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Original article

Interactive effects of waterlogging and irradiance on the photosynthetic performance of seedlings from three oak species displaying different sensitivities (*Quercus robur, Q petraea* and *Q rubra*)

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Summary – Potted seedlings from three oak species (*Quercus robur, Q petraea* and *Q rubra*), known to present different sensitivities to temporary soil hypoxia, were submitted to two contrasted irradiance regimes in a greenhouse (100% and approximately 35% of full greenhouse irradiance) and shortly after to waterlogging for 2–4 weeks. The experiment was repeated for 2 years. Leaf gas exchange, chlorophyll content and fluorescence, leaf water potential and photosynthetic capacity were recorded several times during the stress. Biomass increment was estimated at the end of the stress, or after 3 weeks of drainage. Despite its late application, after completion of elongation and expansion of leaves, shading significantly reduced growth and modified photosynthetic activity. It generally reduced net assimilation rates and increased chlorophyll contents. *Q rubra* seedlings behaved differently: growth decreased and chlorophyll was enhanced as in the two other species but net assimilation rates and photosynthetic capacity were higher in the shade than under full irradiance. This surprising effect probably resulted from high irradiance stress, and was not alleviated by improved fertilization during the second year. Waterlogging induced severe disorders in photosynthesis: net CO_2 assimilation, stomatal conductance, photosynthetic capacity, chlorophyll content and growth declined. In addition, predawn values of photochemical efficiency of photosystem II, which are usually close to the optimum value of 0.83, were significantly decreased. The largest dysfunctions occurred in *Q rubra* seedlings, and the smallest in *Q robur*, which confirms that the number of disorders in photosynthesis is an accurate estimate of the differential sensitivity to waterlogging among genotypes. Shading of the seedlings interacted with waterlogging and limited the extent of induced damage. In particular, stomatal closure and net CO_2 assimilation rates were proportionally less affected under shade. Similarly, predawn leaf water potential presented smaller decreases. The decline of predawn values of photochemical efficiency occured to a much lesser extent under shade. Finally, the behaviour of seedlings during post-waterlogging stress differentiated significantly the species. None of them recov-

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ered completely after 3 weeks. Nevertheless, *Q robur* behaved best, recovering high levels of predawn water potential, slowly reopening stomata and reincreasing net assimilation rates. *Q petraea* recovered with more difficulties: predawn leaf water potential decreased in several plants after the end of waterlogging, and photosynthesis recovered very slowly. *Q rubra* did not recover at all and several individuals died after the end of waterlogging stress.

waterlogging / photosynthesis / oaks / seedlings / root hypoxia / chlorophyll fluorescence

Résumé – Modulation par le niveau d'éclairement de la sensibilité à l'ennoyage de trois espèces de chêne *(Quercus robur, Q petraea* et *Q rubra*). Effets sur la photosynthèse. Des semis en pots de trois espèces de chêne (*Quercus robur, Q petraea* et *Q rubra*), connues pour présenter d'importantes différences de sensibilité à la présence de nappes d'eau temporaires dans le sol, ont été soumis à deux niveaux d'éclairement (100 et 35 % du rayonnement ambiant) dans une serre à Nancy, puis à une période d'ennoyage de plusieurs semaines. Cette expérience a été répétée 2 années de suite. Les échanges gazeux foliaires, les teneurs en chlorophylle, la fluorescence de la chlorophylle, le potentiel hydrique et la capacité de photosynthèse ont été mesurés plusieurs fois au cours de la contrainte. La biomasse accumulée a été estimée à la fin de l'ennoyage, ou après 3 semaines de ressuyage du sol. Malgré son application tardive après l'expansion des feuilles et l'arrêt de croissance en hauteur, l'ombrage a induit des baisses de croissance et d'activité photosynthétique (diminution de l'assimilation nette de CO_2 et augmentation des teneurs en chlorophylles). *Q rubra* a présenté une réponse très différente : alors que la croissance et les teneurs en chlorophylle présentaient les mêmes modifications que dans les autres espèces, l'assimilation nette de CO_2 était plus importante à l'ombre qu'en pleine lumière, et la capacité photosynthétique fortement augmentée. Cette réponse anormale révèle que ces semis de *Q rubra* étaient soumis à un stress lumineux. La fertilisation apportée en deuxième année a permis d'augmenter la photosynthèse, sans toutefois l'amener au niveau des deux autres espèces et sans éliminer la saturation à de faibles éclairements. L'ennoyage a provoqué d'importantes perturbations dans la photosynthèse : l'assimilation nette de CO_2 et la conductance stomatique ont été fortement réduites, la capacité photosynthétique et les teneurs en chlorophylles ont baissé ; des limitations de croissance ont également été constatées. De plus, l'efficience photochimique mesurée en fin de nuit, qui est habituellement proche du niveau optimal d'environ 0,83, a baissé significativement. Les perturbations les plus marquées ont été observées sur les semis de *Q rubra*, et les plus limitées sur ceux de *Q robur*, ce qui confirme la bonne corrélation existant entre perturbations à court terme de la photosynthèse, et sensibilité globale à l'ennoyage. L'ombrage a permis de limiter et de retarder, dans une certaine mesure, l'apparition de ces perturbations. Enfin, le comportement des semis pendant la phase de ressuyage a permis de différencier fortement les espèces. Aucune n'a retrouvé un fonctionnement optimal après 3 semaines de ressuyage. *Q robur* a manifesté la meilleure capacité de récupération, alors que *Q petraea* a présenté de progressives nécroses foliaires, un potentiel hydrique décroissant et des niveaux de photosynthèse très faibles pendant les 3 semaines. Enfin, *Q rubra* a subi une très forte sénescence foliaire et une mortalité non négligeable au cours du ressuyage.

photosynthèse / chênes / ennoyage / hypoxie racinaire / fluorescence de la chlorophylle / jeunes plants

INTRODUCTION

Temporary waterlogging is a common occurrence in soils of temperate forest ecosystems, and as such probably plays an important role as a limiting factor for forest productivity. Lowland forests in northeastern France are particularly prone to temporary waterlogging; they are generally managed as coppice with standards dominated by indigenous oaks, mainly *Quercus robur* L and *Q petraea* Matt Liebl (Becker and Lévy, 1986; Becker et al, 1996). These stands are very difficult to regenerate, owing to poor seedling establishment and survival (Becker and Lévy, 1986). Oak seedlings display large interspecific differences in tolerance to temporary waterlogging. *Q robur* presents a rather high tolerance, and others such as the American *Q rubra* a very high sensitivity; *Q petraea* and *Q palustris* have been shown to be of intermediate sensitivity (Dreyer et al, 1991; Dreyer, 1994). Similarly, observed differences in tolerance to soil hypoxia could at least partly explain gradients in tree distribution in the bottomland plains of the south-eastern United States (Gardiner and Hodges, 1996), and were consistent with the observed distribution of *Nothofagus solandri* and *N menziesii* in New Zealand (Sun et al, 1995). Differences in waterlogging tolerance among woody species probably play a major role in bottomland stands, governing species distribution and forest management (Kozlowski, 1982).

Soil hypoxia resulting from temporary waterlogging rapidly induces very severe disorders in plant growth and physiology, leading to rapid decline and death in the case of the most sensitive species. Severe reductions of growth, important root decay, chlorosis and leaf necroses are common consequences of root hypoxia (Kozlowski, 1982). Stomatal closure and the associated decline of net CO_2 assimilation appears to be a very sensitive and early indicator of waterlogging stress in trees (Zaerr, 1983; Pezeshki and Chambers, 1985, 1986). Moreover, the intensity of the stomatal and photosynthetic responses are correlated to the general sensitivity of the species to waterlogging (Dreyer et al, 1991; Pezeshki, 1993; Dreyer, 1994; Pezeshki et al, 1996). The chain of events leading to stomatal closure and photosynthesis decline is still poorly understood (Dreyer, 1994). Stomatal closure may be induced by root-issued abscisic acid (ABA) (Jackson et al, 1988), but recent investigations showed that the rate of delivery of ABA to shoots was reduced during waterlogging stress (Else et al, 1995). Modified transport of growth regulators or ethylene precursors, and of mineral nutrients (Topa and Cheeseman, 1992; Else et al, 1995) may also contribute to the observed responses. In addition to stomatal closure, waterlogged plants display decreased photosynthetic capacity measured under saturating CO_2 (Dreyer, 1994), reduced rubisco activity (Pezeshki, 1993) and reduced predawn quantum yield of photosystem II (PS II) (Dreyer, 1994). Chlorophyll decay and leaf chlorosis may result after longer periods of waterlogging.

