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Vulnerability of young oak seedlings (*Quercus robur* L) to embolism: responses to drought and to an inoculation with *Ophiostoma quercii* (Georgevitch) Nannf

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Summary — Possible interactions between an infection with *Ophiostoma quercii* and water stress on pedunculate oak (*Quercus robur*) were tested with potted saplings. *O. quercii* was inoculated into the stems of 3-year-old saplings, and a severe drought was imposed for about 40 d. Drought promoted an irreversible decline in total leaf specific conductance of all saplings; direct measurement of losses of hydraulic conductivity in twigs and petioles revealed that a strong embolization occurred in the vessels as soon as minimal leaf water potential decreased below -2.5 MPa. This vulnerability to cavitation on rooted seedlings was in agreement with earlier data obtained on cut branches from the same species left to freely dehydrate; a slight artifact was probably due to the onset of occlusions of embolised vessels in the rooted plants. The presence of fungal spores in the stems did not induce any modification in these water relations on well-watered or stressed seedlings. The role of *O. quercii* in the oak decline symptoms as occurring in Europe may therefore be questioned.

water stress / embolism / oak / *Ophiostoma quercii* / hydraulic conductivity / water relationships / oak decline

Résumé — Vulnérabilité de jeunes semis de chêne pédonculé (*Quercus robur*) à l'embolie : réponses à la sécheresse et à une inoculation avec *Ophiostoma quercii*. Les effets potentiels d'une infection par *Ophiostoma quercii* sur la réponse à la sécheresse de jeunes plants de chêne pédonculé ont été testés. *O. quercii* a été injecté dans le tronc de plants âgés de 3 ans, et une sécheresse intense a été imposée pendant une quarantaine de jours. La sécheresse a provoqué une diminution irréversible de la conductance hydraulique spécifique de tous les plants. Des mesures directes de perte de conductivité hydraulique dans les rameaux et les pétioles ont montré qu'une forte embolie se produisait dès que le potentiel hydrique foliaire était abaissé en dessous de -2.5 MPa. Ce degré

* Correspondence and reprints

Symbols and abbreviations: ψ_{wd} : predawn leaf water potential (MPa); ψ_{wm} : midday leaf water potential (MPa); g_L : leaf specific hydraulic conductance ($\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$); E_T : total transpiration (mmol s^{-1}).

de vulnérabilité à l'embolie était très voisin de celui détecté en laissant des branches d'arbres adultes se dessécher rapidement au laboratoire. Les légères différences observées pour les potentiels hydriques les plus faibles ont pu être dues à des occlusions de vaisseaux se produisant lors de sécheresses de longue durée. La présence de spores d'*O. querci* dans le xylème n'a modifié ni la conductance totale des plants, ni la vulnérabilité des rameaux et des pétioles à la cavitation. Le rôle souvent attribué à ce champignon dans l'induction des dépérissements de chênes en Europe doit être remis en question.

sécheresse / embolie / chênes / *Ophiostoma querci* / conductivité hydraulique / dépérissement

INTRODUCTION

Oak stands in Western and Central Europe are frequently reported to present severe dieback symptoms. In France, pedunculate oak (*Quercus robur* L) is often declining, while sessile oak (*Q. petraea* (Matt) Liebl) seems to exhibit a better resistance; in Central Europe, both species suffer from severe decline. The precise chain of events leading to the onset of these decline processes is still poorly understood. Environmental constraints, and among them repeated periods of water shortage, probably play a major role (Landmann *et al*, 1993). However an involvement of various pathogens has frequently been suspected (Delatour, 1983; Kowalski, 1991). Among the numerous fungi isolated from declining oak trees, those belonging to the group of the Ophiostomatales (*Ascomycotina*) deserve special attention (Delatour, 1986). Indeed, this fungal group comprises a number of strong pathogens like those inducing oak wilt in north-east America (*Ceratocystis fagacearum* (Bretz) Hunt; Gibbs, 1981), or the Dutch-elm disease (*O. novo-ulmi*; Brasier, Sinclair and Campana, 1978). These vascular pathogens severely disorganize the water transport in infected trees (Hall and MacHardy, 1981; Beckmann, 1987).

