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Impact of *Erysiphe alphitoides* on transpiration and photosynthesis in *Quercus robur* leaves

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Abstract Oak powdery mildew, (*Erysiphe alphitoides*) causes one of the most common diseases of oaks. We assessed the impact of this pathogen on photosynthesis and water relations of infected leaves using greenhouse-grown oak seedlings. Transpiration of seedlings infected by oak powdery mildew was also investigated. Altogether, *E. alphitoides* had a low impact on host gas exchange whether at the leaf or whole plant scale. Maximal stomatal conductance of infected leaves was reduced by 20–30% compared to healthy controls. Severely infected seedlings did not experience any detectable change of whole plant transpiration. The reduction in net CO_2 assimilation, A_n, was less than proportional to the fraction of leaf area infected. Powdery mildew reduced both the maximal light-driven electron flux (J_{max}) and the apparent maximal carboxylation velocity (Vc_{max}) although Vc_{max} was slightly more impacted than J_{max}. No compensation for the infection occurred in healthy leaves of partly infected seedlings as the reduced photosynthesis in the infected leaves was not paralleled by increased A_n levels in the healthy leaves of the seedlings. However, *E. alphitoides* had a strong impact on the leaf life-span of infected leaves. It is concluded that the moderate effect of *E. alphitoides* on oak might be related to the small impact on net CO_2 assimilation rates and on tree transpiration; nevertheless, the severe reduction in leaf life-span of heavily infected leaves may lead to decreased carbon uptake over the growth season.

Keywords Powdery mildew · CO_2 Assimilation · Gas exchange

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Introduction

Oak powdery mildew caused by *Erysiphe alphitoides* (formely *Microsphaera alphitoides*) is one of the most common diseases of pedunculate (*Quercus robur*) and sessile (*Q. petraea*.) oaks in Europe. *Quercus robur* is the most susceptible species. *Erysiphe alphitoides* is an exotic pathogen that began spreading in Europe in 1907 (Foex 1941). The disease can be very severe, especially in young regenerations. In young stands, it reduces growth and causes a large seedling mortality (Soutrenon 1998). In mature trees, the disease is generally considered far less damaging although it can reduce tree vigour in conjunction with other factors such as defoliation by insects, and therefore can contribute to tree decline (Thomas et al. 2002, Marçais and Breda 2006). Only young developing

leaves are susceptible to infection by *E. alphitoides* (Edwards and Ayres 1982). Colonisation by the pathogen induces tissue necrosis only when infection occurs very early during leaf development. As the pathogen develops quite late in spring, after the growth of the first flush of oak saplings, the disease is especially prevalent on the second and third flushes that develop between the end of June and August. This mitigates the severity of the disease on large trees. However, the French forest-health survey network recently reported severe infection on mature oak trees and suggested that the pathogen could in some cases trigger tree decline. This appears to be linked with an arrival of the pathogen early in the vegetative season. There was, therefore, a need to analyse the consequences of severe attacks for tree physiology and, in particular, for water use and the ability to assimilate carbon through photosynthesis.

It is well known that foliar pathogens reduce net CO_2 assimilation by leaves (Lakso et al. 1982; Shtienberg 1992; Sabri et al. 1997). This is due to the direct action on photosynthetic processes or to a reduction of stomatal conductance or to both. Indeed, many leaf pathogens induce stomatal closure (Mignucci and Boyer 1979; Niederleitner and Knoppik 1997; Pinkard and Mohammed 2006), although there might be some exceptions (Lakso et al. 1982). A direct effect of the leaf pathogen on photosynthetic processes could be a reduction of carboxylation efficiency due to a possible impact on RUBISCO or on other enzymes of the Calvin cycle (Pennypacker et al. 1990; Niederleitner and Knoppik 1997; Mayr et al. 2001). Leaf pathogens can also alter light interception and photochemistry due to chlorophyll decay and consequently limitation in light-driven electron fluxes (Holloway et al. 1992; Niederleitner and Knoppik 1997; Bassanezi et al. 2002).