In the present work, we intended to further document the disorders induced in forest tree photosynthesis by temporary waterlogging. We addressed in particular the question as to what extent irradiance levels imposed during waterlogging stress could modulate the intensity of the observed responses in species with different sensitivities to root hypoxia. We therefore submitted potted seedlings of three oak species differing largely in sensitivity (*Q robur*, *Q petraea* and *Q rubra*) to a mid-term waterlogging stress (3–4 weeks) under two levels of irradiance in a greenhouse. In addition, we verified whether differences in sensitivity were directly evident at the end of the stress treatment, or could be revealed during recovery after drainage.

MATERIALS AND METHODS

Plant material

Three oak species were used in these experiments: sessile (*Quercus petraea* Matt Liebl provenance Saint-Dizier, Haute-Marne, France), pedunculate (*Quercus robur* L, provenance Manoncourt-en-Woevre, Meurthe-et-Moselle, France), and northern red oak (*Quercus rubra* L, provenance Creutzwald, Moselle, France).

Experiment 1

Acorns were collected during autumn 1993, and were overwintered at –2 °C after fungicide treatment. After removal of the seed coat, they were

Table I. Mean afternoon values (± standard deviation) of microclimate recorded during August 1994 and August 1995 in the greenhouse below a green polyethylene meshed netting (shade), or in full light.

	1994			*1995*		
	Full light		*Shade*	*Full light*		*Shade*
Irradiance (μmol m^{-2} s^{-1})	740 ± 220		250 ± 109	776 ± 227		247 ± 85
		−64%			−68%	
Air temperature (°C)	29 ± 2.6		28.3 ± 2.6	27.7 ± 1.6		27.3 ± 1.7
Vapour pressure deficit (hPa)	20.7 ± 3.5		18.7 ± 2.6	21.6 ± 2.8		20.4 ± 2.4
		−9.5%			−5.5%	

The records were made from LiCor sensors during gas exchange measurements (LiCor 6200; Lincoln, NE, USA).

soaked for 24 h in tap water at ambient temperature, and germinated in 5 L pots filled with a brown clay-loam forest soil. Sixty pots were prepared during 4–6 May 1994, and three acorns from the same species were sown in each, yielding finally 60 seedlings per species. A transparent plastic tubing was fitted tightly to the bottom of each pot, thus making it possible to either drain excess water, or to impose a controlled level of waterlogging when kept vertical. No additional fertilization was provided. Seedlings were grown under ambient climate in a greenhouse near Nancy (north-eastern France), and watered automatically twice daily.

On 4 July, half the pots were placed below a green meshed polyethylene netting (Rekord, Novatex, France) using a completely randomized design. The microclimate below and outside of the netting was recorded during gas exchange measurements, and mean afternoon values were as displayed in table I. On 8 August, after completion of height growth, half the pots in each irradiance regime were waterlogged until soil surface with tap water. The water level was adjusted manually every day to compensate for evaporation. Waterlogging was maintained for 1 month until 2 September, when all seedlings were sampled for biomass analysis. During the course of waterlogging, net CO_2 assimilation rates (A), stomatal conductance to water vapour (g_w), predawn and midday chlorophyll a fluorescence were recorded in situ on all seedlings, approximately once weekly. Leaf disks were collected to measure chlorophyll contents. In addition, oxygen evolution rates were recorded once for each plant during the last week of the waterlogging period, under saturating CO_2 and irradiance to estimate the photosynthetic capacity (A_{max}) of the leaves. Seedlings were harvested thereafter, and biomass and leaf area recorded. The time course of the experiment can be summarized as:

Germination in the greenhouse	*Transfer of half the plants to shade*	*Beginning of waterlogging*	*Ecophysiological measurements*	*End of waterlogging and collection for biomass*
3 May 94	4 July 94	8 August 94	–	2 September 94

Experiment 2

An additional experiment was set up in 1995 to follow the recovery phase after release from waterlogging stress. The protocol used was similar to the preceding, but with a few differences in schedule and treatments. Seedlings were provided during July with 15 g slow release fertilizer Nutricote 100 (N/P/K 13/13/13 + oligo elements).

The plants were transferred to the different shade treatments on 11 August, and waterlogged from 15 August onwards. Pots were drained on 2 September, and left to drain up until 25 September. After the beginning of waterlogging, A and g_w were measured in situ for all seedlings once daily during the first week and once weekly later on. Total chlorophyll contents were recorded five times with a portable spectrophotometer. Predawn leaf water potential was measured at the end of waterlogging and twice during recovery. Seedlings were harvested thereafter for biomass and leaf area. The time course of the experiment can be summarized as:

Germination in the greenhouse	*Fertilization*	*Transfer of half the plants to shade*	*Beginning of waterlogging*	*End of waterlogging*	*Collection of seedlings for biomass*
29 May 95	13 July 95	11 August 95	15 August 95	2 September 95	25 September 95

Techniques

A and g_w were recorded in situ in the afternoon with a portable photosynthesis chamber LiCor 6200 (LiCor, Lincoln, NE, USA) on one leaf of five randomly selected plants per treatment and species (n = 5 plants × 5 dates). Mean values of microclimate during these measurements are listed in table I. Leaf area was measured at the end of the experiments using a DeltaT area meter (DeltaT, Hoddesdon, UK).

Photosynthetic capacity was measured on 10 cm^2 leaf disks in an oxygen electrode (LD2, Hansatech, UK), at 25 °C, under an irradiance of 800 $\mu mol\ m^{-2}\ s^{-1}$ provided by a white halogen lamp (Hansatech, UK), and a gas mixture containing approximately: 76% N_2, 19% O_2 and 5% CO_2. This mixture has been shown to saturate photosynthesis (Chaves, 1991; Ridolfi and Dreyer, 1997). Leaf disks were acclimated to these conditions for 20 min, and O_2 evolution recorded for 3 min after this acclimation. Four to five replicates were measured for each treatment.

Chlorophyll a fluorescence was recorded on the same leaves with a portable modulated fluorometer PAM 2000 (Walz, Effeltrich, Germany) using the standard saturating flash procedure with a leaf clip holder, and on line computation of results with the DA-2000 software from Walz. Care was taken to standardize the position of the fiberoptics with respect to leaves (Bilger et al, 1995). Two records were made:

i) at predawn, photochemical efficiency of dark adapted leaves as:

$$F_v/F_m = (F_m - F_o)/F_m$$

where F_o is the basic fluorescence level, and F_m the fluorescence level induced by a 0.8 s saturating white flash;

ii) under ambient irradiance in the afternoon, immediately after gas exchange measurements, photochemical efficiency of PS II:

$$\Delta F/F_m' = (F_m' - F)/F_m'$$ (Genty et al, 1989)

where F is the steady-state fluorescence under ambient irradiance, and F_m' the fluorescence level induced by a 0.8 s saturating white flash. Non-photochemical quenching was calculated for each individual leaf according to the Stern-Volmer equation:

$$q_{NSV} = F_m/F_m' - 1$$

Predawn leaf water potential was recorded with a pressure chamber on leaves randomly collected in each treatment. Total chlorophyll contents were measured during experiment 1 with disks punched from leaves, immersed in dimethylsulphoxide (DMSO). Chlorophylls were determined spectrophotometrically after 180 min extraction in DMSO at 60 °C, from the optical densities at 648.2 and 664.9 nm, using the regression equations given by Barnes et al (1994). During experiment 2, we used a portable chlorophyll meter SPAD (Minolta, Japan) that measures the optical density in situ on attached leaves at 650 and 940 nm. SPAD values were calibrated against DMSO leaf extracts resulting in the following equation:

$$\text{total chlorophylls } (\mu g\ cm^{-2}) = -22.6 + 3.20 \text{ SPAD units}$$

Biomass was measured after collection of all seedlings at the end of the experiments and divided into lateral roots, tap roots, stems + twigs, and leaves. Total leaf area was measured on fresh leaves with a DeltaT area meter. During the second experiment, we visually assessed the length of living fraction of tap roots. Root biomasses from three seedlings in the same pots were measured together, and the result divided by 3.