Ophiostoma querci (Georgevitch) Nannf has been frequently isolated from declining oak trees (Kowalski, 1991) and is therefore suspected to be involved in the induction of the dieback. To test for this hypothesis, Delatour *et al* (1993) inoculated young

saplings of *Q. robur* with a suspension of conidia, but were unable to detect any foliar symptoms after this inoculation. They nevertheless observed the occurrence of localized bark necroses and conspicuous narrow strips of browning induced in the xylem tissue which were sometimes several 10s of cm long. Moreover, the fungus could be reisolated from these zones even 1 year later. Similar results have been described by Balder (1993) with *O. querci*, *O. stenoceras* (Robak) Melin and Nannf, and *O. proliferum* (Kowalski and Butin) de Rulamort. The length of these discolorations was highly variable among individual trees. These results suggested an important interaction between xylem structure in oaks and the ability of *Ophiostoma* spp to spread in the conducting tissues following an infection, as has been reported for other vascular pathogens (Beckmann, 1987).

However, even if the *Ophiostoma* spp already studied only promoted the occurrence of very limited symptoms of tracheomycosis on oaks under normal water supply, the presence of spores or hyphae inside the xylem could possibly affect tree water relations during drought. Among the mechanisms which could lead to long-term damage, induction of embolism in vessels and the subsequent dysfunctions in water transport could be of major importance. Information concerning vulnerability of oaks to cavitation is increasing. Cochard *et al* (1992) showed that significant embolism appeared as soon as the leaf water potential dropped below -2.5 MPa on branches of *Q. robur* left to dehydrate freely under labo-

ratory conditions, and that almost all vessels were embolised around -3.3 MPa. Measurements made on adult trees in a forest near Nancy during a gradually increasing drought yielded similar results (Bréda *et al*, 1993), and confirmed the good agreement observed by Tyree *et al* (1992a) between embolism induction during drought *in situ* and during rapid dehydration of cut branches. In the present work, we intended to evidence the cavitation induction patterns obtained with rooted saplings during slowly increasing drought. In addition, we tested for potential interactions between the presence of spores and hyphae of *O. quercii* in the xylem and the sensitivity to water stress. In particular, we tested the hypothesis that the presence of spores and hyphae in the xylem vessels could reduce the hydraulic conductivity of our trees, or that they might produce compounds reducing significantly the surface tension of the xylem sap, as reported by Kuroda (1989) who observed that volatile terpenes emitted during the infection of *Pinus thunbergii* by a nematode increased the susceptibility to cavitation.

We therefore inoculated *O. quercii* directly into the xylem of young oaks, and investigated the patterns of dissemination of the fungus in the xylem, comparing it with that simultaneously injected of Indian ink. We then submitted the saplings to water stress by withholding irrigation and followed the total hydraulic conductance from soil to leaves, and the onset of embolism in twigs and petioles.

MATERIAL AND METHODS

Plant material

Three-year-old seedlings of *Q. robur* L were grown in 10 L pots in a peat/sand mixture (50:50 v/v), fertilized with a slow release fertilizer (Nutricote 100, N/P/K 13:13:13, Fertil, Paris), and grown in a glasshouse at the Forestry Research Center of

Champenoux. They were watered every second day. During 1991, bud break and flushing occurred during early March. Seedlings were 170–250 cm high and stem diameter ranged from 0.5 to 1 cm at the inoculation point.

Fungus

The strain of *O. quercii* (Georgevitch) Nannf was isolated from cambial necroses on *Q. petraea* (Matt) Liebl during 1985, at Cerrilly, near Chatillon-sur-Seine (north-eastern France; Morelet, 1992), and stored on wood pieces at 4°C (Delatour, 1991). The inoculum was prepared from cultures grown during about 1 month on petri dishes (Difco malt agar 3%, 25°C), which produced large amounts of conidia (*Hyalodendron* and *Pesotum* stages). Washing each culture with 15 ml sterilized water yielded a high density of spores (about 10^8 ml⁻¹) adjusted to 10^6 m⁻³. The diameter of conidia was investigated using microfiltration; no conidia were smaller than 0.45 µm, but many passed 0.8 µm filters.

Inoculation

A micro-perfuse connected to teflon tubing containing the conidia suspension was used to inject the suspension directly into the xylem of the annual growth ring. The absorption was entirely passive, with no additional pressure.

