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Compared sensitivity of seedlings from 3 woody species (*Quercus robur* L, *Quercus rubra* L and *Fagus silvatica* L) to water-logging and associated root hypoxia: effects on water relations and photosynthesis

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Summary – Seedlings of *Quercus robur*, *Q. rubra* and *Fagus silvatica* were submitted to a period of partial (water table at 6 cm below ground) or total water-logging for 4 weeks. Important disorders were induced by the latter treatment in growth (root decay, partial leaf wilting), water relations (decreased predawn water potential) and photosynthesis (stomatal closure, reduced net assimilation rates, lowered O₂ evolution under saturating CO₂ and irradiance, and limited photochemical efficiency of PS II). It has been concluded that the observed stomatal closure was accompanied by strong disorders at chloroplast level, which happened without visible water-logging-induced deficiencies in mineral nutrient supply. Reactions to partial water-logging were much more limited. *F. silvatica* displayed the strongest disorders in response to both treatments, *Q. robur* showed only slight stress effects in response to partial water-logging and *Q. rubra* had intermediate behaviour. These observations are in agreement with the reported differences in sensitivity to water-logging of adult trees in the stand. The precise chain of events leading to these disorders in the shoots of water-logged seedlings remains to be elucidated.

stomatal conductance / hydraulic conductance / mineral nutrition / photochemistry / photosystem II

Abbreviations. ψ_{wm} and ψ_{wd} : midday and predawn leaf water potential (MPa); *PFD*: photon flux density ($\mu\text{mol m}^{-2} \text{s}^{-1}$); *A*: net CO₂ assimilation rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$); g_w : leaf conductance to water vapour ($\text{mmol m}^{-2} \text{s}^{-1}$); c_i : intercellular concentration of CO₂ ($\mu\text{mol mol}^{-1}$); Δw : leaf to air difference in vapour mole fraction; g_L : specific hydraulic conductance from soil to leaves ($\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$); *F*₀, *F*_m and *F*'₀, *F*'_m: basal and maximal fluorescence after dark adaptation and 10 min at 220 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively; *F*/*F*_m: photochemical efficiency of PS II in dark-adapted leaves; $\Delta F/F_m'$ and F_v/F_m' : photochemical efficiency of PS II and of open PS II centres after 10 min at a given irradiance (220 or 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$); *qp*: photochemical quenching of fluorescence; *A*_{max}: maximal rate of photosynthetic O₂ evolution under 5% CO₂ and 800 $\mu\text{mol mol}^{-1}$ irradiance ($\mu\text{mol O}_2 \text{m}^{-2} \text{s}^{-1}$); *C*: control treatment; *PF*: partially water-logged treatment; *F*: completely water-logged treatment.

Résumé – Sensibilité de jeunes plants de chêne pédonculé (*Quercus robur* L), de chêne rouge d'Amérique (*Q. rubra* L) et de hêtre (*Fagus silvatica* L) à l'ennoyage et l'hypoxie racinaire : effets sur la photosynthèse et les relations hydriques. De jeunes plants de chêne pédonculé (*Quercus robur* L), de chêne rouge d'Amérique (*Q. rubra* L) et de hêtre (*Fagus silvatica* L) ont été soumis à un ennoyage total (nappe affleurant en permanence à la surface des pots) ou partiel (nappe à 6 cm sous le niveau du sol) pendant 4 sem. Le premier traitement a fortement perturbé la croissance des plants en provoquant une importante mortalité racinaire. Des dysfonctionnements majeurs ont aussi été constatés sur les parties aériennes : diminution du potentiel hydrique de base, fermeture des stomates, limitation de l'assimilation nette de CO_2 et de la capacité photosynthétique (mesurée par le dégagement d' O_2 en conditions de CO_2 et d'éclairement saturants), réductions irréversibles de l'efficacité photochimique du photosystème II. Le second a provoqué des réactions plus limitées. D'importantes différences interspécifiques ont été constatées ; *F. silvatica* a présenté la plus grande sensibilité, avec des nécroses foliaires très étendues, et des réductions massives de la capacité photosynthétique dans les 2 traitements, alors que *Q. robur* n'a que peu réagi à l'ennoyage partiel. Ces résultats sont en accord avec les observations sur les exigences écologiques de ces espèces en peuplement. Enfin, elles démontrent que les désordres imposés à la photosynthèse par l'ennoyage sont dus à la conjonction d'une fermeture des stomates et d'importants dysfonctionnements au niveau cellulaire, qui n'ont pas été induits par une dégradation de la disponibilité en éléments minéraux, les concentrations totales en N, P, K, Ca, Mg, S mesurées au niveau foliaire n'ayant que peu changé au cours des traitements.

conductance stomatique / conductance hydraulique / nutrition minérale / photochimie / photosystème II

INTRODUCTION

Temporary water-logging is a very common occurrence in the plain forests of north-eastern France. Oak stands in particular frequently grow on soils with temporary high water tables, which produce gleyic or pseudo-gleyic accumulation layers in the soil profile (Becker and Lévy, 1986). Water-logging has both direct (poor growth) and indirect consequences (shallow rooting predisposing trees to summer water stress) for tree growth and productivity (Becker and Lévy, 1986). Oak species present different sensitivities to this constraint: *Quercus robur* is known to exhibit a lower sensitivity than *Q. petraea* to direct effects of soil hypoxia, but also to display some difficulties in coping with periods of drought following water-logging (Becker and Lévy, 1986; Lévy *et al*, 1986). *Q. rubra*, which is now widely afforested in France, is suspected to be

even more water-logging intolerant than both the indigenous species (Belgrand, 1983). *Fagus silvatica* is known to be strongly intolerant, and never occurs on soils in which temporary water tables occur.