Treatment effects were assessed using a three factorial ANOVA (factor species, irradiance level and waterlogging) with the software Statview II. Comparisons between means were based on Fisher's PLSD at 0.05.

RESULTS

Biomass and growth

During year 1, large differences in growth parameters were observed among species (fig 1). The largest biomass was observed in *Q robur* and *Q rubra*, whereas *Q petraea* presented only a third of this value. This was also particularly visible in the fine root

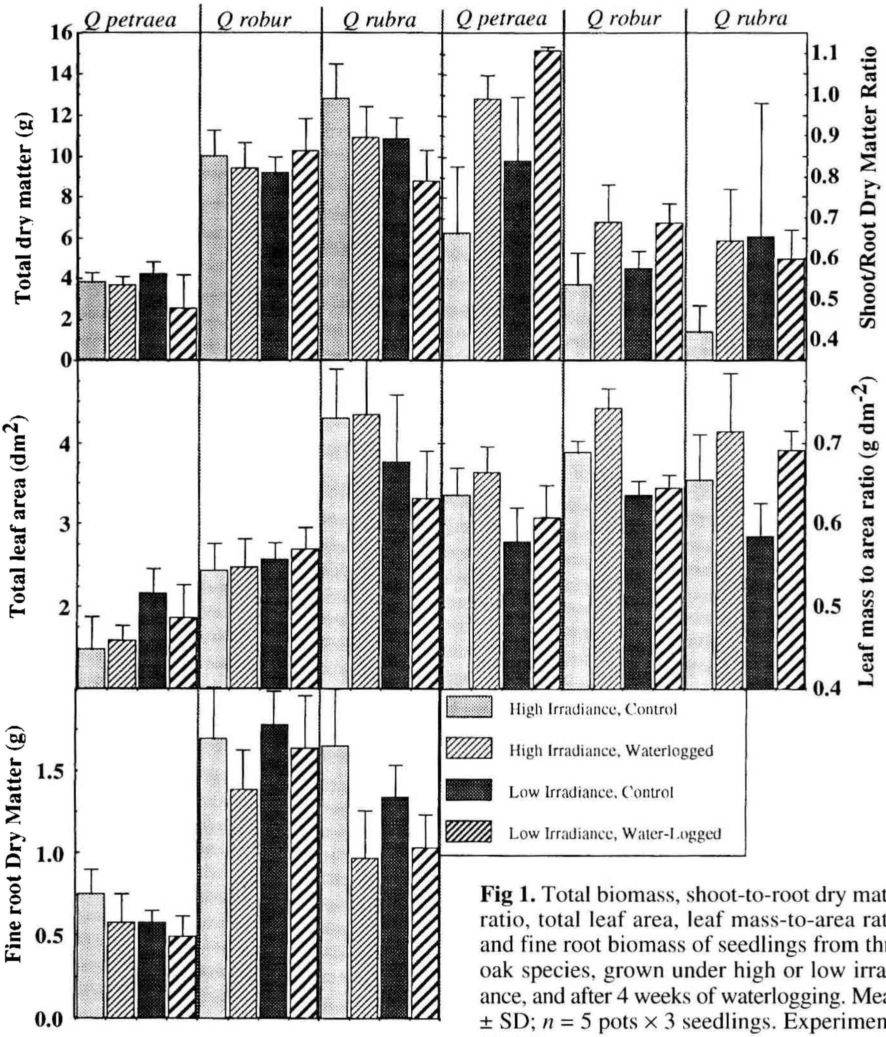

Fig 1. Total biomass, shoot-to-root dry matter ratio, total leaf area, leaf mass-to-area ratio, and fine root biomass of seedlings from three oak species, grown under high or low irradiance, and after 4 weeks of waterlogging. Means ± SD; n = 5 pots × 3 seedlings. Experiment 1 (1994).

mass. In addition, *Q robur* and *Q rubra* bore relatively more roots to shoots than *Q petraea* (lower shoot-to-root ratio). *Q rubra* displayed the largest leaf area with a small number of very large leaves. Leaf mass-to-area ratio presented only limited interspecific differences: *Q robur* displayed slightly larger values than the two other species. Such features correspond to the frequently described growth patterns of *Q robur*, *Q petraea* and *Q rubra* seedlings (see, eg, Dreyer, 1994). As expected, shading induced only limited effects on growth owing to the late date of application (beginning of July, when approximately two growth flushes were fully installed); nevertheless, it reduced significantly the leaf mass-to-area ratio in all species, and increased the shoot-to-root ratio.

Waterlogging induced some disorders in growth (fig 1 and table II). Total biomass and total leaf area were not significantly reduced, owing to the late application of the stress. The shoot-to-root ratio increased significantly in almost all cases, revealing probably root growth cessation and even a severe fine root decay. Surprisingly, waterlogging resulted also in a significant increase in leaf mass-to-area ratio in almost all cases. The largest effects were recorded in *Q rubra*, with visible leaf senescence and important root decay, which was not accounted for in the biomass estimates. No interaction was detected between waterlogging and the level of irradiance on most of the variables tested (table I).

The results from the second experiment were essentially the same. Only a few differences were noted. Total biomass was reduced in all seedlings owing to late germination. At the end of the waterlogging period, leaf necroses and wilting occurred in *Q rubra* and to a lesser extent in *Q petraea*. The fraction of seedlings affected by these

Table II. Results from an analysis of variance testing effects of species (Sp), growth irradiance (I) and waterlogging (WL) on seedling biomass, shoot-to-root ratio, fine root biomass, total leaf area, and leaf mass-to-area ratio (*n* = 20 per species): experiments 1 and 2 (1994 and 1995).

Factor	*Biomass*	*Shoot/root*	*Fine roots*	*Leaf area*	*Leaf mass/area*
Experiment 1					
Sp	0.0001	0.0001	0.0001	0.0001	0.0002
I	0.0174	0.0174	NS	NS	0.0001
Sp × I	0.030	NS	NS	0.0005	NS
WL	0.009	0.0001	0.0001	NS	0.0001
I × WL	NS	NS	NS	NS	NS
Sp × WL	0.026	NS	NS	NS	0.423
Sp × WL × I	NS	NS	NS	NS	NS
Experiment 2					
Sp	0.0001	0.0001	NS	0.0001	0.0001
I	0.0017	NS	0.0097	NS	0.0007
Sp × I	0.0217	NS	NS	NS	NS
WL	0.0001	0.0001	0.0001	0.0001	NS
I × WL	0.0001	0.0003	0.0018	0.0029	NS
Sp × WL	0.0036	NS	0.0048	NS	NS
Sp × WL × I	NS	NS	NS	NS	NS

Not significant (NS): $P > 0.05$.

Table III. Fraction of seedlings from three oak species grown under high or low irradiance that presented significant leaf necroses or abscission after the end of waterlogging, or following 10 or 20 days of drainage.

Time	Q petraea		Q robur		Q rubra	
	High	*Low*	*High*	*Low*	*High*	*Low*
End of waterlogging	5%	5%	0%	0%	44%	47%
10 days of drainage	22%	11%	5%	0%	75%	53%
20 days of drainage	25%	16%	8%	0%	92%	73%

disorders increased readily during the post-waterlogging period (table III). In *Q rubra*, this led to shedding of an important fraction of the leaves. Root decay was much greater in waterlogged seedlings as a result of the 3-week drainage with important decreases in biomass. In addition, necroses occurred and the fraction of necrotic tap root, measured after drainage, was small in *Q robur*, larger in *Q petraea*, and very large in *Q rubra* (fig 2). Irradiance interacted significantly: the decay was larger under full irradiance in *Q petraea* and *Q robur*, but not in *Q rubra* where tap roots were heavily affected in both cases (table IV).