Experiment 1

Patterns of dissemination of the fungus in the xylem tissue following injection were analysed on 48 trees using suspensions of conidia mixed with sterile Indian Ink (5% dilution, Steadler, Marsmatic 745R; sterilisation: 20 min at 120°C). Prior to the use of this mixed suspension, we tested for potential effects of Indian ink and latex paint, another dye frequently used in water relation studies, on conidial viability (24 h incubation at 25°C). The ink/conidia mixture (0.1 ml) was injected during April 1991 into 48 trees at 50 cm below the upper limit of the 1990 growth flush. Spread of the fungus inside the xylem was observed through re-isolation from cut segments of stems. Stems were disinfected with alcohol, debarked, and

sliced into 1 cm segments. Each segment was placed on a malt/agar medium containing 50 mg L⁻¹ of both penicillin and streptomycin. Different injection procedures were tested: (1) half of the injections (24) were made under water to avoid wounding induced cavitation, and half in air, and (2) in each group 18 trees were injected at dawn and 6 at midday with about -1.5 MPa water potential. Reisolation was made after 2-3 h, and delayed by 24 h on half of the trees.

Assessment of vessel length

Vessel lengths were measured in 8 seedlings using the technique described by Zimmermann and Jeje (1981) adapted to oaks by Cochard and Tyree (1990). A solution of blue pigment (latex paint) was diluted 100/1 in water and passed through a 5 µm filter. The eluate was perfused through stem segments from the distal end, at an over-pressure of 0.015 MPa during 24 h. Perfusions were applied at 4 different locations: 5 cm above, and 5, 20, 50 cm below the contact zone between 2 successive growth cycles; 2 saplings were used for each of these treatments. The number of vessels filled with pigments was counted under a dissecting microscope every 2.5 cm. Only vessels included in the current year's (1991) wood with a diameter above 20 µm were taken into account. The statistical procedure of Zimmermann and Jeje (1981) was used to estimate vessel length distribution.

Experiment 2

Total hydraulic conductance during drought was measured on 16 seedlings grown in individual 10 L pots. They were inoculated during May with repeated injections at about 10 points all along the upper 70 cm of the stem to ensure a satisfactory dispersal of conidia all over the xylem (inoculated trees), or injected in the same way with sterile water (control trees). After 2 months of incubation, 4 treatments were defined: (1) water-stressed and inoculated with *O. quercus*; (2) water-stressed and non-inoculated; (3) well-watered and inoculated; and (4) well-watered and non-inoculated (control). Two successive cycles of drought were imposed, each lasting about 10-15 d. Pots were weighed every second day and either the total amount (controls) or half of

the lost water (water stress) was added during the first drought cycle. During the second, pots were left to dry out freely. Predawn (ψ_{wd}) and midday (ψ_{wm}) leaf water potentials were measured on one leaf of every tree during 6 sunny days with a pressure chamber, before dawn, and between 12 and 1 pm UT, respectively. Losses of weight were recorded for each plant between 11 AM till 1:30 pm UT (Sartorius IB31000P balance, ± 0.1 g). Due to the large leaf area of the saplings, soil evaporation was considered to be negligible and the loss of weight was recorded as the diurnal maximal rate of transpiration (E_t). Total leaf area (LA) of each tree was estimated at the end of the experiment with a planimeter (ΔT Devices, UK). These measurements allowed the computation of a specific soil to leaf hydraulic conductance as reported by Cohen *et al* (1983), Granier and Colin (1990) and Reich and Hinckley (1989) as:

$$g_L = E_t / \{LA (\psi_{wd} - \psi_{wm})\}$$

g_L : specific soil-to-leaf hydraulic conductance (mmol m⁻² s⁻¹ MPa⁻¹); E_t : maximal transpiration (mmol s⁻¹); LA: leaf area (m²); and ψ_{wd} and ψ_{wm} : predawn and minimal leaf water potential (MPa); in this equation ψ_{wd} was used as an estimate of the soil water potential.