The effects of water-logging on woody species have frequently been analysed (Kozłowski, 1982). Water-logging induces soil hypoxia and decreases redox-potential (Gambrell *et al*, 1991) which may impair root metabolism (Konings and Lambers, 1991), decrease nitrogen availability through denitrification (Drew, 1983), and promote the accumulation of toxic species such as reduced manganese or iron cations (Gambrell *et al*, 1991). Root dysfunctions in turn induce marked stress effects on shoots. Reduced root hydraulic conductance (Andersen *et al*, 1984; Harrington, 1987; Smit and Stachowiak, 1988) has sometimes been reported to promote decreases in leaf water potential (Zaerr, 1983; Osonubi and

Osundina, 1987). Stomatal closure and associated decreases in net CO₂ assimilation are now considered as general responses to root anoxia (see, for instance Dreyer *et al.*, 1991; Pezeshki, 1991; Reece and Riha, 1991; Topa and Cheeseman, 1992). Reductions in growth, appearance of leaf necroses and decreases in leaf nutrient contents have also been frequently described (Colin-Belgrand *et al.*, 1991; Drew, 1991).

The physiological mechanisms leading to these disorders in shoot behaviour are poorly understood. It is now widely accepted that the decreases in leaf water potential due to reduced hydraulic conductance do not form the trigger mechanism leading to stomatal closure during water-logging, and that hormonal signals must be involved. Root issues abscisic acid (ABA) is thought to be this signal during water-logging (Bradford, 1983; Jackson and Hall, 1987; Zhang and Davies, 1987). Large amounts of ethylene are issued during root hypoxia and seem to induce some of the growth reactions like the appearance of root aerenchyma (Jackson, 1985; Voesenek *et al.*, 1992), but an involvement in stomatal behaviour and photosynthesis regulation remains to be demonstrated. There are still many open questions about how leaf photosynthesis is impaired. Water-logging-induced decreases of net CO₂ assimilation rates (*A*) have been reported to occur at constant or even increasing values of intercellular CO₂ concentrations (*c_i*) (Pezeshki and Sundstrom, 1988; Smith and Ager, 1988; Dreyer *et al.*, 1991; Vu and Yelenovski, 1991), which would mean that photosynthetic processes other than the diffusion of CO₂ through stomata are impaired. Calculation of *c_i* in stressed leaves may lead to artefacts due to potential non-uniform stomatal closure (Terashima *et al.*, 1988), and the above results need therefore to be confirmed by independent methods of analysis. Moreover, the site of primary limita-

tions of photosynthesis during water-logging stress has still to be identified.

In the present work, we analyse photosynthetic functions of potted seedlings from the 3 cited tree species during periods of water-logging using gas exchange measurements to assess stomatal conductance and net CO₂ assimilation rates, photosynthetic oxygen evolution under high CO₂ concentrations, saturating irradiance to estimate maximal photosynthesis, and finally chlorophyll-a fluorescence to monitor photochemical efficiency of PS II.

MATERIAL AND METHODS

Plant material

Acorns of *Q robur* L and *Q rubra* L were collected under selected individual trees in the Forêt Domaniale de Manoncourt en Woëvre (Meurthe et Moselle, eastern France) and in the Forest of Schopperten (Moselle, eastern France) during Autumn 1989 and kept over-winter in a cold chamber at -1°C. Sixty seedlings of each species were grown in a sandy loam in 5 L, 25-cm-deep pots from spring 1990 onwards in a glasshouse at INRA Champenoux, under natural illumination (irradiance ≈ 70% of external; minimal temperature over winter 10°C, maximal temperature during summer 30°C; manual watering; fertilisation with 30 g slow release fertiliser per plant on June 12, 1990, Nutricote 100, N/P/K 13/13/13 + oligo elements). Sixty *F silvatica* L saplings (1-year-old saplings from Office National des Forêts, Clairlieu nursery near Nancy, seed collected in the Forêt Domaniale de Haye) were planted into similar pots during February 1990 and grown under the same conditions. At the end of 1990, mean heights and stem diameters were: 473 ± 7.3 and 7.58 ± 0.24 mm, 331 ± 7.1 and 6.48 ± 0.23 mm, 356 ± 4.05 and 6.67 ± 0.38 mm for *Q robur*, *Q rubra* and *F silvatica*, respectively.