Water relations

Predawn leaf water potential (Ψ_{wp}) in all control seedlings was between –0.1 and –0.2 MPa at the end of the waterlogging period, independently of irradiance and experiment (fig 3). During the first experiment, a significant but limited decrease was observed in response to waterlogging in *Q*

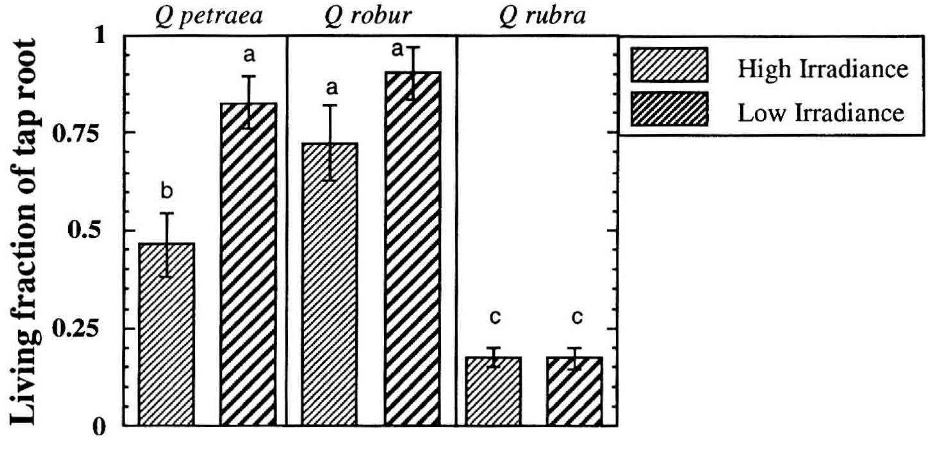

Fig 2. Living fraction of the tap root of seedlings of three oak species, grown under high or low irradiance, after 2 weeks of waterlogging and 3 weeks of drainage. Only waterlogged seedlings are presented (no root decay observed in controls). Total length of the tap root was close to 20 cm, limited by the size of the pots. Means ± SD; $n = 18$. Experiment 2 (1995).

Table IV. Net assimilation rate of seedlings from three oak species after 3 weeks of waterlogging, expressed as a fraction of the values recorded on control seedlings. Experiment 1 and 2.

Irradiance	Q petraea		Q robur		Q rubra	
	High	*Low*	*High*	*Low*	*High*	*Low*
Experiment 1	0.49	0.59	0.45	0.72	0.09	0.20
Experiment 2	0.15	0.31	0.22	0.70	0.22	0.32

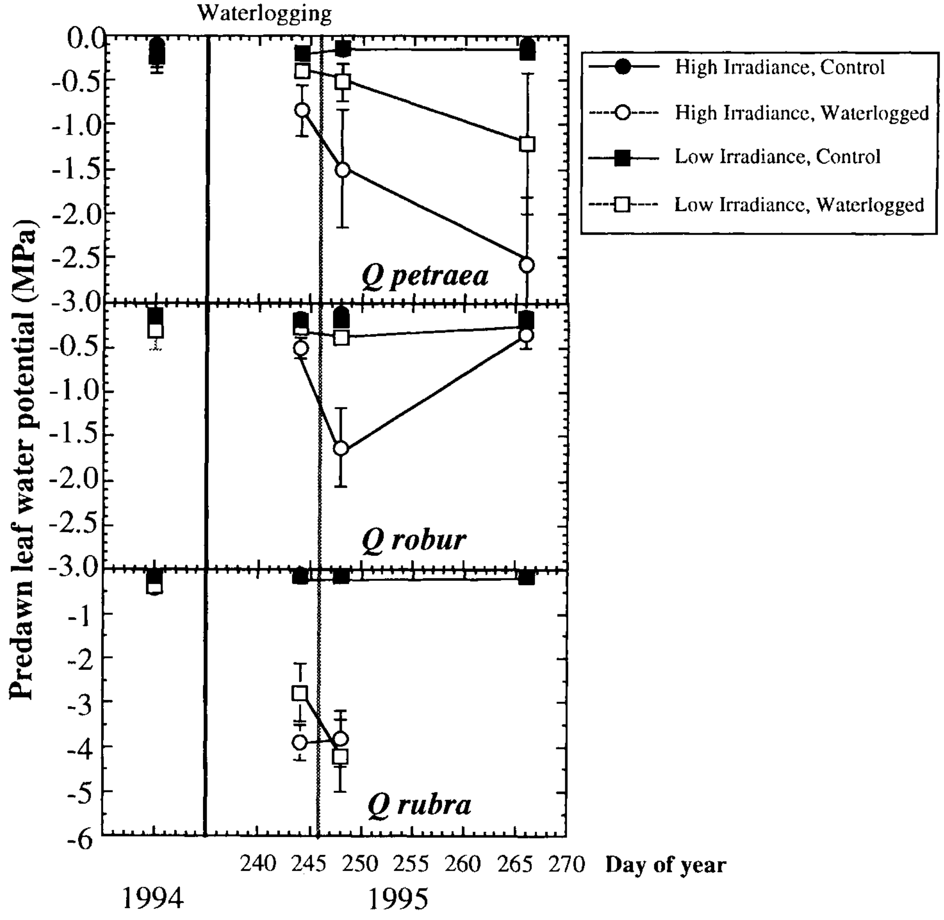

Fig 3. Predawn leaf water potential recorded on seedlings of three oak species, grown under high or low irradiance, at the end of a 4-week period of waterlogging (1994 and 1995) and during the course of drainage (1995). Means ± SD; $n = 5$. Owing to important leaf necroses, no measurement could be made on *Q rubra* at the end of the recovery phase.

robur under low irradiance. The largest decrease was detected in *Q rubra*, where Ψ_{wp} approached –0.4 MPa, revealing dysfunctions in water absorption. During the second experiment, Ψ_{wp} declined more significantly at the end of waterlogging, with severe decreases in *Q rubra* and smaller ones in *Q petraea* and in *Q robur* (fig 3). Large interspecific differences were observed during drainage: in *Q rubra*, owing to leaf necroses, measurements of Ψ_{wp} could not be made; in *Q petraea*, Ψ_{wp} decreased continuously with a large interindividual variability (recovery in several individuals, and necroses in others); in *Q robur*, the recovery was almost total after 3 weeks. Irradiance modulated this time course in the two latter species, with decreases being larger under high than low irradiance. In *Q rubra*, waterlogging resulted in similar and severe decline under both microclimates.

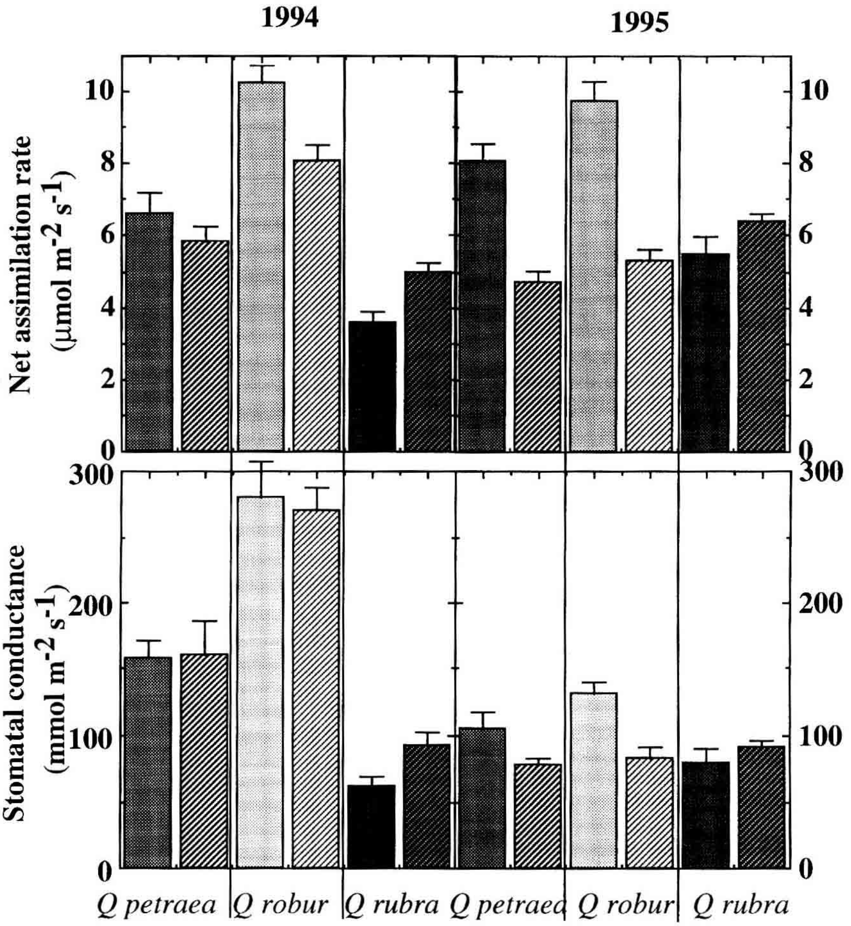

Fig 4. Net CO_2 assimilation rates (*A*) and stomatal conductance to water vapour (g_w) in seedlings of three oak species, grown under high or low irradiance. Means ± SD; *n* = 5 seedlings × 6 measurement dates per treatment. Experiments 1 (1994) and 2 (1995). Dotted columns: high irradiance; striped columns: low irradiance.

