Quercus robur* seedlings grown in split-root containers with two compartments and submitted to the following treatments: control (WW); half-droughted (WD); unwatered (DD); half root system severed (RE). Arrow indicates beginning of treatment (n = 5 except for RE after day 216 where n = 3); * indicates significant differences from controls.

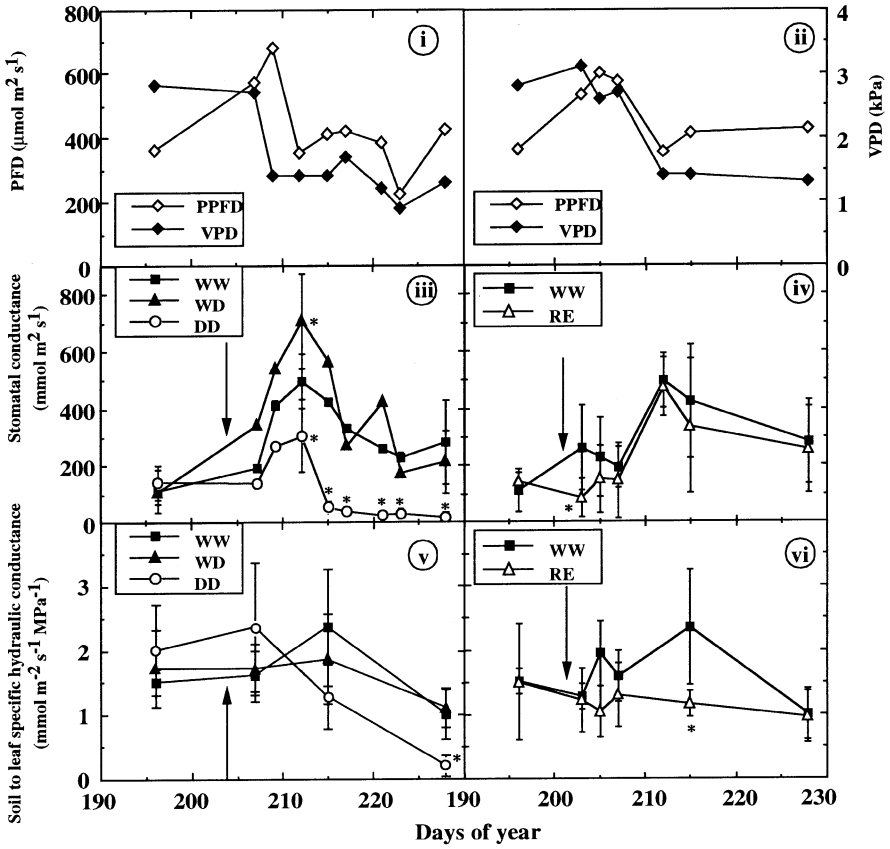

Figure 2: Time course of (i) and (ii) photosynthetic photon flux density (PPFD) and vapor pressure deficit (VPD); (iii) and (iv) stomatal conductance (g_s); and (v) and (vi) soil to leaf specific hydraulic conductance (g_L) of young *Quercus robur* seedlings grown in split-root containers and submitted to the following treatments: control (WW); half-droughted (WD); unwatered (DD); half root system severed (RE). Arrows indicate beginning of treatment. Data for stomatal conductance represent mean ± SD of three measurements times five plants. Data for hydraulic conductance represent mean ± SD of five replicates (three for RE after ABA analysis on Day 216). The SD have been omitted in a few graphs for clarity. An asterisk indicates significant differences from controls.

within the expected range of values for small oaks (Reich and Hinckley 1989, Simonin et al. 1994, Figure 2v).

Severing half of the root system (RE treatment) had no effect on Ψ_b (Table 2 and Figure 1), or g_L (Figure 2vi), hence g_s displayed lower values two days after excision, and then recovered rapidly to control values (Figure 2iv).