Experimental design

An external transparent tubing was connected to the bottom of the pots, allowing a precise con-

tol of the water table. Forty-eight seedlings were selected in each species, and randomly distributed into 3 treatments: control (C); partially flooded to 6 cm below soil surface (PF); and completely flooded (F). Water-logging was initiated on May 25 1991 with tap water; the level of the water table was controlled every day. Under such conditions, O_2 partial pressure is expected to drop in a few days to well below the critical oxygen pressure for root tip growth (around 20 kPa) or even for older root maintenance (5 kPa) (Saglio *et al.* 1984; Drew, 1991) and soil redox potential to decrease to -100 to -200 mV (Gambrell *et al.* 1991). The characteristic smell for methane production was detected in our pots after approximately 8–10 d of water-logging. Sapling responses to these conditions were tested every week on 3 randomly selected individuals in each species \times treatment. Midday leaf water-potential (ψ_{wm}) was measured in the greenhouse on the selected individuals, which were thereafter transported into a growth chamber with following climate: temperature 22/16°C; 16/8 h day/night; irradiance around 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Chlorophyll-a fluorescence and oxygen evolution at 5% CO_2 were measured the following morning, and gas exchange monitored on 2 leaves per plant in the afternoon, after at least 4 h of illumination. Predawn leaf water potential (ψ_{wm}) was measured during next morning and all saplings were harvested for biomass, chlorophyll content, and nutrient composition determination. The whole procedure was repeated every week from week 1 to 4 after beginning of water-logging. Twelve saplings had been analysed per species and treatment at the end of the experiment.

Photosynthesis and water relations

ψ_{wm} and ψ_{wd} were measured on 2 leaves per individual with a pressure chamber. Gas exchange was monitored with a portable photosynthesis chamber Li Cor 6200 on 2 leaves per plant. Mean values \pm confidence intervals of microclimate during measurements were as follows: PFD: $310 \pm 2 \mu\text{mol m}^{-2} \text{s}^{-1}$; leaf temperature: $26.1 \pm 0.2^\circ\text{C}$; CO_2 concentration in air: $422 \pm 2 \mu\text{mol mol}^{-1}$; leaf to air difference in water vapour (Δw): $20.0 \pm 0.4 \text{ Pa kPa}^{-1}$. Leaf area was determined with a ΔT planimeter. Results were computed as in Ball (1987) (net assimilation rate, A , $\mu\text{mol m}^{-2} \text{s}^{-1}$; leaf conductance to water vapour g_w , $\text{mmol m}^{-2} \text{s}^{-1}$; and intercellular concentration

of CO_2 , c_i , $\mu\text{mol mol}^{-1}$), and presented as A/c_i relationships (Guehl and Aussenac, 1987). We computed the specific (*ie* related to leaf area) hydraulic conductance from soil to leaves as: $g_L = g_w \cdot \Delta w (\psi_{wd} - \psi_{wm})$, according to Reich and Hinckley (1989).

Chlorophyll fluorescence and oxygen evolution

Chlorophyll-a fluorescence from photosystem II (PS II) was recorded at wavelengths around 690 nm at ambient temperature with a pulse amplitude modulated fluorometer (PAM 101, Walz, Germany), using the procedure described by Epron and Dreyer (1992). Leaf disks (20 mm diameter) were punched from seedlings kept in darkness overnight, and inserted into a temperature-controlled leaf-disk holder (22°C). Initial fluorescence (F_0) was determined with a pulsed light-emitting diode ($< 10 \text{ mW m}^{-2}$) at a frequency of 1.6 kHz; maximal fluorescence (F_m) was obtained with an oversaturating flash of white light (0.7 s; 4 000 $\mu\text{mol m}^{-2} \text{s}^{-1}$; Schott KL 1 500N FRG). The optimal photochemical efficiency of PS II, *eg* after complete relaxation in the dark (Krause and Weis, 1984; Genty *et al.* 1987), was estimated from the ratio $F_v/F_m = (F_m - F_0)/F_m$. After 10 min exposure to actinic white light (Schott KL1500, FRG, 220 $\mu\text{mol m}^{-2} \text{s}^{-1}$), an additional white light flash allowed computation of the photochemical efficiency of PS II according to Genty *et al.* (1989) from $\Delta F/F_m'$ where $\Delta F = F_m' - F$ (F : level of steady-state fluorescence, and F_m' , fluorescence during a saturation). Basic fluorescence (F_0) was recorded immediately after switching off actinic illumination, and used to compute the photochemical efficiency of open PS II reaction centres as: $F_v'/F_m' = (F_m' - F_0)/F_m'$. Photochemical quenching, *ie* the fraction of open PS II reaction centres, was computed according to Genty *et al.* (1989) as: $q_p = (F_v'/F_m') / (\Delta F/F_m')$. A second period of induction was imposed immediately thereafter (10 min, 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$), and the same parameters recorded.