Experiment 3

Loss of hydraulic conductivity of twigs and petioles during drought was examined on 80 seedlings (same substrate, same pots, same height, but 2-3 seedlings grown in each pot) were used for the same treatments as in *Experiment 2*. Drought was imposed as in *Experiment 2*, and ψ_{wd} measured every second day on one of the individuals in each pot. Watering was controlled to maintain midday leaf water potential (ψ_{wm}) above -3.3 MPa during the first cycle, and no watering was supplied during the second period of drought. The technique developed by Sperry *et al* (1988), and described in detail by Cochard *et al* (1992) for oak trees was used to monitor loss of hydraulic conductivity. ψ_{wm} was measured between 11 am and 1 pm UT and the pot rewetted to stop any further induction of embolism. During the following morning, 5 twigs and 10 petioles were cut off under water from the upper crown of the same seedling. Twigs were recut into 2 cm long segments under water. Petioles were prepared in the same way, and a segment of the leaf mid-rib included whenever the petiole was less than 2 cm

long. This procedure was repeated during the experiment on 8 well-watered and 15 water-stressed for both the inoculated and control treatments. Embolism was computed as the loss of conductivity, *ie* as:

$$(k_m - k_i) / k_m$$

where $k = F / P$

where k_i is the actual conductivity ($\text{mg s}^{-1} \text{MPa}^{-1}$), measured immediately on the sample with a 65 cm head of degassed distilled water containing 0.1% HCl (pH 2); this step was performed as quickly as possible to avoid passive resaturation of the xylem; k_m is the maximal conductivity, measured after resaturation of the samples by repeated flushes of a perfusion solution at 0.1 MPa; a single flushing of 15–20 min was usually enough to fully restore maximal conductivity; for strongly embolized samples, 2 periods of 15 min each were used; F is the actual flow of degassed water through the sample (kg s^{-1}), monitored with a balance (Mettler, $\pm 0.01 \text{ mg}$); l is the length of the sample (m), usually 2 cm; and P is the pressure applied to the water (MPa).

Maximal conductivity (k_m) was used to calculate the leaf specific conductivity of individual petioles ($= k_m / \text{LA}$, $\text{mg s}^{-1} \text{MPa}^{-1} \text{m}^{-1}$, with LA: leaf area).

RESULTS

Vessel lengths

Distributions of vessel lengths showed following features (fig 1): (1) vessel lengths measured from the top of these 2.5 m high saplings sometimes reached values as high as 80 or even 110 cm; (2) mean vessel length increased from top to bottom of the stem; (3) no discontinuity appeared between both growth flushes (1990 and 1991); and (4) more than half of the vessels 5 cm above the connection were over 5 cm long.

Fungus dispersal in the stem

O. quercii could be reisolated in continuous sequences from 37 trees and in discontin-

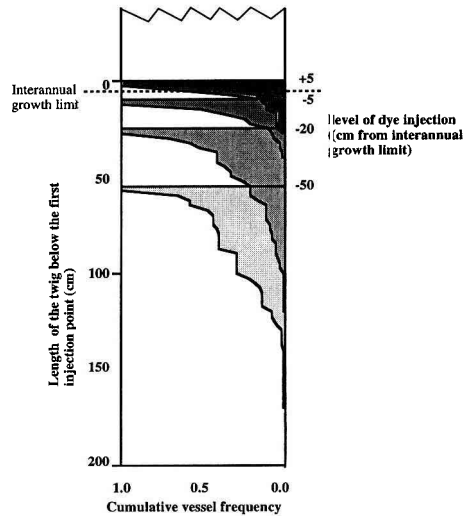


Fig 1. Cumulated frequencies of vessel lengths in the current year wood of 3-year-old *Q. robur* saplings, as measured through dye injection at 4 different locations in the stem: 5 cm above and 5, 20 and 50 cm below the contact between current and previous year shoots. The 4 corresponding distributions were represented on the same figure.

uous sequences in the 11 remaining from the injection point till a maximal distance varying between 7 and 46 cm (mean 28.7 ± 10.7 cm, very regular distribution with a kurtosis of -1.114 and a skewness of -0.022). The maximal spread was identical whether the injection had been made under water or not (respectively 29.9 ± 10.9 and 27.4 ± 10.6 cm, Fisher PLSD non-significant at 5%). No significant effect of any of the other injection procedures could be observed: injections at predawn, while water potential was high, were followed by the same pattern of distribution of the conidia in the stems than injections made at mid-day; furthermore, reisolation after 2 h yielded the same maximal distance of spread than those made after a 24 h delay. The only difference was related to the speed of absorp-

tion of the inoculum: the delay for the complete absorption of the 0.1 ml suspension varied between a few seconds (injection at midday) and a few minutes (injection at dawn).