Photosynthesis

A general analysis of recorded values of gas exchange (fig 4) revealed large interspecific differences under control conditions: *Q robur* displayed highest, *Q rubra* lowest and *Q petraea* intermediate values of net CO_2 assimilation rates (A). Surprisingly, *Q rubra* displayed higher values of A in the shade than under full irradiance. This observation indicates a limitation of photosynthetic capacity in the latter case, which was confirmed by the higher values of O_2 evolution under saturating CO_2 and 500 µmol m^{-2} s^{-1} PFD in shade leaves (A_{max}, fig 5). Stomatal conductance followed similar interspecific patterns, paralleling *A*. Nevertheless, in *Q rubra* leaves grown under full irradiance, g_w was not the limiting factor for *A*, as demonstrated by the low photosynthetic capacity measured at 5% CO_2 (A_{max}), which remained close to the rates measured under ambient CO_2. In the other species, and in *Q rubra* under shade, A_{max} was close to 14 µmol m^{-2} s^{-1} (fig 5). It may therefore be argued that differences in g_w were the main cause for the observed differences in *A* between *Q petraea* and *Q robur* under full irradiance, and among the three species in the shade. The observations made during the second year essentially confirmed these results (fig 4). *Q rubra* again displayed higher values of *A* when grown under low irradiance.

As expected, waterlogging induced a general and rather large decrease in g_w and *A* in all species under both irradiance regimes; the decrease was already maximal a few days after treatment begin, and was maintained until the end of the waterlogging during the two years (see example from experiment 2 in fig 6). We therefore pooled data from the last measurement days during waterlogging to compute a global loss of *A* and g_w (table IV). During the second year, the levels reached at the end of waterlogging were lower than during year 1. Despite this discrepancy, the responses of

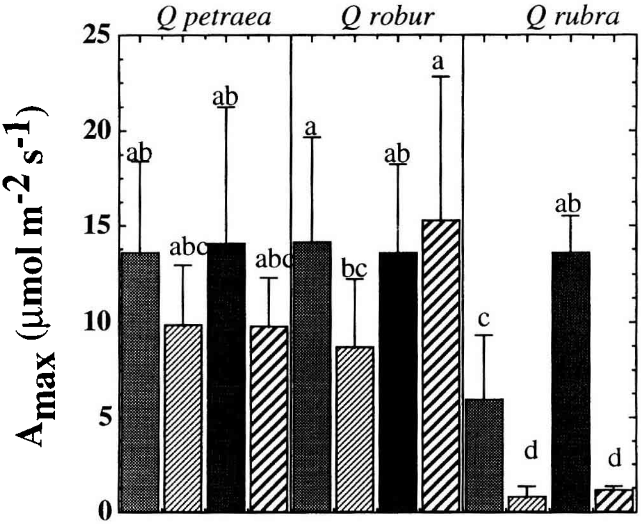

Fig 5. Photosynthetic capacity estimated from oxygen evolution under saturating irradiance (800 µmol m^{-2} s^{-1}) and 5% CO_2 (A_{max}). Measurements on leaf disks from seedlings of three oak species, grown under high or low irradiance, at the end of a 4-week waterlogging treatment. Means ± SD; *n* = 5. Experiment 1 (1994). Key as in figure 1.

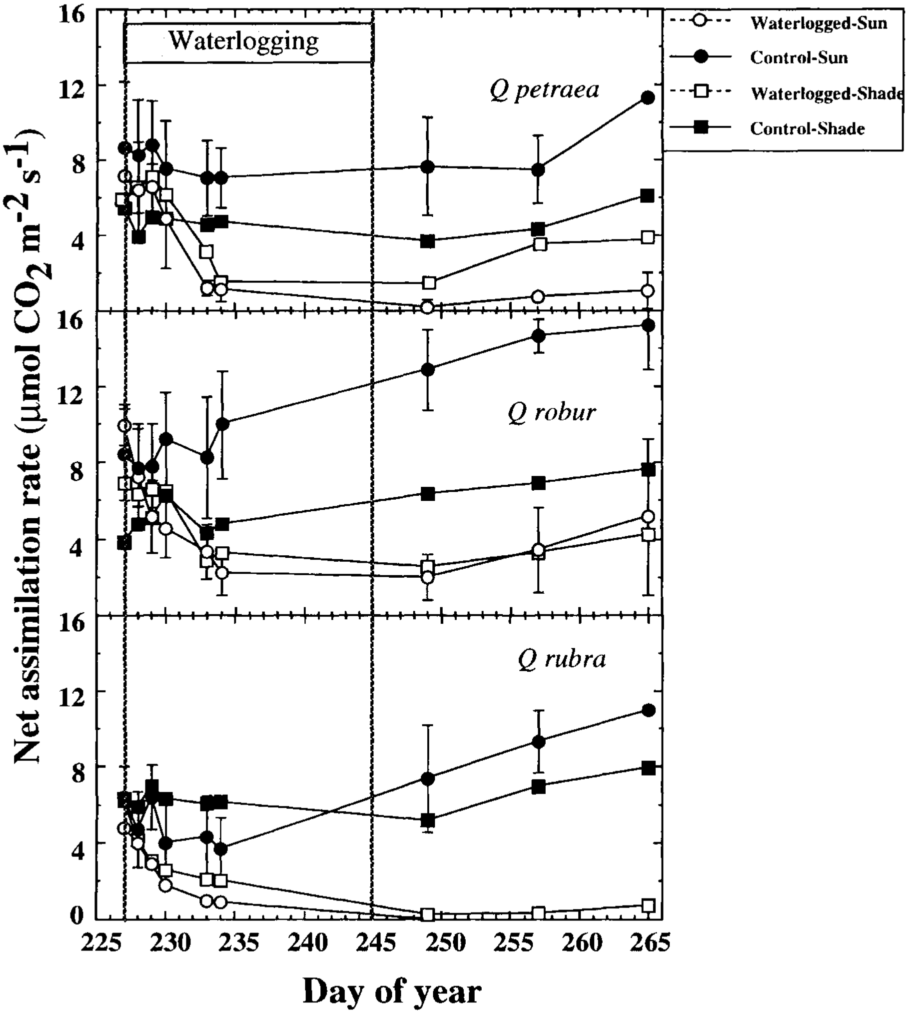

Fig 6. Time course of net CO_2 assimilation rates (A) in seedlings of three oak species, grown under high or low irradiance, and submitted to a 4-week period of waterlogging. Means ± SD; $n = 5$. Experiment 2 (1995). Lines indicate beginning and end of waterlogging; symbols as in figure 3.

the different species remained similar: i) larger decreases were recorded on high irradiance than on shade leaves in the three species; ii) the smallest decreases were observed in *Q robur*, and the largest in *Q rubra*. Stomatal closure was probably not the unique cause of the photosynthesis decline, as A_{max} also decreased, although not significantly in *Q robur* and *Q petraea*, but very severely in *Q rubra* (fig 5). The drainage during experiment 2 resulted in a very slow recovery of A in *Q robur* under both irradiance levels and in *Q petraea* under the low one. No recovery was observed in *Q rubra*, nor in *Q petraea* under high irradiance (fig 6).