Volumetric soil water content in dry compartments gradually declined to minimal values of 5.4% and 4.3% in the WD- and DD-treated plants, respectively, at the end of the experiment. Drought caused a gradual decrease in predawn leaf water potential (Ψ_p) in DD-treated plants, but had no detectable effect on WD leaf water potential (Table 2, Figure 1). Up to Day 214, WD-treated plants showed larger values of g_s than the controls (WW); thereafter, these differences decreased (Figure 2iii). In DD-treated plants, g_s declined with respect to that of WW-treated plants from Day 211 on, that is, after volumetric soil water content had decreased to around 9%. After Day 215, stomatal closure of the DD-treated plants was almost

Table 2. Soil water content in the dry compartment, predawn leaf water potential and whole-plant transpiration measurements taken at the beginning and end of the experiment on seedlings of *Quercus robur* with roots split between two containers. Treatments: WW, controls; WD, drought in a single compartment; DD, drought in both compartments; RE, roots severed in one compartment. Values are the means ± SD. Within rows, different letters indicate significant differences ($P < 0.05$, ANOVA followed by Fisher's PLSD test); $n = 5$ (WW, WD, DD) or $n = 3$ (RE).

	WW	WD	DD	RE
Soil water content (%)				
Initial		17.8 ± 3 a	17.8 ± 2 a	
Final		5.4 ± 2 b	4.3 ± 1 b	
Predawn leaf water potential (MPa)				
Initial	–0.30 ± 0.1 a	–0.17 ± 0.1 a	–0.25 ± 0.1 a	–0.2 ± 0.1 a
Final	–0.20 ± 0.1 a	–0.25 ± 0.1 a	–1.90 ± 0.6 b	–0.2 ± 0.1 a
Transpiration (mmol m^{-2} s^{-1})				
Initial	2.17 ± 0.3 a	2.80 ± 0.4 a	3.30 ± 0.2 a	1.8 ± 0.2 a
Final	1.00 ± 0.2 a	1.33 ± 0.4 a	0.13 ± 0.1 b	1.3 ± 0.3 a

complete (Figure 2iii). A gradual decrease of g_L in response to drought paralleling the decrease in g_s was observed in these plants (Day 215), but was only significant on Day 228. No significant difference in g_L was detected between WD- and WW-treated plants during the whole experiment, except on Day 215 (Figure 2v).

Shoot and root growth

At the end of the experiment, almost all oak seedlings had completed four growth flushes and were about 80 cm tall. No treatment-induced differences in final height and diameter were detected among WW-, RE-, WD- and DD-treated plants (Table 3). Whole plant biomass was significantly reduced in WD, DD, and RE treatments. Because plants were subjected to drought after completion of the third shoot flush, no differences in average leaf area or leaf dry mass of Flushes 1, 2 or 3 were observed among treatments (data not shown). Average area of individual leaves from Flush 4 was markedly reduced with respect to WW in all treatments (–35% for DW, –55% for DD and –72% for RE). Total leaf dry mass on Flush 4 was similar to WD, DD or RE treatments and lower than in the WW treatment (Table 3).

Total root biomass was significantly lower in WD-, DD- and RE-treated plants than in WW-treated plants (Table 3). An examination of values from single compartments revealed important differences among treatment effects (Figure 3). The DD treatment reduced total root biomass compared to the WW treatment. In WD-treated plants, root biomass was severely reduced in the dry compartment, even to a larger extent than in DD-treated plants, whereas in the wet compartment, root accumulation was similar to that in WW-treated plants. Severing half of the root system (RE) resulted in significantly higher root biomass in the remaining compartment than in WW-treated plants. Random heterogeneity among compartments in WW-treated plants was relatively important; the mean difference in individual plants was 17.6 g ± 6.65 (significantly different from 0 at $P = 0.004$), i.e., 66% of single compartment biomass. This difference in means was much lower in DD-treated plants because biomass accumulation was less (4.55 g ± 4.81, $P = 0.102$). However, it is unlikely that observed differences between wet and dry compartments in WD-treated plants (20 g greater in the wet compartment) could be ascribed solely to such a heterogeneity, as compartments were randomly chosen before drought.

The WW-treated plants showed sustained root elongation throughout the experiment (Figure 4). After two weeks in dry soil, root elongation had almost stopped in DD-treated plants. Root elongation was slowed in the dry compartment of WD-treated plants, and enhanced in the wet compartment (+38% total length on Day 228 compared to control values). Elongation of the remaining roots of RE-treated plants was increased (+66% in total length two weeks after excision and +133% at the end of the experiment as compared with WW-treated plants).