In fact, when analysing in parallel spread of spores and of Indian ink, we observed very similar values of maximal extent for both the fungus (28 ± 10.7 cm) and the dye (27.4 ± 9.1 cm). Moreover, a very good correlation between both values was detected (fig 2). In a few cases, the extent of dye was slightly lower, which was probably related to the difficulty of detecting pigmentation when only very few vessels were stained. We concluded that the main factor controlling the extent of the fungus was probably the dimension of vessels into which conidia were injected, and that the mechanism governing the spread of these conidia was a passive diffusion similar to that of particulate ink.

The survival of the fungus in the inoculated trees was shown through reisolutions during following year made on 3 inoculated

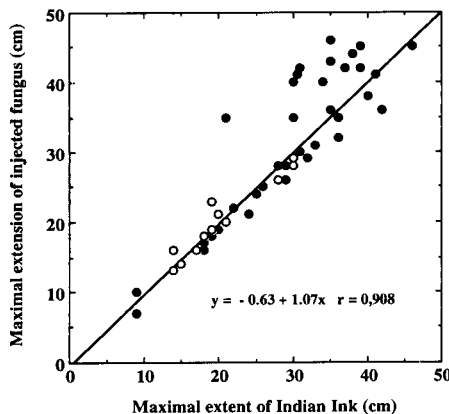


Fig 2. Relationship between the maximal spread of spores of *O quercus* (as observed with sequential reisolation from 1 cm long segments) and that of Indian ink particles in the xylem of young *Q robur* following the injection of a mixture of spores and ink at the base of the stems. Injections were made either at predawn (●) or at midday (○) on transpiring plants.

seedlings. As already observed by Delatour *et al* (1992), the fungus remained present in the xylem tissue in which it was injected but did not spread further. It nevertheless induced browning symptoms in the xylem, which were already detected at the end of the drought experiments, that is 2 months after inoculation, and again 1 year later (data not shown).

Total soil-to-leaf hydraulic conductance (g_L) in response to drought and inoculation

Reduced watering imposed a rapid decline of ψ_{wd} , which reached low values of about -2 MPa after 18 d. Complete rewatering allowed a recovery to high values around -0.5 MPa in less than 2 d. The second drought cycle yielded even stronger reductions to -3.8 MPa (fig 3c). ψ_{wm} decreased approximately from -2 to -3 MPa during the first drought cycle; rehydration yielded only a slight recovery, and finally the second drought cycle resulted in a decline to -3.8 MPa. The difference between ψ_{wd} and ψ_{wm} remained high during periods of adequate water supply, but decreased strongly during the stress.

Control trees showed almost constant values of g_L with no significant difference between inoculated and non-inoculated trees (fig 3a). Mean values were 1.03 and 1.02 $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$ for inoculated and controls, respectively, which were not statistically different (Fisher PLSD, 5%).

The value of g_L declined rapidly to very low values during the first drought cycle (fig 3b); it recovered only partially after rewatering, and decreased again during the second cycle. The lowest values reached during drought were around 0.25 $\text{mmol m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$. No significant difference between inoculated and control saplings could be detected during this stress evolution (Fisher PLSD, 5%).

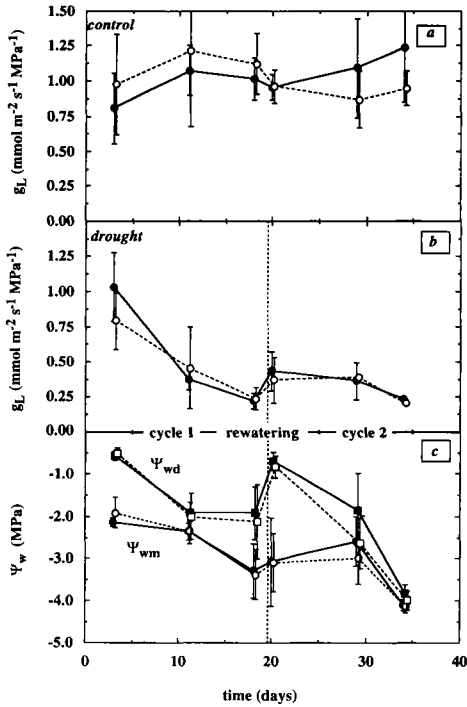


Fig 3. Water relations of inoculated (●) and non-inoculated (○) saplings of *Q. robur* during 2 successive drought cycles: time-course of leaf specific hydraulic conductance (g_L) of well-watered (a) and drought-exposed (b) saplings, and of mid-day (ψ_{wm}) and predawn (ψ_{wd}) leaf water potentials of drought-exposed saplings (c). Vertical bars indicate standard error.