It is known that *E. alphitoides* does reduce carbon assimilation in leaves (A_n) and translocation of carbohydrates from infected leaves to the rest of the plant (Hewitt and Ayres 1975, 1976). However, to properly assess the impact of oak powdery mildew on whole-tree physiology, we need to know the relationship between severity of leaf infection and physiological dysfunction such as reduction of A_n or altered transpiration, which has not yet been studied. Fraction of leaf area infected is usually not an efficient estimate of the magnitude of pathogen impact on leaf CO_2 assimilation (Shtienberg 1992; Bassanezi et al. 2002; Robert et al. 2004). Bastiaan (1991) introduced the concept of virtual lesions to assess the impact of a pathogen on leaf physiology. A larger impact than expected from lesion size could be caused by a toxin or by an accumulation of carbohydrates within the infected leaves due to altered translocation (Wright et al. 1995). In contrast, increased photosynthesis in the healthy areas of infected leaves, caused by altered sink-source relationships, could explain the occurrence of a smaller than expected impact of lesion size. Such a compensation for infection by the healthy parts of the seedlings could also occur at whole plant level, with increased photosynthesis in healthy leaves. This was described on spruce trees infected by rust (Mayr et al. 2001).

Foliar pathogens often alter leaf stomatal conductance (Niederleitner and Knoppik 1997; Pinkard and Mohammed 2006). *Erysiphe alphitoides* increased transpiration of infected oak leaves (Hewitt and Ayres 1975). However, little is known about the impact of foliar pathogens on whole-plant water use. Such an impact on transpiration could be very detrimental because of the frequency of *E. alphitoides* and to the fact that water shortage during drought is one of the main causes of oak decline.

We therefore tested the following hypotheses to document the physiological impact of controlled levels of infection with *E. alphitoides* on leaves of *Q. robur* seedlings: (1) Reductions in net CO_2 assimilation rates (A_n) are proportional to the fraction of leaf area infected; (2) Heavy infection results in a compensating increase of A_n in the non-infected leaves; (3) Heavy infection leads to altered transpiration at the leaf and whole sapling levels. These questions were addressed with seedlings that were manually infected with suspensions of spores of the fungus.

Materials and methods

Plant material

The experiments were conducted with 1 year-old *Q. robur* seedlings grown from acorns (15–25 cm high) collected in the Amance forest (close to Nancy, Meurthe-et-Moselle, NE France) except when otherwise stated. Plants were grown in an outdoor nursery, in 5 l pots (1/1, v/v sand-peat potting mix) and transferred

during the winter preceding the experiment to a greenhouse. Nutrients were supplied at planting in the form of 2 g l^{-1} Nutricote 100 (N/P/K 13/13/13 plus micronutrients) and pots were watered daily.

Inoculation and infection rating

Inoculation was performed by applying *E. alphitoides* spores with a thin paintbrush onto the upper face of expanding leaves when they had reached about 70–90% of their final area. The spores were produced on infected oak seedlings shaken one day before the experiment to eliminate the presence of old spores. The oak powdery mildew used for inoculation was a population from the Amance forest (Meurthe-et-Moselle, NE France) maintained on *Q. robur* seedlings in the greenhouse. Depending on the experiment, we either tried to produce homogeneously-infected oak seedlings, with different levels of infection (see below) or seedlings with leaves at different levels of infection. For this purpose, we applied the following dry-inoculation treatments: (i) no inoculation and protection for 5 days of the leaves with cellophane bags allowing gas exchange, in order to prevent cross-infections; (ii) one inoculation spot towards the centre of the leaf with a very thin brush (no. 4); (iii) 3–4 inoculation spots scattered on the leaf with a medium brush (no. 8); (iv) inoculation of the entire leaf surface with the medium brush. We designed the inoculation procedure so as to obtain leaves with each of the four treatments on the same branch. Non-inoculated seedlings protected from infection with cellophane bags and placed in the same conditions as inoculated ones were used as controls.

Two to 3 weeks after inoculation, leaves were rated for infection on a 0–4 scale: 0, no infection; 1, one to three lesions of mildew and < 25% of leaf area infected; 2, > 3 lesions or 25–50% of the leaf surface infected; 3, > 50% of leaf surface infected; 4, leaf with necrosis and/or abnormal growth with infection-related deformation. At this time, lesions were sporulating. Studied leaves were selected *a posteriori* to represent infection classes 0 to 3. Leaves in class 4 were not included in the study because the major impact of *E. alphitoides* on those leaves was to drastically reduce their functional area, and to lead to the formation of small and distorted leaves. To assess the fraction of leaf area infected, contours of the leaves and of the infected areas were recorded at the end of each experiment on a plastic sheet and the total leaf and infected area were computed with a DeltaT video leaf-area meter (DeltaT, Hoddesdon, UK).