Table V. Chlorophyll content (mg dm^{-2}) in leaves of control and waterlogged seedlings from three oak species grown under two irradiance regimes.

Irradiation	*1994*			*1995*		
	Q petraea	Q robur	Q rubra	Q petraea	Q robur	Q rubra
Controls						
High irradiance	5.27 ± 0.26 ^{a}	4.66 ± 0.209 ^{b}	2.17 ± 0.085 ^{c}	5.60 ± 0.68 ^{a}	5.40 ± 0.91 ^{a}	3.87 ± 0.63 ^{c}
Low irradiance	6.25 ± 0.30 ^{d}	5.76 ± 0.283 ^{d}	3.22 ± 0.123 ^{e}	5.67 ± 0.67 ^{a}	5.38 ± 0.80 ^{a}	4.39 ± 0.81 ^{b}
Waterlogged (after 2 weeks)						
High irradiance	4.85 ± 1.21 NS	4.53 ± 1.09 NS	1.99 ± 0.41 NS	5.75 ± 0.50 NS	4.72 ± 0.96 *	3.23 ± 0.99 *
Low irradiance	6.04 ± 1.29 NS	6.2 ± 1.56 NS	2.99 ± 0.64 *	6.13 ± 0.79 NS	5.04 ± 0.69 *	3.82 ± 0.91 *

Results from two experiments are measurements made after DMSO extraction (1994) or in situ with a SPAD (1996). Mean values ± standard error (n = 20–26). Different letters among controls from a given year indicate statistically significant differences, and NS or * refers to significance of the waterlogging effects respectively to corresponding control.

Chlorophyll content

Chlorophyll content, expressed on a leaf area basis, was significantly modulated by species and irradiance (table V). Higher levels were recorded under low than under high irradiance. *Q petraea* in general displayed highest values among the three species. Very low levels were detected in *Q rubra*. Waterlogging resulted in a limited decrease in chlorophyll content with no self-evident time course; pooled data after week 1 of treatment showed a limited decrease under both irradiance regimes, which was not significant in each individual species owing to high interindividual variability.

Chlorophyll contents measured with the SPAD (table V) were close to those recorded during the previous year in control seedlings of *Q petraea* and *Q robur*, confirming the validity of in situ measurements. In *Q rubra*, the contents were higher than for 1994, as a result of fertilization, but remained still lower than in the two other species. Irradiance levels induced no significant increase, owing to late application of the contrasted regimes. Two weeks of waterlogging resulted in a significant decrease of the contents in *Q rubra* and *Q robur*, under both irradiance levels, but not in *Q petraea*. Owing to heavy leaf necrosis and shedding in *Q rubra* and *Q petraea*, no measurements were made later.

Predawn photochemical quantum yield of PS II (F_v/F_m)

Predawn values of the photochemical quantum yield of PS II (F_v/F_m) displayed sig-

Table VI. Predawn values of photochemical quantum yield of photosystem II measured on control seedlings from three oak species, grown under two irradiance regimes.

	Q petraea	Q robur	Q rubra
F_v/F_m High irradiance	0.791 ± 0.005 ^{b}	0.805 ± 0.002 ^{a}	0.780 ± 0.003 ^{c}
F_v/F_m Low irradiance	0.817 ± 0.003 ^{d}	0.827 ± 0.002 ^{c}	0.820 ± 0.003 ^{d}

Data pooled over the whole experiment (means ± SE). $^{a-d}$ Different letters indicate significantly different values, Fischer's PLSD, n = 5 leaves × 9 measurement dates.

nificant differences among species and irradiance in control plants (table VI). Values in shade leaves were generally high and close to the theoretical optimum (around 0.83). They were slightly but significantly lower in sun leaves, the largest difference being observed with *Q rubra*. Waterlogging induced a severe decline of F_v/F_m down to 0.6 in *Q rubra* sun leaves (fig 7), with lowest values recorded 1 week after the begin-

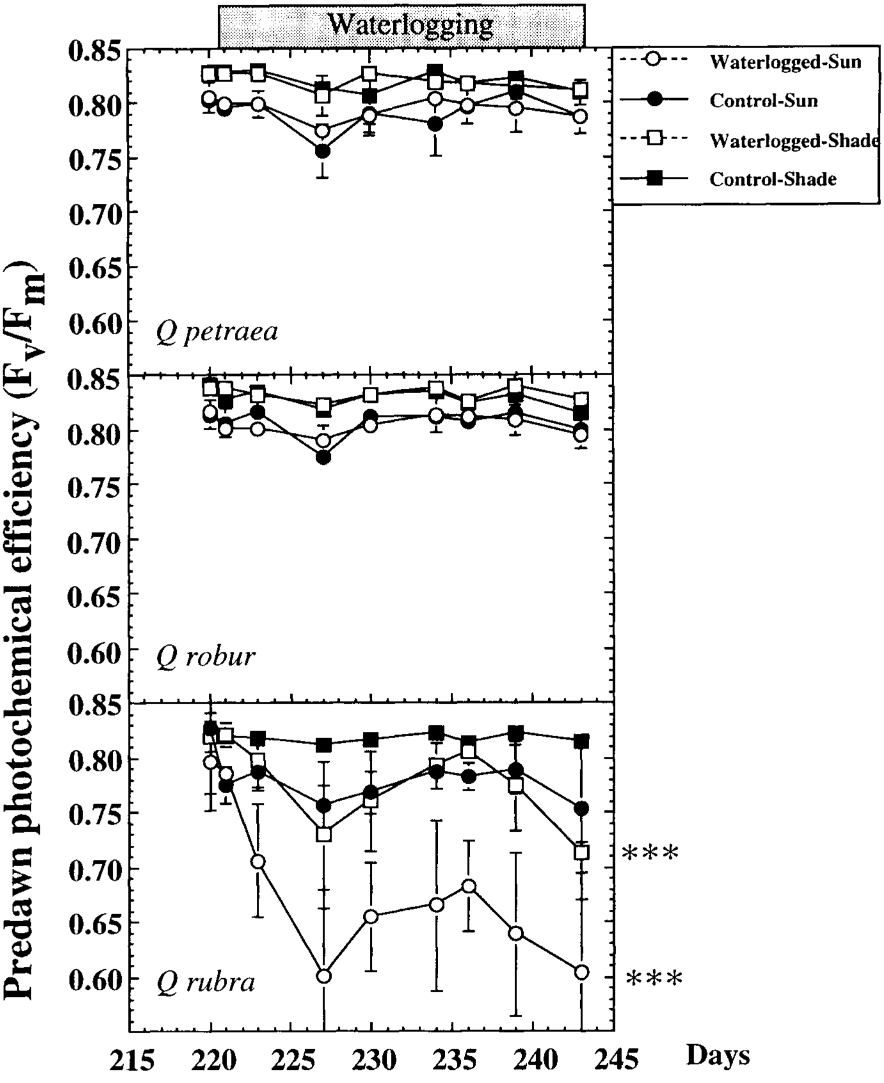

Fig 7. Time course of predawn photochemical quantum yield of photosystem II (F_v/F_m) recorded in seedlings from three oak species grown under two irradiance regimes and submitted to waterlogging. Mean ± SD; $n = 5$; *** denotes a highly significant effect of waterlogging.