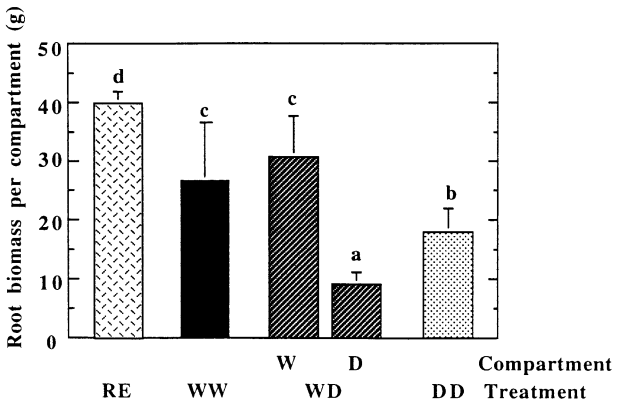

Figure 3. Root dry weight per compartment of young *Quercus robur* seedlings grown in split-root containers and submitted to the following treatments: control (WW); half-droughted (WD); unwatered (DD); half root system severed (RE). Measurements were made at the end of the drought. Each plant compartment was measured separately. Means ± SD of five replicates ($n = 5$ for WD-, DD-, WW- and $n = 3$ for RE-treated plants). Different letters indicate significant differences at $P < 0.05$.

Table 3. Shoot height, diameter at the root collar and biomass of seedlings of *Quercus robur* with roots split between two containers. Treatments: WW, controls; WD, half-droughted; DD, unwatered; RE, roots severed in one compartment. Total biomass and mean individual area are indicated for leaves expanded during the 4th growth flush, i.e., during drought treatment or following root severing. Values are means ± SD. Within rows, different letters indicate significant differences ($P < 0.05$, ANOVA followed by Fisher's PLSD test); $n = 5$ (WW, WD, DD) or $n = 3$ (RE).

Growth parameter	WW	WD	DD	RE
Height (m)	0.89 ± 0.2 a	0.76 ± 0.2 a	0.65 ± 0.3 a	0.74 ± 0.1 a
Diameter (mm)	15.3 ± 1 a	13.4 ± 1 a	13.0 ± 2 a	14.0 ± 1 a
Whole plant biomass (g)	110.3 ± 19 a	80.0 ± 15 b	71.5 ± 10 b	82.7 ± 10 b
Root dry weight (g)	53.5 ± 8 a	38.2 ± 8 b	36.1 ± 5 b	40.1 ± 2 b
Shoot dry weight (g)	56.7 ± 13 a	41.8 ± 8 b	35.4 ± 5 b	42.5 ± 9 ab
Leaf dry weight (g), Flush 4	11.3 ± 3 a	7.5 ± 1 b	6.3 ± 2 b	6.5 ± 4 b
Average leaf area (mm^2), Flush 4	3630 ± 596 a	2364 ± 689 b	1636 ± 367 c	1009 ± 482 d

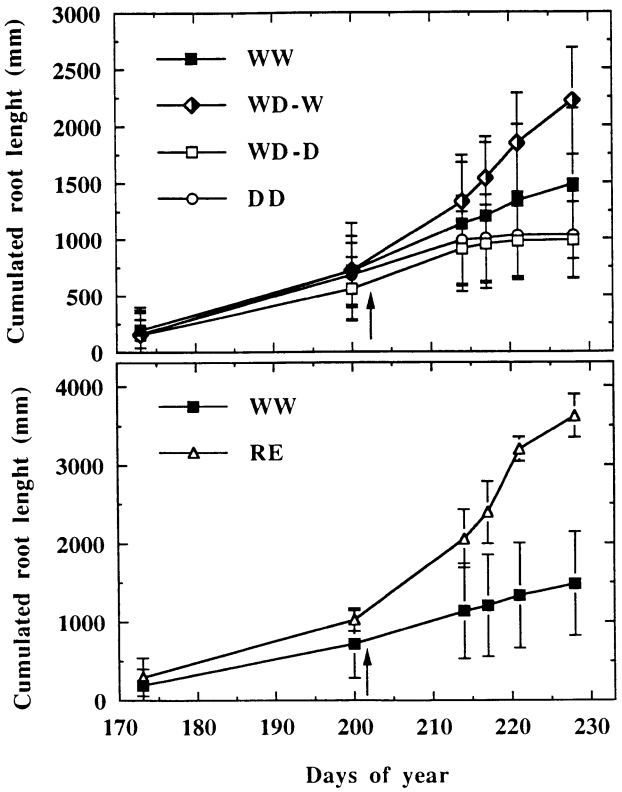

Figure 4. Time course of total root length of young *Quercus robur* seedlings grown in split-root containers and submitted to the following treatments: control (WW); half-droughted (WD); unwatered (DD); half root system severed (RE). Arrows indicate beginning of treatment. Each plant compartment was measured separately. Points represent means ± SD; $n = 5$ (WD, WW and DD), and $n = 3$ (RE).