Experiment 1: transpiration of whole plants

Sixty 2 year-old oak seedlings were randomly assigned to 4 inoculation treatments: (i) non-inoculated controls; (ii) two spore inoculation spots on each leaf with a thin brush (no. 4); (iii) four spore inoculation spots on each leaf with a medium brush (no. 8); (iv) inoculation of the entire surface of all leaves with the medium brush. Plants were rated for infection 4 weeks after inoculation, with each leaf rated according to the above description. The seedling infection rating was computed as the mean of all leaf ratings. Forty-one seedlings with infection ratings from 0 to 3.5 were then selected for the study.

The potted seedlings were transferred to a climate chamber at a temperature of 25°C with a 17 h photoperiod and an irradiance in the PAR of 200 µmol m^{-2} s^{-1}. A fully randomised experimental design was used. Each pot was weighed twice a day, in the morning and at the end of the afternoon, 5 days in a row. Irrigation was provided daily in the evening after weighing the pots. On the fifth day, leaves were sampled, dried for 48 h at 60°C and then weighed. Leaf area was measured on a sample of 10 leaves per seedling in order to compute specific leaf areas, i.e., area per unit dry weight (SLA). Total leaf area of each seedling was computed as the product of total leaf dry weight and SLA.

Experiment 2: stomatal conductance and leaf transpiration *in situ*

Stomatal conductance and transpiration were measured on leaves from the four milder infection classes (0 to 3) on twelve 1–2 year-old *Q. robur* seedlings with a LiCor 1600 null balance porometer (LiCor, Lincoln, Nebraska). About 2–8 leaves were measured on each seedling (sample sizes of 20, 6, 13 and 11, respectively, for ratings of 0, 1, 2 and 3). Whenever possible, a representative leaf of each infection class was measured on each seedling. The measurements were made outdoors during a sunny summer afternoon (13 h 30–14 h 30), and transpiration and stomatal conductance recorded on both sides of the leaves to test whether infection induced water losses from the adaxial surface (*Q. robur* is hypostomatal).

Experiment 3: photosynthesis of infected leaves

The photosynthesis of mildew-infected leaves was measured under laboratory conditions with a LiCor 6400 portable photosynthesis chamber (LiCor, Lincoln, Nebraska), on leaves infected 3–4 weeks earlier. Ten infected and eight healthy seedlings were measured. For infected seedlings, 1–2 leaves of each of the four milder infection classes (0 to 3) were measured while only one leaf was measured on healthy seedlings. Healthy and infected seedlings were measured alternatively. All together, 53 leaves were measured (13, 9, 11 and 12 leaves, respectively, for ratings of 0, 1, 2 and 3 on diseased seedlings and 8 leaves on healthy seedlings).

The 6 cm^2 leaf chamber was clamped randomly onto the leaf, not taking into account mildew infection. Leaf temperature was controlled at 24°C, relative humidity maintained between 60% and 70%, and incident photosynthetic photon flux density set at 1000 µmol m^{-2} s^{-1}. The 20 min sequence was started at a CO_2 concentration of 400 µmol mol^{-1} to fully activate photosynthesis, followed by 3 min periods at each of the following CO_2 concentrations (in the order, 300, 250, 200, 150, 100, 50, 600, 900, 1200, 1500 and 1800 µmol mol^{-1}). Three measurements at 20 s intervals were made at the end of each 3 min period. After 1800 µmol mol^{-1} CO_2, the leaf was left in the dark at 400 µmol mol^{-1} CO_2 for 25 min to measure leaf respiration.