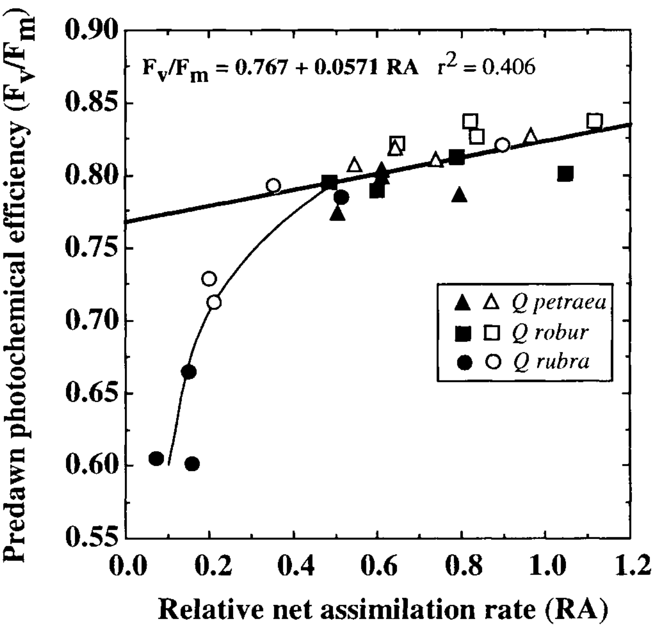

Fig 8. Relationship between mean values of F_v/F_m and net CO_2 assimilation rates measured during the day before, and expressed as a fraction of control values. Each point is the mean of five measurements. Solid symbols: high irradiance; open symbols: shade. Same data as in figure 7.

ning of the treatment. A smaller decline was recorded in shade leaves of the same species. No decrease was observed in either of the other species. Interestingly, as shown in figure 8, the most significant decrease occurred mainly when diurnal photosynthesis was reduced to less then 40% of the controls; this threshold was only crossed by *Q rubra* seedlings after 1 week of waterlogging. Moreover, as shown in table VII, the decline in F_v/F_m was due to a severe decrease in F_m, while F_o either remained constant, or increased slightly.

Table VII. Effects of waterlogging on the predawn values of basic (F_o) and maximal (F_m) fluorescence of *Q rubra* leaves.

	F_o	F_m
Control		
Low irradiance	1.00 ± 0.05^a	1.00 ± 0.05^a
High irradiance	0.95 ± 0.06^b	0.79 ± 0.09^c
Waterlogged		
Low irradiance	1.08 ± 0.07^c	0.86 ± 0.13^b
High irradiance	0.97 ± 0.18^b	0.51 ± 0.14^d

Values computed as a fraction of the corresponding mean of control plants grown in the shade, and pooled over the waterlogging period (means ± SD). $^{a-d}$ Different letters indicate significantly different values, Fisher's PLSD; n = 6 days × 5 seedlings.

Photochemical efficiency during the afternoon

Time courses of photochemical efficiency recorded during the afternoon under the actual irradiance ($\Delta F/F_m'$) presented very distinct features in the three species (fig 9). As expected, values measured on shade plants (and under low irradiance) were high and close to 0.75. They were much lower in sun plants and under higher irradiance. In addition, the highest values among sun plants were detected in *Q robur*, followed by *Q petraea* and *Q rubra* (0.62, 0.55 and 0.48, respectively, with the exclusion of day 227 when irradiance was higher). The interspecific ranking of these values corresponds to that for net assimilation rates. Waterlogging resulted in very limited decreases in leaves

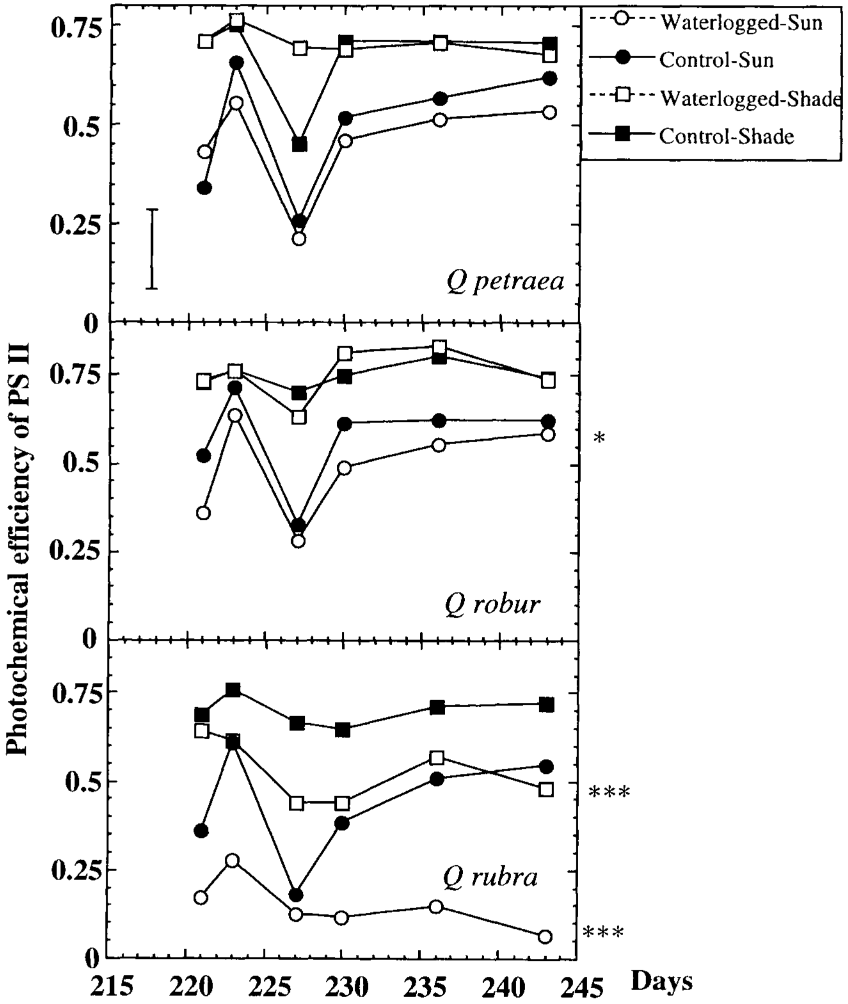

Fig 9. Time course of photochemical efficiency of photosystem (PS II) ($\Delta F/F_m'$) recorded during the afternoon under actual irradiance, in seedlings from three oak species grown under two irradiance regimes and submitted to waterlogging. Mean values; $n = 5$; *** denotes highly significant effect of waterlogging. Vertical bar denotes mean value of (±) SD.

of *Q robur* and *Q petraea*, but induced large declines in *Q rubra* under the two irradiance levels; again, the decrease in $\Delta F/F_m'$ was proportional to that of net assimilation rates.

Decreases in photochemical efficiency of PS II were accompanied as expected by increases in non-photochemical quenching (q_{NSV}, fig 10). In general, all treatments

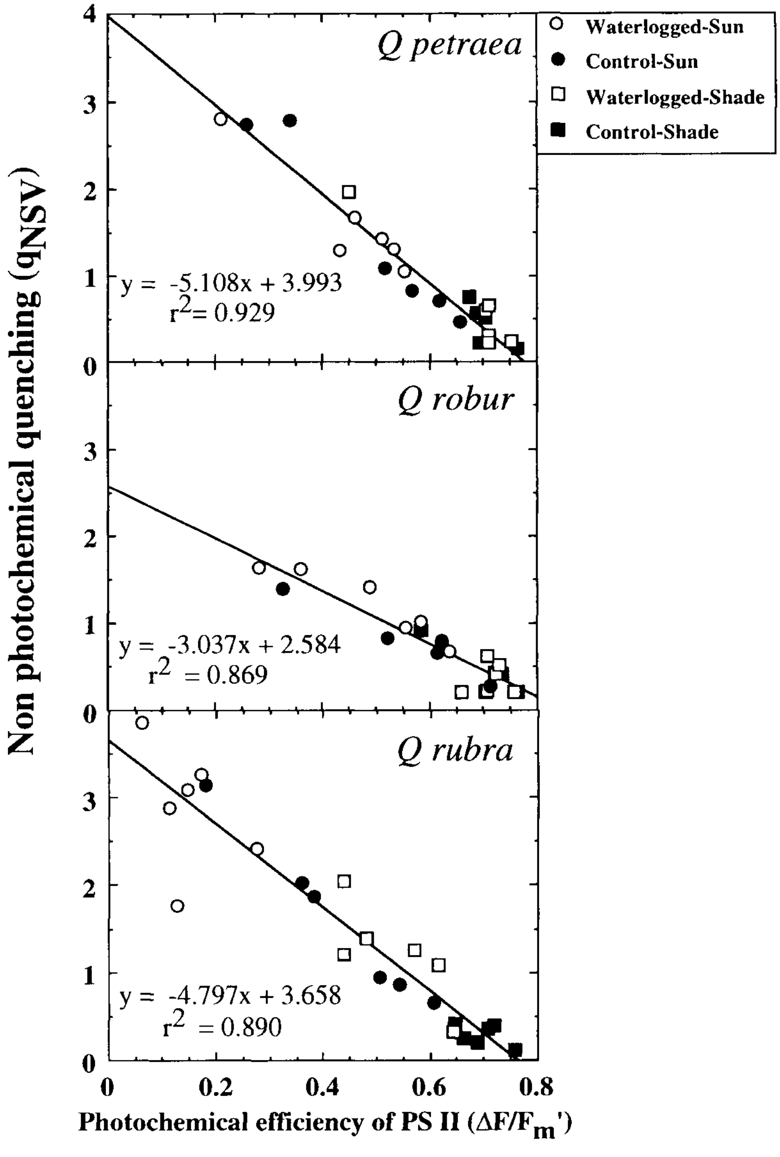

Fig 10. Correlation between photochemical efficiency of photosystem (PS II) ($\Delta F/F_m'$) and non-photochemical quenching (q_{NSV}) recorded under growth irradiance in seedlings from three oak species grown under two irradiance regimes and submitted or not to waterlogging. Mean ± SD; $n = 5$.