ABA and ABA-GE concentrations in leaves, roots and xylem sap

ABA concentrations in xylem sap and tissues displayed several distinct features (Table 4): (i) ABA concentrations in leaf tissues and root apices did not increase in response to any treatment; ABA-GE concentrations were large in the leaves (an order of magnitude larger than ABA), but below the detection threshold of our ELISA test in root tips; (ii) there were sometimes large differences among individuals in ABA concentrations in xylem sap $[ABA]_{xyl}$; in particular, $[ABA]_{xyl(shoot)}$ in the WW treatment was above 200 nM in three plants and below 100 nM in three other plants; for other treatments and $[ABA]_{xyl(roots)}$, variability was much lower; ABA-GE in all xylem sap samples remained below the detection threshold of the ELISA test; (iii) in all treatments there was a poor correlation between $[ABA]_{xyl}$ in roots and shoots as computed from ($[ABA]_{xyl(shoot)} = 0.43\ [ABA]_{xyl(roots)} + 50.9$, $r^2 = 0.240$, $n = 17$) as a result of three divergent WW-treated plants that had high values of $[ABA]_{xyl(shoot)}$. Excluding these values significantly improved the correlation ($[ABA]_{xyl(shoot)} = 0.48\ [ABA]_{xyl(roots)} + 13.6$, $r^2 = 0.754$, $n = 14$); (iv) comparison between DD- and WW-treated plants revealed significantly higher values of $[ABA]_{xyl(roots)}$ (approximately threefold), but not of $[ABA]_{xyl(shoot)}$ in DD-treated plants because of high variability in WW-treated plants; (v) no significant difference in $[ABA]_{xyl(roots)}$ and $[ABA]_{xyl(shoot)}$ was observed between WD- and WW-treated plants; however, $[ABA]_{xyl(shoot)}$ in WD-treated plants was close to the three lowest values in WW-treated plants; in all cases, $[ABA]_{xyl}$ of WD-treated plants was significantly lower than that of DD-treated plants; (vi) total apparent flux of ABA was computed on a leaf area basis from total water

Table 4. Concentration of ABA in the xylem sap $[ABA]_{xyl}$ extracted from stem and roots, and in leaf tissues and root apices of young *Quercus robur* seedlings grown in split-root containers. Treatments: WW, controls; WD, drought in a single compartment; DD, drought in both compartments; RE, roots severed in one compartment. Measurements made at end of treatment. Means ± SD. Within rows, different letters indicate significantly different values at $P < 0.05$ (nonparametric test of Kruskall and Wallis).

	Compartment	WW ($n = 6$)	WD ($n = 4$)	DD ($n = 4$)	RE ($n = 3$)
Stem $[ABA]_{xyl}$ (nM)		148.4 ± 106 ab	27.2 ± 13 b	142.8 ± 59 a	35.5 ± 15 b
Root $[ABA]_{xyl}$ (nM)	Dry		82.3 ± 16 a	275.5 ± 77 b	29.9 ± 9 a
	Wet	71.8 ± 41 a	85.2 ± 37 a		
Leaf ABA concentration ($pmol\ g_{FW}^{-1}$)		79.6 ± 42 a	48.6 ± 11 a	39.8 ± 19 a	79.6 ± 16 a
ABA in root apices ($pmol\ g_{FW}^{-1}$)	Dry		24.0 ± 10 a	57.6 ± 28 a	
	Wet	31.1 ± 15 a	23.3 ± 8 a		18.1 ± 6 a
Leaf ABA-GE ($pmol\ g_{FW}^{-1}$)		1159 ± 996 a	572 ± 236 a	961 ± 229 a	1452 ± 237 a
		($n = 3$)	($n = 4$)	($n = 4$)	($n = 3$)
Total ABA flux to leaves ($pmol\ m^{-2}\ s^{-1}$) computed from stem $[ABA]_{xyl}$		3.33 ± 1 a	0.58 ± 0.3 b	0.31 ± 0.3 b	1.07 ± 0.4 b
Total ABA flux to leaves ($pmol\ m^{-2}\ s^{-1}$) computed from root $[ABA]_{xyl}$		2.36 ± 1.33 a	1.80 ± 0.1 ab	0.50 ± 0.4 b	0.89 ± 0.2 ab

consumption determined gravimetrically 1 day before extraction, and from either $[ABA]_{xyl(shoot)}$ or $[ABA]_{xyl(roots)}$; it was between 0.5–3 pmol m^{-2} s^{-1} in all treatments with one exception (computation based on $[ABA]_{xyl(shoot)}$ in WW-treated plants). In both cases, total ABA flux was lower in DD-treated than in WW-treated plants, because of very low transpiration rates.