A direct plot of g_L against ψ_{wd} (fig 4) showed that the observed declines appeared at rather high values of ψ_{wd} (around -1 MPa). Stronger stress intensities only induced limited additional depression of g_L .

Embolism in twigs and petioles

During the whole experiment, loss of hydraulic conductivity remained very low in well-watered saplings, and no significant inoculation-related difference appeared on twigs or petioles (table I). Embolism strongly

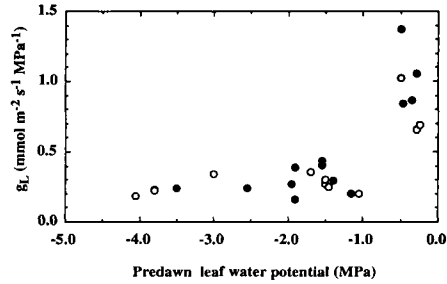


Fig 4. Total leaf specific conductance (g_L) of drought-exposed saplings of *Q. robur* as a function of predawn leaf water potential (ψ_{wd}). The data are the same as in figure 2b, but each point represents an individual measurement. Data obtained the day following rewatering were omitted.

increased after the 2 periods of water stress and yielded 60% loss of conductivity, but again no difference was detected in relation to inoculation (table I).

We plotted all measured values of embolism against the lowest values of ψ_{wm} experienced by the saplings prior to the measurement (fig 5). Two major observations could be drawn from these vulnerability curves: (1) curves from inoculated and non-inoculated saplings overlapped completely showing that no effect of the inoculation with *O. quercii* was detected on petioles or twigs; and (2) twigs and petioles displayed approximately the same vulnerability to embolization.

Finally, we compared these vulnerability curves obtained on petioles from potted saplings dehydrated at a rather slow rate (40 d) with those from branches rapidly dehydrated (a few hours, data obtained with adult *Q. robur*, Cochard *et al*, 1992, fig 6b). Both curves showed strong similarities, with cavitation beginning around -2.5 MPa and increasing steeply around -3 MPa. The second half of the curves diverged: on potted saplings, losses of conductivity remained significantly lower than on cut branches at low water potentials.

Table I. Loss of hydraulic conductivity detected in twigs and petioles of saplings of *Q robur* inoculated or not with *Q quercu* (a) well-watered controls; (b) after 2 periods of strong drought (1 = complete loss of conductivity).

	Petioles	Twigs
Irrigated <i>n</i> = 9		
Inoculated	0.075 ± 0.02 ^a	0.17 ± 0.04 ^a
Control	0.045 ± 0.01 ^a	0.09 ± 0.03 ^a
Droughted <i>n</i> = 9		
Inoculated	0.58 ± 0.07 ^b	0.61 ± 0.05 ^b
Control	0.643 ± 0.059 ^b	0.645 ± 0.061 ^b

Same letters in a column indicate absence of difference (Fisher PLSD, 5%).

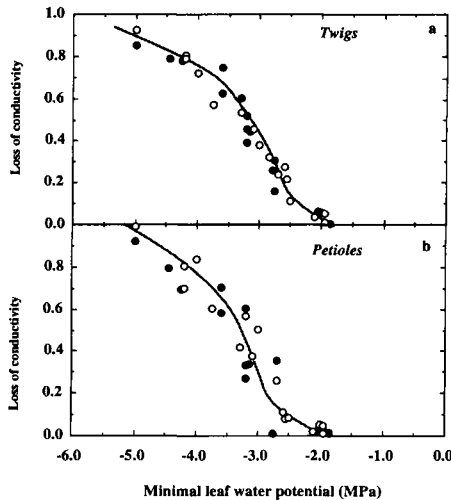


Fig 5. Vulnerability to cavitation as expressed by losses of conductivity vs minimal leaf water potential; (a) twigs and (b) petioles from *Q robur* saplings, inoculated (●) or not (○) with *Q quercu*. Each point is the mean of 5 (twigs) or 10 (petioles) measurements made on a unique sapling. Lines were eye-fitted.