Maximal photosynthetic O_2 evolution rate (A_{max} , $\mu\text{mol O}_2 \text{ m}^{-2} \text{s}^{-1}$) was measured on a second leaf disk with an oxygen electrode assembly (LD2 MK2, Hansatech, UK, 22°C, $N_2 + O_2 + CO_2$: 80/15/5%). A period of 20 min induction under an irradiance of 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Light Unit LS2, Hansatech, UK) was provided before the measurements.

Specific leaf weight (g dm^{-2} , oven-dried at 85°C for 24 h) and total chlorophyll content (3 ml DMSO, incubation at 60°C during 90 min, optical densities measured at 663 and 645 nm, according to Hiscox and Israeltam, 1979) were determined on the same disks.

Leaf nutrient content

Saplings were separated into leaf, stem and root compartments, and oven-dried at 85°C for dry-weight determination. Total mineral content was measured as follows. Samples were crushed (Tecator-Cyclotec 1093 Sample Mill) and total nitrogen was measured with an autoanalyzer Technicon after mineralization with H_2SO_4 and H_2O_2 , and all other elements (S, P, Mn, Mg, Ca, K) were quantitated with an ICP (Jobin Yvon JY 438 Plus) after a mineralization with HClO_4 and H_2O_2 .

Statistical analysis of results

Due to the rapidity of reactions to water-logging, many parameters were strongly modified during week 1, but showed no significant evolution from week 1 to week 4. We therefore pooled the data together, and analysed them as a factorial design (3 species \times 3 treatments \times 12 individuals). Results are presented as means \pm standard error of the mean.

RESULTS

Growth and external signs of water-logging stress

Flooding was imposed during a period of active growth in all species. The height growth of *F. silvatica* was slow (due to strong ramification and sympodial growth in this species). Growth was completely stopped on all species by the total (F) and partial (PF) water-logging treatments. Visual symptoms induced by water-logging were very different among species. In *F. silvatica*, F induced visible signs of leaf necrosis after 1

week (brown spots at leaf margins); these necroses, together with brown spots along vascular bundles, progressively spread over the whole leaf laminae in the following weeks. Surprisingly, after 3 weeks, new growth was initiated, and short shoots with tiny, vitreous leaves were formed, while the primary foliage decayed progressively. No epinasty was observed. Root systems displayed a strong decay with no lenticels and no new root formation. PF induced analogous symptoms with less severity and a week's delay; roots survived in the upper, unflooded soil layer but no additional root growth occurred there. New leaf formation was slightly more intense than in F.

Oaks displayed very different symptoms. F saplings never showed leaf necrosis, but sometimes very strong epinasty after 2 weeks. On a few individuals, epinastic leaves dried out very rapidly during days with high VPD and temperature (around 30°C). Root systems of *Q. robur* and *Q. rubra* behaved differently. While F induced almost the same intensity of root decay, with no appreciable growth, no lenticels and no adaptive feature to water-logging, PF allowed growth of new roots in the non-flooded soil layer on *Q. robur* alone, and none at all on *Q. rubra*. Newly grown roots were thick, non-ramified and white along their whole length. Their formation began from the third week onwards. This observation is in agreement with that made earlier by Colin-Belgrand *et al* (1991) on the same species.

Figure 1 displays the total biomass of the saplings. Reductions in root biomass were very significant due to root decay in the F treatments. Shoot biomass was less affected, and only a fraction of the leaves completely dried out.

Water relationships

Water relationships were strongly affected by the water-logging (table I). Significant

decreases in predawn (ψ_{wd}) and midday (ψ_{wm}) leaf water potentials were recorded in F in *Q. robur* and *Q. rubra*, while PF promoted only limited effects. Because of reductions in leaf area, the estimated specific soil to leaf hydraulic conductance (g_L) showed no significant decrease in PF, and F even induced a slight increase of g_L in *Q. robur*. Strong leaf decay in *F. silvatica* in F impaired the water relationship measurements.

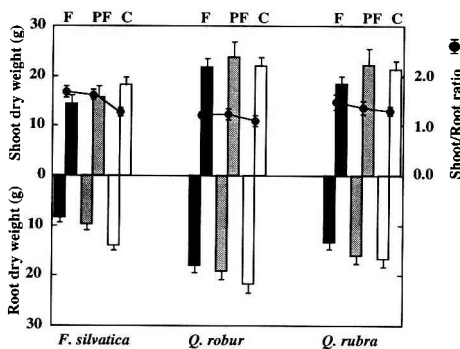


Fig 1. Shoot and root biomass accumulated at the end of the experiment, and shoot to root ratio, for seedlings of *Q. robur*, *Q. rubra*, and *F. silvatica*, as influenced by a period of water-logging, F and PF: complete and partial (to 6 cm below soil level) water-logging during 1–4 weeks; C: control plants.