Discussion

A gradual decrease in soil water content to 20% of field capacity affected both water relations and seedling growth of pedunculate oak (*Quercus robur*). Predawn leaf water potential declined, and stomatal conductance, leaf expansion and root growth were severely reduced. It has been assumed that such responses are partly induced by metabolic signals produced by roots, in particular by ABA transported from drying roots to shoots (see review by Davies et al. 1994). We investigated this assumption by studying split-root seedlings subjected to four treatments (WW, well-watered controls; DD, drought in both root compartments; WD, drought in only one compartment; and RE, half the roots severed). Plants in the DD treatment showed: (i) no increases in ABA concentrations of leaf tissues and root tips, and (ii) significant (threefold) increases in root xylem sap ABA concentrations $[ABA]_{xyl}$.

Similar results showing no increase in ABA concentration for water stressed leaves have been observed before (Zhang et al. 1987 and Davies et al. 1990); however, contrary results showing significant ABA increases have also been reported (Neales et al. 1989, Khalil and Grace 1993, Gallardo et al. 1994). Based on previous studies, it can be stated that: (i) root signaling and stomatal closure are commonly described as occurring before any change in total leaf ABA; and (ii) an increase in total ABA in root apices is not a prerequisite to ABA delivery via transpiration flux on the basis of a pH driven ABA redistribution model (Daeter et al. 1993). Similarly, large changes in apoplastic ABA can occur in response to pH drifts in leaf tissues without any new ABA synthesis (Hartung and Slovik 1991).

Xylem sap concentrations of ABA in controls were within the range of values reported for well-watered trees; i.e., generally between 50 and 250 μmol m^{-3} (Loveys et al. 1987, Wartinger et al. 1990, Khalil and Grace 1993, Bertrand et al. 1994). Triboulot et al. (1996) found similar values in adult trees and Scuiller (1990) found slightly higher values in *Q. petraea* L. ex Liebl. and *Q. robur* seedlings. The drought-induced increase in ABA remained moderate in DD-treated plants, similar to that observed by Khalil and Grace (1993) in *Acer pseudoplatanus* L., but much lower than for *Prunus dulcis* (Mill.) D.A. Webb (Wartinger et al. 1990) or *Pinus sylvestris* L. or *Picea abies* (L.) Karst. (Jackson et al. 1995). Despite the increase in $[ABA]_{xyl}$, ABA fluxes from roots to leaves in DD-treated plants were lower or similar to those in control plants. Similar results were reported by Tardieu et al. (1993), Jackson et al. (1995), and Triboulot et al. (1996), whereas Schurr et al. (1992) reported a sevenfold increase of ABA flux to leaves in *Helianthus annuus* L. Whether ABA delivery rate to leaves or concentration in the xylem sap arriving at leaves is more significant for control of stomatal aperture has recently been investigated by Gowing et al. (1993) and Trejo et al. (1993, 1995). These authors suggest that ABA concentration in the vicinity of guard cells was probably lower than in xylem sap, and that mesophyll cells exerted some control on the rate of ABA flux from xylem vessels to guard cells, probably through differential trapping and metabolization, or through recycling to the phloem (as modeled by Hartung and Slovik 1991). Simply, leaf tissues significantly modulate the apparent sensitivity of stomatal conductance to $[ABA]_{xyl}$. Tardieu and Davies (1992) showed that the apparent sensitivity of stomata to $[ABA]_{xyl}$ was increased when leaf water potential decreased. Correia and Pereira (1995) demonstrated that the apparent sensitivity of lupin stomata varied during drought. Furthermore, adaxial and abaxial stomata of amphistomatous leaves are known to respond differently to exogenous ABA (see Lancaster et al. 1977, Pemadasa 1981, Henson and Turner 1991, Correia and Pereira 1995, Ridolfi et al. 1996). Thus, the reduction or stability of ABA flux to shoots accompanying drought-induced stomatal closure shows either: (i) a limited role of ABA from roots in stomatal control during drought; or (ii) changes in apparent stomatal sensitivity to $[ABA]_{xyl}$ during the course of drought. A low sensitivity would be expected at the end of our experiment.