aligned on the same relationship in a given species, showing that decreased efficiency was obtained with similar increases in q_{NSV} independently of the treatment. The $\Delta F/F_m'$ – q_{NSV} relationship was different in *Q robur* as compared to the two other species: similar declines of photochemical efficiency were obtained with a smaller increase in non-photochemical quenching in the former.

DISCUSSION

Effects of irradiance on the photosynthetic performance of leaves of the three species

Seedlings from the three oak species displayed significant differences in photosynthetic performance, similarly to what had been reported during earlier comparative studies. In general, seedlings of *Q robur* present larger stomatal conductance, higher net assimilation rates and photosynthetic capacities than *Q petraea* and *Q rubra* (Epron et al, 1993; Dreyer, 1994). The low performance of *Q rubra* is probably related to low chlorophyll contents. Fertilization during the second experiment resulted in improved performance of seedlings from this species, together with increased chlorophyll contents; nevertheless, the levels attained by the two other species were not reached. Such differences should not be regarded as reflecting general specific traits; they are probably largely influenced by soil conditions in the pots. As such they reveal a large plasticity of *Q robur* seedlings with respect to soil conditions, and a low tolerance of *Q rubra* seedlings to limitations in soil fertility.

Despite the fact that shading was imposed late, after completion of elongation, it had a significant influence on photosynthetic performance in all species. In *Q robur* and *Q petraea*, it induced significant increases in chlorophyll content and reduced slightly the photosynthetic capacity of the leaves. In general, such responses correspond to the known acclimation to shade, and resemble those occurring when leaf ontogeny takes place under contrasted irradiance. The fact that such changes occurred even with adult leaves illustrates the large plasticity of the photosynthetic apparatus with respect to irradiance. Shading increased slightly but significantly the predawn value of photochemical efficiency of PS II (F_v/F_m). The physiological significance of this finding will be discussed below. Finally, as expected, shading reduced dry matter increment in both species in almost all cases.

For both years, shading had very positive effects on *Q rubra*: it unexpectedly increased net assimilation rates and photosynthesis. The extent and the direction of these changes clearly demonstrate that, under full irradiance, control seedlings suffered from chlorosis and high irradiance stress, which was partly relieved by shading. The difference in predawn photochemical efficiency (F_v/F_m) between controls and shaded plants was larger than in two of the other species. This can be interpreted as resulting from high irradiance stress due to low assimilation rates even under saturating irradiance. The fact that in sun leaves both basic (F_0) and maximal fluorescence (F_m) were lower, may be interpreted, according to Butlers model (Butler, 1978), as resulting from the onset of long lasting non-photochemical fluorescence quenching, rather than of severe dysfunctions in photochemical reaction centres. Additional measurements would be needed to confirm this point. The observed differences between shade and sun leaves in the two other species are more difficult to interpret: they may result from changes in the balance between antenna and photosystem core chlorophylls. A detailed discussion on this issue lies beyond the aims of our experiment.

Effects of waterlogging on growth and photosynthetic performance of leaves: comparison among species

Waterlogging induced disorders in growth and photosynthesis similar to what had already been described with oak species (Dreyer et al, 1991; Dreyer, 1994; Gardiner and Hodges, 1996). These disorders included stomatal closure and reduced rates of net CO_2 assimilation by leaves. Such effects could have been produced by a root-issued signal inducing stomatal closure, such as ABA (Jackson et al, 1988), but recent analyses revealed that the amount of ABA transported to shoots was reduced during waterlogging (Else et al, 1995). Enhanced export from roots of the ethylene precursor 1-aminocyclopropane-I-carboxylicacid (ACC) could be another signal for photosynthesis reduction (Else et al, 1995); however, Pezeshki et al (1996) found no increase in ethylene production by waterlogged oak seedlings, whereas they detected a significant one in the highly tolerant *Taxodium distichum*. In addition, it is unlikely that the observed disorders were only the consequences of long-lasting stomatal closure. In particular, decreases of F_v/F_m were almost never observed during drought stress. During water stress photosynthesis is limited mainly by stomatal closure (Epron and Dreyer, 1992), and decreases of F_v/F_m occur only when diurnal assimilation rates are already reduced to nil (Epron *et al*, 1993). Short-term drought never resulted in reductions of maximal photosynthetic capacity to the extent of those recorded with waterlogging (Chaves, 1991; Epron and Dreyer, 1993).

The extent of decreases in F_v/F_m was significantly modulated by shading. Decreases were much lower in the shade than under full irradiance. Moreover, under both irradiance regimes, severe declines in F_v/F_m occurred only when net assimilation rates during the day before, were already reduced to one-third of the initial level. We may hypothesize that root-hypoxia had probably no direct damaging effect on photochemistry, but that the observed declines of F_v/F_m resulted from photoinhibition occurring in leaves where net assimilation was already severely affected. This situation is to some extent similar to that observed during water stress, where photochemistry is only affected after complete cessation of net CO_2 assimilation (Epron et al, 1993). Nevertheless, short-term effects of water and hypoxia stress on photosynthesis probably differ very significantly: the former is mainly mediated via stomatal closure (no effects on photosynthetic capacity), while the latter seems to be due to a combination of both stomatal closure and direct effects on photosynthetic carbon reduction cycles, as revealed by the low photosynthetic capacities. In addition, the recovery from short-term drought stress is usually much faster than the one observed here.

The discrepancy among species, in the relationship between photochemical efficiency of PS II ($\Delta F/F_m'$) and non-photochemical quenching (q_{NSV}) was a surprising finding. The fact that in *Q robur* declines in $\Delta F/F_m'$ of similar amplitude than in the two other species were obtained with smaller increases in q_{NSV} means, in the frame of Butlers model, that the efficiency of open centres decreased less, and in turn that the extent of the centre closure was larger. The generality of this observation and its significance in the ecophysiological performance of the *Q robur* seedlings remain to be clarified.

Interactions between waterlogging and irradiance during the stress on biomass increment were limited; significant ones were only detected in the second experiment, where post-waterlogging stress probably amplified the effects of waterlogging. Reductions of biomass were proportionally higher in full irradiance plants in all species. Similarly, fine root dry matter was less reduced by waterlogging under shade, and tap root decay was of lesser extent in shaded *Q petraea* and *Q robur* (in *Q rubra*, very important decay occurred under both conditions). Decreases in predawn leaf water potential were larger under full irradiance than in the shade, and more interestingly, in *Q petraea* the post-waterlogging decrease was of lesser extent in the shade plants.

Post-water logging recovery discrimination of species sensitivity to stress

Post-waterlogging stress is a well-known consequence of the period of hypoxia: the recovery of higher O_2 concentrations in the rhizosphere results in severe oxidative stresses, which amplify the damage directly induced by hypoxia. This was also the case with oak seedlings, in which root decay occurred and predawn water potential went on decreasing in the sensitive species *Q rubra*, but also in the much less sensitive *Q petraea*.

A practical consideration may be stated as a conclusion. Testing the sensitivity of diverse species to soil hypoxia may rely on a quantitative and rapid assessment of disorders induced in photosynthesis during the initial steps (a few days) of the waterlogging stress. Nevertheless it should also take into consideration the sensitivity to post-hypoxic stress, which may reveal further dysfunctions and differences in tolerance.

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