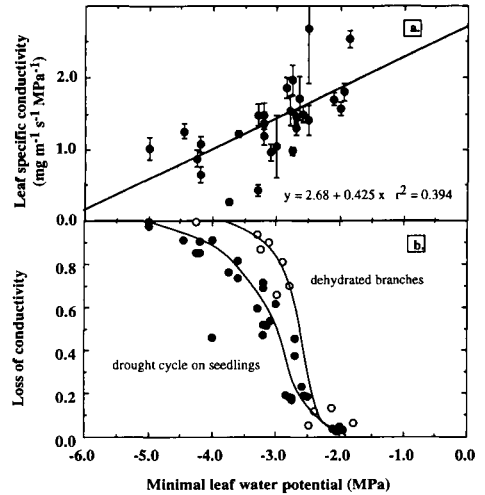


Fig 6. Relationship between minimal values of leaf water potential and (a) total leaf specific hydraulic conductivity (LSC) measured on excised petioles; linear regression is indicated; and (b) loss of hydraulic conductivity corrected for reductions in LSC (same data as in figure 5b) (●). Data obtained earlier on cut and dehydrating branches have been added for comparison (Cochard *et al*, 1992) (○).

This discrepancy could be explained by the fact that, on slowly dehydrating potted saplings, embolized vessel could be progressively plugged and therefore unable to refill under pressure during our measurements. This would lead to underestimates of maximal conductivity (k_m) and is a consequence of drought-induced loss of conductivity. Calculated values of leaf specific conductivities (k_m/LA) decreased significantly with the lowest values of ψ_{wm} (fig 6a), which can only be the consequence of decreases in k_m with increasing duration of drought. We corrected our data for this artefact, using the regression coefficient between minimal potential and k_m , and obtained the new vulnerability curve displayed in figure 6b, which is similar to that obtained with cut branches.

DISCUSSION

Water relations of oak saplings submitted to drought

Vessel length distribution in current year's wood of *Q robur* saplings was in agreement with the results obtained by Cochard and Tyree (1990) with mature *Q rubra* and *Q alba*.

The same maximal length of about 1 m was observed, and the distribution along the growing axis was similar, with the shortest vessels located near the distal end of the current year's shoot, and the longer ones in the earlier growth segments.

Total specific hydraulic conductance of trees (g_L) is a good parameter describing the overall efficiency of water extraction from soils and transport to the shoots (Reich and Hinckley, 1989; Granier and Colin, 1990; Bréda *et al*, 1993). The values obtained here were in close agreement with those reported for oaks by Reich and Hinckley (1989). The g_L decreased in response to drought, similarly to what had been reported in many species (Granier *et al*, 1989; Bréda *et al* 1993). Such decreases may be due either to changes in the hydraulic properties at soil–root interface or to xylem embolism with strong stresses. Under natural conditions they were mainly ascribed to reversible reductions of hydraulic conductivity at the soil–root interface (Bréda *et al*, 1993). In the present study, the decline in g_L could only be partially reversed by rewatering. This poor recovery may be partly ascribed to the fact that vessel embolization participated in the decrease in g_L , under such low leaf water potentials, as has been demonstrated by direct measurements of losses of conductivity in twigs and petioles.

The vulnerability to cavitation evidenced by our measurements of loss of hydraulic conductivity on twigs and petioles during a

drought was very similar to that measured with different oak species (Cochard *et al*, 1992): cavitation began at leaf water potentials around -2.5 MPa. Oaks display an intermediate response between vulnerable species like *Juglans regia* (Tyree *et al*, 1992b), or *Populus deltoides* (Tyree *et al*, 1992), and more resistant ones like *Juniperus virginiana* or *Cedrus atlantica* (Tyree and Ewers, 1991; Cochard, 1992).

We clearly showed that twigs and petioles displayed very similar vulnerabilities to embolism, as had already been shown on several different oak species (Cochard *et al*, 1992). Oaks present no hydraulic segmentation based on differential susceptibility to cavitation, while some species like common walnut (*Juglans regia*) do; in the latter petioles are much more vulnerable than twigs (Tyree *et al*, 1992b).