Information about the role of root-to-shoot signaling in drought responses was gained from plants in the WD treatment, where only half the root system was subjected to drought. Split-root experiments have often been conducted to establish that stomatal closure could be controlled by a nonhydraulic signal issued from the drying part of the root system (Blackman and Davies 1985, Zhang et al. 1987, Neales et al. 1989, Gowing et al. 1990, Bano et al. 1993, Khalil and Grace 1993). Our results did not support this general scheme. Soil water contents at the end of the experiment were similar in the dry compartments of both WD- and DD-treated plants (5.4% and 4.3%, respectively) but the WD treatment did not reduce stomatal conductance or total water consumption, as observed by Gallardo et al. (1994). Water absorption in the well-watered compartment was sufficient to sustain transpiration rate during gradual drought increase in the other WD compartment, likely because of: (i) high hydraulic efficiency of roots in the wet compartment; (ii) compensatory root growth in the wet compartment; and (iii) increased watering frequency to sustain absorption in a reduced soil volume. A dilution of ABA exported from drying roots in the overall transpiration flux, may explain the lack of an increase of $[ABA]_{xyl}$ in the shoot, and the absence of stomatal closure. Exposing a larger fraction of the root system to drought may result in increased ABA delivery to shoots (Ebel et al. 1994). Nevertheless, this dilution of ABA in the transpiration flux could not be confirmed: no increase in ABA concentration was detected in root tips and xylem sap extracted from drought-treated roots of WD-treated plants, despite the severe water stress that led to complete cessation of elongation in this compartment.

On the other hand, growth of WD-treated plants was only slightly affected by the drought treatment (decrease in leaf

expansion, and increase in root elongation in the wet compartment). It has been argued that leaf elongation is more sensitive to soil drying and to potential nonhydraulic signals than stomatal conductance. Saab and Sharp (1989) observed no stomatal closure and a 20% reduction of leaf growth in maize seedlings grown in split-root pots. Similarly, Ebel et al. (1994) detected significant effects on leaf growth in *Sorghum* with no change in stomatal conductance. Such effects have also been detected in field experiments (Sadras et al. 1993, with sunflower). Severing half the root system (RE treatment) resulted in similar effects as the WD treatment (reduction in leaf expansion and growth enhancement of remaining roots) plus a transitory decline of stomatal conductance. The responses were greater, because of the sudden change in root/shoot ratio, and cannot be explained by a positive nonhydraulic signal. This similarity between drought and root severing treatments is an additional argument against the occurrence of root signaling effects in the WD treatment.

In conclusion, our study of oak seedlings grown in split-root devices yielded indefinite results: root signalling (ABA efflux from roots) probably occurred when both root compartments were drying out, but could not be demonstrated in the case where only one compartment was depleted of water. Oak seedlings display plasticity in their response to drought, extracting water efficiently from a reduced volume of soil, and increasing root growth in the well-watered soil compartment in response to localized drought. No significant release of ABA was detected in drying roots although root growth was inhibited. Efflux of ABA is probably controlled not only by the local water status of root tips, but also by a more integrated process occurring at the whole-root level.

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References

Bano, A., K. Dörffling, D. Bettin and H. Hahn. 1993. Abscisic acid and cytokinins as possible root-to-shoot signals in xylem sap of rice plants in drying soil. Aust. J. Plant Physiol. 20:109–115.

Bertrand, A., G. Robitaille, P. Nadeau and R. Boutin. 1994. Effects of soil freezing and drought stress on abscisic acid content of sugar maple sap and leaves. Tree Physiol. 14:413–426.

Blackman, P.G. and W.J. Davies. 1985. Root-to-shoot communication in maize plants and the effects of soil drying. J. Exp. Bot. 36:39–48.

Correia, M.J. and J.S. Pereira. 1995. The control of leaf conductance of white lupin by xylem ABA concentrations decreases with the severity of water deficits. J. Exp. Bot. 46:101–110.

Cornish, K. and J.A.D. Zeevaart. 1985. Abscisic acid accumulation by roots of *Xanthium strumarium* L. and *Lycopersicon esculentum* Mill. in relation to water stress. Plant Physiol. 79:653–658.