General effects of water-logging on photosynthesis

The effects of water-logging on A is shown through A vs c_i relationships in figure 2. Important interspecific differences appeared. *Q. robur* displayed both highest values of A in controls and very limited decreases in

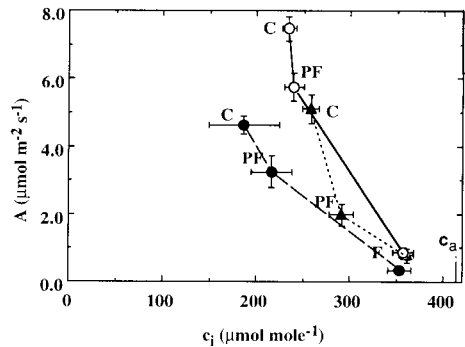


Fig 2. Effects of water-logging on net CO_2 assimilation rates of seedlings of *Q. robur* (O), *Q. rubra* (●) and *F. silvatica* (▲) presented as a function of calculated intercellular CO_2 mole fraction (c_i). Each point is the mean of a given treatment (C: control; PF: partially flooded; F: flooded; $n = 12$). c_a : CO_2 mole fraction in the atmosphere. Duration of water-logging: 1–4 weeks.

Table I. Effects of water-logging treatments (C: control; F: completely water-logged; PF: partially water-logged) on predawn (ψ_{wd}) and midday leaf water potential (ψ_{wm}), and on the root-to-shoot specific hydraulic conductance (g_L) of seedlings from 3 species.

Species	Treatment	ψ_{wd} MPa	ψ_{wm} MPa	g_L $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$
<i>Q. robur</i>	C	-0.33 ± 0.66 a	-1.68 ± 0.12 a	2.33 ± 0.23 a
	PF	-0.27 ± 0.04 a	-1.11 ± 0.11 a	3.08 ± 0.33 a
	F	-1.76 ± 0.34 b	-2.37 ± 0.32 b	5.39 ± 2.77 b
<i>Q. rubra</i>	C	-0.47 ± 0.07 a	-1.25 ± 0.12 a	1.23 ± 0.17 a
	PF	-0.54 ± 0.04 a	-1.31 ± 0.14 a	0.94 ± 0.12 a
	F	-1.55 ± 0.21 b	-1.64 ± 0.18 a	0.99 ± 0.36 a
<i>F. silvatica</i>	C	-0.23 ± 0.06 a	-1.42 ± 0.12 a	1.61 ± 0.29 a
	PF	-0.28 ± 0.10 a	-1.00 ± 0.12 a	1.45 ± 0.38 a
	F	-0.77 ± 0.34 a	—	—

* Mean values \pm standard errors of the mean, pooled over the 4 weeks of treatment, $n = 12$, Fischer PLSD.

** Strong leaf decay did not allow measurements on *F. silvatica* in the F treatment.

response to PF. *Q. rubra* was characterised by low A and c_i in controls, and by a stronger decline in PF, and finally, *F. silvatica* displayed low A in controls and the steepest decrease in PF. All 3 species responded to F by large reductions in A which reached values below $1 \mu\text{mol m}^{-2} \text{s}^{-1}$. Declines in A were accompanied by increases in c_i , suggesting that stomatal closure was not the only cause of these decreases in photosynthetic activity.

O_2 evolution rates at saturating CO_2 (A_{max}) and total chlorophyll content decreased in response to PF and F as compared to controls (fig 3) with the exception of *Q. rubra*, where controls displayed very low A_{max} . Total chlorophyll contents in the leaf disks were highest in *Q. robur*, lowest in *F. silvatica*. A_{max} was highest in control *Q. robur*, and very low and close to A in all other species and treatments.

Photochemical efficiency of PS II

Important species-related differences appeared in some of the fluorescence parameters (table II). In particular, *F. silvatica* displayed the highest basic fluorescence

(F_0), and lowest values for maximal fluorescence (F_m), and for photochemical efficiencies of dark-adapted PS II (F_v/F_m), PS II ($\Delta F/F_m'$), and open reaction centres (F_v'/F_m') at $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ PFD. On dark-adapted leaves, basic fluorescence F_0 was constant with water-logging, displaying slight increases only in *Q. robur*. Maximal fluorescence F_m decreased only slightly in F treatments for *Q. robur* and *Q. rubra*. In contrast, *F. silvatica* reacted very strongly, presenting steep declines in both treatments. The photochemical efficiency of PS II in dark-adapted leaves (F_v/F_m) declined significantly in the F treatment, *F. silvatica* displaying the sharpest decline and *Q. robur* the most limited. The relationship between weekly A and F_v/F_m (fig 4) was very different among species: in both oaks F_v/F_m remained above 0.7 even while A had decreased to almost zero, while in *F. silvatica* F_v/F_m was low even in controls and decreased at higher A . After 10 min induction at $800 \mu\text{mol m}^{-2} \text{s}^{-1}$, the photochemical efficiency of PS II ($\Delta F/F_m'$) of control plants was lowest in *F. silvatica*, and highest in *Q.*

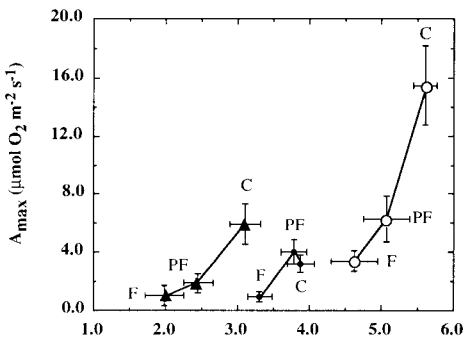


Fig 3. Compared values of oxygen evolution rates at 5% CO_2 (A_{max}) and total chlorophyll contents in seedlings of *Q. robur* (O), *Q. rubra* (●) and *F. silvatica* (▲) as a function of the imposed treatment; F: completely flooded; PF: partially flooded; C: control. Duration of water-logging: 1–4 weeks, $n = 12$. Means \pm standard error.