The good agreement between the results obtained with our saplings dehydrating over a relatively long period and earlier data obtained by Cochard *et al* (1992) on excised branches, showed that the cavitation-inducing processes are probably of the same nature *in situ* and on cut branches. This observation confirms the suitability of the latter method, as already shown by Tyree *et al* (1992a). Apart from this rather good agreement between both methods concerning the water potential inducing onset of cavitation (around -2.5 MPa), a significant discrepancy appeared for stronger deficits: the loss of conductivity was more progressive in potted saplings, and 50% loss was reached at about -3.2 MPa, while for severed branches it had already been reached -2.6 MPa. A very likely explanation for this lies in the fact that during gradually increasing drought, embolization may rapidly become irreversible, due to vessel plugging. This leads to artefacts in the estimate of loss of conductivity with Sperry's resaturation technique. The decrease of the leaf-specific conductivity of petioles with increasing drought duration and intensity we

evidenced in this work is a good argument for this hypothesis. Nevertheless, a correction of our data based on the assumption that leaf specific conductance should be constant in the absence of drought, did not completely overcome the differences. They could also be partly due to differences among juvenile and adult trees, but no information is yet available on age-related changes of vulnerability in current year wood of trees.

Effects of an inoculation with *O quercus* on water relations

Our results brought some insight into the mechanisms leading to the initial dispersal of spores of *O quercus* following a direct inoculation. A rapid dispersal of conidia at a rate and a distance very similar to that observed for ink particles was observed in the xylem of our potted saplings. Underwater injection, aimed at avoiding wounding-induced cavitation, did not modify it as compared to direct injection. Furthermore, neither the rate of transpiration nor the leaf water potential prevailing during injection had any effect on this dispersal. In fact, the initial dispersal of the conidia appeared to be a passive process very similar to that occurring with particles of Indian ink, and was probably mainly controlled by the length of the vessels into which both were injected. Pit membranes impeded their transport into adjacent vessels, as their pores probably do not exceed 0.17 μm diameter (Ewers and Fisher, 1989). The few discrepancies that we observed between the dispersal of ink particles and fungus reisolation were probably due to the higher sensitivity of the latter method. Similar observations have already been reported by Mace *et al* (1971) for *Fusarium oxysporum*.

Reisolations made after 1 year showed that the fungus remained viable, but still sequestered in the same stem segments

(Delatour *et al*, 1993), thus demonstrating that the initial dispersal was not followed by any further spread into adjacent vessels. This point clearly distinguished *O quercus* from *O novo-ulmi* which spreads readily all over the xylem of young elms after a few days (Delatour *et al*, 1993).

Despite this poor ability to colonize xylem tissues, *O quercus* survived in the stems of young oaks and induced browning symptoms which were detected just 2 months after inoculation. This observation confirms many earlier observations of brown spots in the xylem of inoculated trees and seedlings (Przybyl and Delatour, personal communication). The presence of living conidia in the xylem tissue and vessels could induce potential synergistic effects with drought. Two complementary observations following massive injection at several heights in the stem showed that no such effects occurred: (1) the total leaf-specific hydraulic conductance (g_L) was not modified on well-watered or stressed plants by the presence of the fungus; and (2) cavitation induction occurred at exactly the same rate in both cases.

Vascular pathogens have been described to induce losses of hydraulic conductivity in young seedlings of *Q rubra* inoculated with *Ceratocystis fagacearum*; in this case, vessels became non-conductive and tyloses, gum and material depositions were observed (Jutte, 1977). In seedlings of *Ulmus americana* inoculated with *O ulmi*, Newbanks *et al* (1983) detected rapid embolization along a 10 cm segment of stem above the wound. No such direct occlusion of vessels was observed in our trees, as leaf-specific hydraulic conductance was not significantly modified. Another potential action of vascular pathogens has been hypothesized: the emission of compounds decreasing the surface tension of xylem sap and as a consequence increasing the susceptibility to cavitation. Such hypothetical effects would only act under high tensions in

the xylem, *ie* during periods of water stress. This hypothesis was considered and discussed by Kuroda (1989) who suspected that volatile terpenes induce cavitation in the case of *Pinus thunbergii* infected with the pine wood nematode (*Bursaphelenchus xylophilus*). For *O. quercii*, we observed no direct vessel occlusion as the total soil-to-leaf hydraulic conductance remained constant. Nor did we detect increased susceptibility to cavitation in uninfected twigs. As a result, water stress did not lead to enhanced disorders in inoculated vs non-inoculated saplings. We must therefore conclude that *O. quercii* is unable to induce any hydraulic dysfunction in young oaks even during a strong drought. This observation, added to the increasing number of reports of the low pathogenicity of *Ophiostoma* species on adults trees *in situ* in Europe (Balder, 1992) reinforces the hypothesis of the absence of implication of this fungus in the oak decline processes reported.

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