Creelman, R.J., H.S. Mason, R.J. Benson, J.S. Boyer and J.E. Mullet. 1990. Water deficit and abscisic acid cause differential inhibition of shoot versus root growth in soybean seedlings. Plant Physiol. 92:205–214.

Daeter, W., S. Slovik and W. Hartung. 1993. The pH gradients in the root system and the abscisic acid concentration in xylem and apoplastic saps. Philos. Trans. R. Soc. Lond. B 341:49–56.

Davies, W.J., T.A. Mansfield and A.M. Hetherington. 1990. Sensing of soil water status and the regulation of plant growth and development. Plant Cell Environ. 13:709–719.

Davies, W.J. and J. Zhang. 1991. Root signals and the regulation of growth and development of plants in drying soil. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42:55–76.

Davies, W.J., F. Tardieu and C.L. Trejo. 1994. How do chemical signals work in plants that grow in drying soil? Plant Physiol. 104:309–314.

Ebel, R.C., A.J.W. Stodola, X. Duan and R.M. Augé. 1994. Nonhydraulic root-to-shoot signalling in mycorrhizal and non-mycorrhizal sorghum exposed to partial soil drying or root severing. New Phytol. 127:495–505.

Else, M.A., W.J. Davies, P.N. Whitford, K.C. Hall and M.B. Jackson. 1994. Concentrations of abscisic acid and other solutes in xylem sap from root systems of tomato and castor-oil plants are distorted by wounding and variable sap flow rates. J. Exp. Bot. 45:317–323.

Gallardo, M., N.C. Turner and C. Ludwig. 1994. Water relations, gas exchange and abscisic acid content of *Lupinus cosentinii* leaves in response to drying different proportions of the root system. J. Exp. Bot. 45:909–918.

Gollan, T., J. Passioura and R. Munns. 1986. Soil water status affects the stomatal conductance of fully turgid wheat and sunflower leaves. Aust. J. Plant Physiol. 13:459–464.

Gollan, T., U. Schurr and E.-D. Schulze. 1992. Stomatal response to drying soil in relation to changes in the xylem sap composition of *Helianthus annuus*. I. The concentration of cations, anions, amino acids in, and pH of the xylem sap. Plant Cell Environ. 15:551–559.

Gowing, D.J.G., W.J. Davies and H.G. Jones. 1990. A positive root-sourced signal as an indicator of soil drying in apple, *Malus* × *domestica* Borkh. J. Exp. Bot. 41:1535–1540.

Gowing, D.J.G., H.G. Jones and W.J. Davies. 1993. Xylem-transported abscisic acid—the relative importance of its mass and its concentration in the control of stomatal aperture. Plant Cell Environ. 16:453–459.

Hartung, W. and S. Slovik. 1991. Physicochemical properties of plant growth regulators and plant tissues determine their distribution and redistribution: stomatal regulation by abscisic acid leaves. Tansley Review No. 35. New Phytol. 119:361–382.

Henson, I.E. and N.C. Turner. 1991. Stomatal responses to abscisic acid in three lupin species. New Phytol. 117:529–534.

Jackson, G.E., J. Irvine, J. Grace and A.M. Khalil. 1995. Abscisic acid concentrations and fluxes in droughted conifer saplings. Plant Cell Environ. 18:13–22.

Khalil, A.M. and J. Grace. 1993. Does xylem sap ABA control the stomatal behaviour of water stressed sycamore (*Acer pseudoplatanus* L.) seedlings? J. Exp. Bot. 44:1127–1134.

Label, P., N. Imbault and M. Villar. 1994. ELISA quantitation and GC-MS identification of abscisic acid in stigma, ovary and pedicel of pollinated poplar flowers (*Populus nigra* L.). Tree Physiol. 14:521–530.

Lancaster, J.E., J.D. Mann and N.G. Porter. 1977. Ineffectiveness of abscisic acid in stomatal closure of yellow lupin, *Lupinus luteus* var. Weiko III. J. Exp. Bot. 28:184–191.

Loveys, B.R., S.P. Robinson and W.J.S. Downton. 1987. Seasonal and diurnal changes in abscisic acid and water relations of apricot leaves (*Prunus armeniaca* L.). New Phytol. 107:15–27.

Munns, R. and R.W. King. 1988. Abscisic acid is not the only stomatal inhibitor in the transpiration stream of wheat plant. Plant Physiol. 88:703–708.