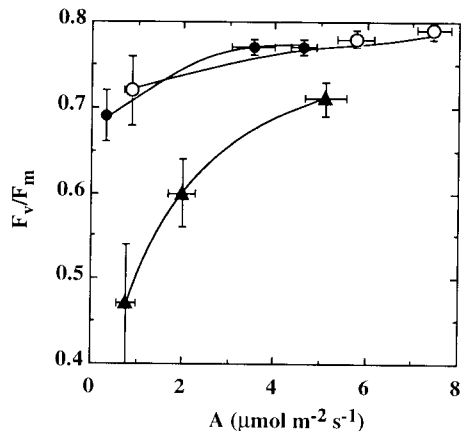


Fig 4. Relationship between net assimilation rates (A) and PS II photochemical efficiency of dark-adapted leaves (F_v/F_m) of *Q. robur* (O), *Q. rubra* (◆) and *F. silvatica* with or without root hypoxia. Duration of water-logging: 1–4 weeks, $n = 12$. Means \pm standard error.

robur. This is in agreement with the other observations on the photosynthetic activity of the 3 species. Flooding induced reductions in $\Delta F/F_m'$ were steepest for *F. silvatica* and only moderate in *Q. robur*.

Finally, we analysed the relationship between $\Delta F/F_m'$ and the photochemical efficiency of open PS II centres (F_v'/F_m') and the photochemical quenching of fluorescence (q_p) measured at both 220 and 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ irradiance (fig 5). All species and irradiance levels aligned on the same curves, which showed that the decline in $\Delta F/F_m'$ was always accompanied by simultaneous decreases in q_p , indicating a decrease in the fraction of open PS II reaction centres, and in F_v'/F_m' , indicating an increase in thermal deexcitation of PS II.

Nutrient contents in the leaves

Results of the mineral nutrient quantifications are shown in table III. Strong species-related differences appeared in the leaves, *F. silvatica* displaying lowest contents in N, P

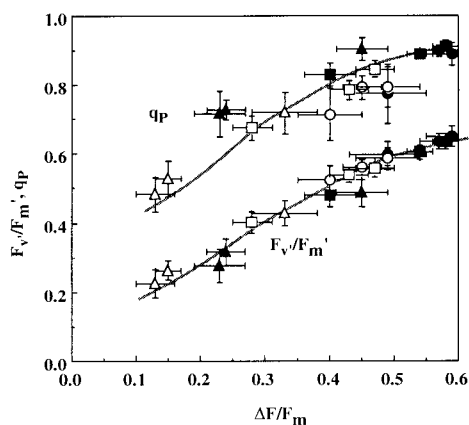


Fig 5. Relationship between photochemical efficiency of PS II ($\Delta F/F_m'$) of leaves of *Q. robur*, (\bullet, \circ), *Q. rubra* (\blacksquare, \square) and *F. silvatica* ($\blacktriangle, \triangle$) after 10 min induction at 220 (black symbols) or 800 (open symbols) $\mu\text{mol m}^{-2} \text{s}^{-1}$ and photo-chemical quenching (q_p) and photochemical efficiency of open reaction centres (F_v'/F_m') under different intensities of water-logging. Duration of water-logging: 1–4 weeks, $n = 12$. Means \pm standard error.

Table II. Effects of water-logging on the photosynthetic functions of leaves from 3 species as measured with chlorophyll fluorescence.

Species	Treatment	F_o	F_m	F_v/F_m	$\Delta F/F_m'$	F_v'/F_m'
<i>Q. robur</i>	C	12.1 ^{a+}	58.0 ^{a+}	0.788 ^{a+}	0.488 ^{a+}	0.587 ^{a+}
	PF	12.6 ^{ab}	58.7 ^a	0.784 ^a	0.452 ^a	0.560 ^a
	F	13.9 ^b	54.3 ^a	0.716 ^b	0.405 ^a	0.525 ^a
<i>Q. rubra</i>	C	13.6 ^{a*}	59.7 ^{a+}	0.772 ^{a+}	0.430 ^{a+}	0.538 ^{a+}
	PF	13.5 ^a	60.0 ^a	0.773 ^a	0.473 ^a	0.558 ^a
	F	14.5 ^a	53.1 ^a	0.694 ^b	0.280 ^b	0.400 ^b
<i>F. silvatica</i>	C	13.8 ^{a*}	49.0 ^{a*}	0.707 ^{a*}	0.331 ^{a*}	0.429 ^{a*}
	PF	14.7 ^a	40.3 ^{ab}	0.603 ^b	0.146 ^b	0.263 ^b
	F	13.9 ^a	33.4 ^b	0.467 ^b	0.127 ^b	0.226 ^b

F_o , F_m : basic and maximal fluorescence of dark-adapted leaves; F_v/F_m , PS II photochemical efficiency of dark-adapted leaves; $\Delta F/F_m'$ and F_v'/F_m' , photochemical efficiency of PS II and open reaction centres after 10 min at 800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PFD. Fischer PLSD was used to assess significance of differences among treatments in a given species (same letters: not significant) or among species in the controls (same symbol: not significant)

Table III. Mineral nutrient content in leaves (N, P, K, Mg, Ca, Mn, S, mg dm⁻² leaf area) or roots (Mn_{roots}, mg g⁻¹ DM) of young seedlings of *Q robur*, *Q rubra* and *F silvatica* submitted to water-logging for 1–4 weeks.

Species	Treatment	LSW	N	P	K	Mg	Ca	Mn	S	Mn _{roots}
<i>Q robur</i>	C	0.51	18.0 ^a	0.77 ^a	4.63 ^a	1.10 ^{ab}	2.05 ^a	2.14 ^a	1.29 ^a	0.650 ^a
	PF	0.51	15.3 ^b	0.64 ^b	4.14 ^b	0.99 ^a	1.63 ^b	1.67 ^b	1.18 ^a	0.683 ^{ab}
	F	0.48	18.3 ^b	0.65 ^b	4.35 ^a	1.17 ^b	2.05 ^a	1.82 ^{ab}	1.22 ^a	0.791 ^b
<i>Q rubra</i>	C	0.51	14.4 ^a	0.59 ^a	5.07 ^a	0.90 ^a	2.72 ^a	2.06 ^a	0.94 ^a	0.605 ^a
	PF	0.48	12.2 ^a	0.55 ^a	4.45 ^a	0.85 ^a	1.79 ^b	1.25 ^b	0.82 ^a	0.505 ^{ab}
	F	0.50	13.8 ^a	0.61 ^a	5.36 ^a	1.05 ^b	2.43 ^a	1.73 ^a	1.05 ^b	0.625 ^b
<i>F silvatica</i>	C	0.47	16.9 ^a	0.59 ^a	3.07 ^a	0.97 ^a	3.00 ^a	2.85 ^a	1.12 ^a	1.075 ^a
	PF	0.45	15.1 ^a	0.45 ^a	3.84 ^a	1.08 ^a	3.25 ^a	2.57 ^a	1.12 ^a	1.041 ^a
	F	0.43	16.1 ^a	0.49 ^a	4.87 ^b	1.35 ^b	4.59 ^b	3.31 ^b	1.71 ^b	1.160 ^a
Interspecies			***	**	*	***	***	***	***	***

C: control; PF: partial water-logging; F complete water-logging LSW: leaf specific weight (g dm⁻²). The same letters indicate non-significantly different values among each species. Interspecific differences are assessed in the last line (* 5%, ** 1%, *** 0.0%)

and K per leaf area and *Q robur* the highest in N, P, Mg. Significant disorders occurred in response to water-logging in the former species, with increases in almost all elements for the F treatment, probably due to internal recycling after leaf wilting. These increases were much lower in PF. In both oak species, the effects of water-logging were much more limited, and only few changes could be observed. No clear Mn accumulation was detected in leaves or roots.

DISCUSSION AND CONCLUSION

Effects of water-logging on growth and nutrient concentrations

Water-logging-induced decay of root systems is a common feature among woody species (see Colin-Belgrand *et al*, 1991, for

a review). The observed decay was severe in the completely water-logged (F) treatment. None of the expected specialised root-adaptation criteria, such as aerenchyma development and adventitious root growth, were detected. The occurrence of some adventitious roots in *Q robur* after 3 weeks in the partially water-logged (PF) treatment showed that the intensity of the hypoxia induced by F rather than the limited duration of the experiment were responsible for this lack of appearance of adventitious roots. The ranking of sensitivity among the tested species as inferred from the intensity of disorders in growth was in agreement with the generally known sensitivities to water-logging. *F silvatica* is known to be strongly flood-sensitive, while *Q robur* is expected to be rather insensitive (Belgrand, 1983; Lévy *et al*, 1986). Root decay and leaf necroses were the worse in the former species.

Drew (1991) hypothesised that one of the major effects of root hypoxia on shoot

physiology could be mediated by decreased nutrient assimilation and translocation, in particular N, K and P. For instance Drew and Sisworo (1979) observed reductions of N content in barley to 2/3 of the initial concentrations. Colin-Belgrand *et al* (1991) obtained significant decreases in the N content and no effect on any other element in oak saplings. In the present study we observed declines for a few elements in PF, but not in F. Accumulation of toxic reduced cations like Fe^{2+} or Mn^{2+} is sometimes suspected to be another deleterious consequence of hypoxia (Gambrell *et al*, 1991). We observed a slight accumulation of total Mn in oak roots, but it is unlikely that such low concentrations can be really toxic. In general, all nutrient contents measured in our saplings were largely above generally accepted deficiency thresholds.