Neales, T.F., A. Masia, J. Zhang and W.J. Davies. 1989. The effects of drying part of the root system of *Helianthus annuus* on the abscisic acid content of roots, xylem sap and leaves. J. Exp. Bot. 40:1113–1120.

Passioura, J.B. 1988. Root signals control leaf expansion in wheat seedlings growing in drying soil. Aust. J. Plant Physiol. 19:565–576.

Pemadasa, M.A. 1981. Abaxial and adaxial stomatal behaviour and responses to fusicoccin on isolated epidermis of *Commelina communis*. New Phytol. 89:373–384.

Reich, P.B. and T.M. Hinckley. 1989. Influence of predawn water potential and soil to leaf hydraulic conductance on maximum daily leaf conductance in two oak species. Funct. Ecol. 3:719–726.

Ridolfi, M., M.L. Fauveau, P. Label, J.P. Garrec and E. Dreyer. 1996. Responses to water stress in an ABA-unresponsive hybrid poplar (*Populus koreana* × *trichocarpa* cv Peace). 1. Sensitivity of stomata to closure stimuli. New Phytol. 134:445–456.

Saab, I.N. and R.E. Sharp. 1989. Nonhydraulic signals from maize roots in drying soil: inhibition of leaf elongation but not stomatal conductance. Planta 179:466–474.

Sadras, V.O., F.J. Villalobos, E. Fereres and D.E. Wolfe. 1993. Leaf responses to soil water deficits: comparative sensitivity of leaf rate and leaf conductance in field-grown sunflower (*Helianthus annuus* L.). Plant Soil 153:189–194.

Schurr, U., T. Gollan and E.-D. Schulze. 1992. Stomatal response to drying soil in relation to changes in the xylem sap composition of *Helianthus annuus*. II. Stomatal sensitivity to abscisic acid imported from the xylem sap. Plant Cell Environ. 15:561–567.

Scuiller, I. 1990. Exploration de la variabilité des comportements écophysiologiques de semis de chênes blancs européens soumis à la sécheresse. Thèse de l'Université de Nancy I, Nancy, France, 124 p.

Simonin, G., H. Cochard, C. Delatour, A. Granier and E. Dreyer. 1994. Vulnerability of young oaks (*Quercus robur*) to embolism during water stress and after an inoculation with *Ophiostoma querci*. Ann. Sci. For. 51:493–504.

Tardieu, F., J. Zhang, N. Katerji, O. Bethenod, S. Palmer and W.J. Davies. 1992. Xylem ABA controls the stomatal conductance of field grown maize subjected to soil compaction or soil drying. Plant Cell Environ. 16:413–420.

Tardieu, F. and W.J. Davies. 1992. Stomatal response to abscisic acid is a function of current plant water status. Plant Physiol. 98:540–545.

Tardieu, F., J. Zhang and D.J.G. Gowing. 1993. Stomatal control by both [ABA] in the xylem sap and leaf water status—a test of a model for droughted or ABA-fed field grown maize. Plant Cell Environ. 16:413–420.

Trejo, C.L. and W.J. Davies. 1991. Drought-induced closure of *Phaseolus vulgaris* stomata precedes leaf water deficit and any increase in xylem ABA concentration. J. Exp. Bot. 42:1507–1516.

Trejo, C.L., W.J. Davies and L.M.P. Ruiz. 1993. Sensitivity of stomata to ABA: an effect of the mesophyll. Plant Physiol. 102:497–502.

Trejo, C.L., A.L. Clephan and W.J. Davies. 1995. How do stomata read abscisic acid signals? Plant Physiol. 109:803–811.

Triboulot, M.B., M.L. Fauveau, N. Breda, P. Label and E. Dreyer. 1996. Stomatal conductance and xylem-sap abscisic acid (ABA) in adult oak trees during a gradually imposed drought. Ann. Sci. For. 53:207–220.

Wartinger, A., H. Heilmeier, W. Hartung and E.-D. Schulze. 1990. Daily and seasonal courses of leaf conductance and abscisic acid in the xylem sap of almond trees (*Prunus dulcis* (Mill.) D.A. Webb) under desert conditions. New Phytol. 116:581–587.

Watts, S., J.L. Rodriguez, S.E. Evans and W.J. Davies. 1981. Root and shoot growth of plants treated with abscisic acid. Ann. Bot. 47:595–602.

Zhang, J., U. Schurr and W.J. Davies. 1987. Control of stomatal behavior by abscisic acid which apparently originates in the roots. J. Exp. Bot. 38:1174–1181.