Water relations of water-logged saplings

The fact that PF induced only limited changes in predawn leaf water potential (ψ_{wd}) has previously been shown for oak seedlings (Dreyer *et al*, 1991). Complete water-logging induced a very strong decline in ψ_{wd} , as reported earlier by Zaerr (1983) and Osonubi and Osundina (1987). Such a decline may be considered as an additional index for extensive root decay in the F seedlings. Soil hypoxia is known to cause rapid decreases in root hydraulic conductivity (Everard and Drew, 1987; Harrington, 1987; Smit and Stachowiak, 1988). Surprisingly, despite the observed root decay, our estimates of the overall soil-to-leaf specific hydraulic conductance (g_L) did not decrease in PF, and even increased slightly in F. The g_L values we calculated with control and PF seedlings were in agreement with those generally reported for oaks (see Dreyer *et al*, 1993, for a review). However the maintenance of g_L in F may only be explained by the decline in the transpiring

leaf area due to leaf decay, or by artefacts due to the use of predawn water potential as an estimate of soil water potential. Direct measurements of root hydraulic conductivity would be needed to solve this question.

Photosynthesis under water-logging stress

Important stomatal closure occurred in all treatments and species in response to water-logging. This observation has been widely reported for many species and intensities of root hypoxia (see, for instance, Childers and White, 1942; Lewty, 1990; Dreyer *et al*, 1991). Such stomatal closure strongly limits CO_2 influx into the mesophyll and therefore net assimilation rates (A) of water-logged plants. However the observed decreases occurred at increasing values of intercellular CO_2 mole fraction (c_i), as previously reported by Dreyer *et al* (1991), Vu and Yelenosky (1991) and Pezeshki (1991). In addition, photosynthetic O_2 evolution measured at 5% CO_2 and under saturating light (A_{max}), and photochemical efficiency of PS II were all depressed. This latter observation opposes many results obtained with drought stress, where photosynthesis decreases occur at constant A_{max} and photochemical efficiency (see review by Chaves, 1991, and Epron and Dreyer, 1993, for an example with oaks). We may conclude that in the case of water-logging, important dysfunctions are induced at chloroplast level. This supports earlier observations (Bradford, 1983).

Two hypotheses are generally put forward to explain reductions in photosynthetic performance during water-logging: (1) reduced mineral supply to leaves in particular N and P (Drew, 1991); and (2) toxic compounds produced by anaerobic metabolism in the roots. The decrease in chlorophyll content observed here in response to water-logging in all species was

analogous to the reported chlorosis occurring in many cereals (Drew, 1991). The absence of really significant decreases in total nitrogen or phosphate levels in the leaves opposes the hypothesis that nutrient imbalance was the cause of the reduced photosynthetic performance. Disorders in mineral nutrition probably act during long-term and partial water-logging in forest stands.

The observed decreases in PS II efficiency of dark-adapted leaves (F_v/F_m) were due to decreases in maximal fluorescence (F_m) at almost constant basic fluorescence (F_o). Reversible decreases in photochemical efficiency are a common feature in oaks under natural irradiance, but they vanish after a few hours of darkness (Epron *et al.*, 1992). Irreversible decreases were still apparent after a night of recovery and were accompanied by increases in F_o which were until now, only observed on seedlings submitted to drought during several days, on which net assimilation rates were already zero (Epron *et al.*, 1993). The same applied in our case to *Q. robur* and *Q. rubra*, with the exception that F_o remained constant. In *F. silvatica*, morning values of photochemical efficiency decreased before photosynthesis was completely impaired. Photochemical efficiency of PS II after a 10 min activation ($\Delta F/F_m'$) was also decreased by water-logging. This decline was due to simultaneous reductions of the efficiency of open PS II centres (F_v'/F_m') and the fraction of open centres as estimated by the photochemical quenching q_p (Genty *et al.*, 1989; Baker, 1991). Interestingly, the same balance between $\Delta F/F_m'$, F_v'/F_m' and q_p was detected on all species: a given stress-induced reduction of PS II activity ($\Delta F/F_m'$) was obtained by an analogous down-regulation of the efficiency of open PS II centres and a decrease of the fraction of open centres. These observations clearly support the hypothesis of important disorders induced in the photosynthesis at the chloroplast level,

in addition to the limitation of CO_2 diffusion due to stomatal closure. They nevertheless do not provide any clear evidence of these disorders. Direct effects at PS II level may be suspected, but are not confirmed by an increase in F_o . A limitation of the photosynthetic reduction cycle resulting in turn in a down-regulation of photochemistry could also be suggested. Solving this point and detecting potential toxic species issued by roots under hypoxia clearly needs further experiments.

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