Net CO_2 assimilation rate, A, was assumed to be related to C_i, the intercellular CO_2 concentration within the leaf, by the following minimum relationship (Farquhar et al. 1989):

$$A = (1 - 0.5/\tau.Ci) \times \min\left\{W_j, W_c\right\}$$

where:

$$W_c = \frac{C_i}{C_i + K_c(1+O/K_o)} \times Vc_{max}$$
$$W_j = \frac{C_i}{4\times(C_i+O/\tau)} \times (\alpha \times Q) \times \sqrt{\frac{1}{1+\left((\alpha^2\times Q^2)/J_{max}^2\right)}}$$

and O, τ, α, Q, K_c and K_o being either known constants or parameters with a known value at a given temperature. The fitting of the relationship was done using the procedure NLIN of SAS and enabled us to estimate Vc_{max} et J_{max}. As we used intercellular CO_2 concentration as a basis for the computation, and not the chloroplastic one, and as it is known that internal resistance to CO_2 transfer in oaks is not negligible, these estimates have to be considered as apparent values integrating a transfer component; real Vc_{max} (and to a lesser extent real J_{max}) are probably larger (Ethier and Livingston 2004).

At the end of each experiment, leaves were sampled and their total area and the fraction of leaf area infected by mildew were assessed as stated above. Leaves were then dried at 60°C for 48 h and ground (with a ball-mill for 1.5 min) after determination of their dry weight. Carbon and nitrogen concentrations were measured with an elemental analyser (Thermo Quest NA 1500 NCS, Carlo Erba, Italy) from 2–3 mg of powder for each sample.

Experiment 4. Shedding of *E. alphitoides* infected leaves under natural conditions

In order to assess whether infection by oak powdery mildew decreased leaf life-span, 100 *Q. robur* seedlings with leaves at different infection levels were sampled in a natural regeneration of the Amance Forest (Meurthe-et-Moselle, NE France). Seedlings were growing below the canopy of parent trees. Seedlings were sampled so as to obtain an even distribution of leaves with similar infection levels over the 10×10 m plot. Two leaves of the second flush (formed at the beginning of July) were sampled per seedling. Altogether, 54 healthy leaves (infection rating of 0), 45 leaves rated 1, 50 leaves rated 2, 26 leaves rated 3 and 23 leaves rated 4 were sampled. The leaves were observed every 3–4 days from mid-July to mid-September to detect leaf decay and shedding. Leaf life-span was estimated using a survival analysis with the Kaplan-Meier non-parametric method, using the leaf infection index as an independent variable. The only censure occurring in the data was for leaves that had not been shed by mid-September. The model was fitted with the SAS procedure PHREG (SAS/STAT 8.1, SAS Institute Inc., Cary, NC).

Statistical analyses

The relationship between seedling transpiration and infection level was analysed with a mixed model using SAS. First order autoregressive covariance structure was assumed for transpiration during successive days. Two different types of analyses were performed. First, we compared leaf characteristics (C and N contents, stomatal conductance, respiration in

the dark, Vc_{max} and J_{max}) of healthy leaves from either entirely healthy seedlings or partly infected ones, in order to detect possible compensation processes induced by infection. This was done with a Student t-test. Second, the same leaf traits were compared among the different leaf infection levels (ratings 0 to 3). This was done by variance analysis taking the infection index as a fixed effect and the seedlings as a random factor, using the procedure 'mixed' of SAS.

Results

The relationship beween infection index and the fraction of leaf area infected by *E. alphitoides* was, as expected, very strong. The mean fraction of infected leaf area was respectively 15.7%±2.4%, 32.7%±2.9% and 69.0%±16.7% for indices 1, 2 and 3 respectively (sample sizes respectively of 19, 22 and 22, confidence intervals given).

Impact of *E. alphitoides* on seedling water relations

Despite the large range of infection levels reached on leaves of the tested seedlings, no significant relationship could be detected between the transpiration of whole seedlings recorded during 5 days and the level of infection by oak powdery mildew (Fig. 1, F=1.99, P=0.166). A more specific analysis showed that daily transpiration was not related to infection level at any time (F=0.97, P=0.3302) and that no time * infection index interaction could be detected (F=0.42, P=0.7943).

No transpiration flux was detected with a null balance porometer during experiment 2 on the adaxial leaf surface whatever the infection level, despite the visible presence of powdery mildew on this side of the leaf. In contrast, a large transpiration flux was detected whatever the infection level on the abaxial face of the leaf. Infection had a significant impact on stomatal conductance, decreasing it by 15–30% for leaves with infection indices of 1–3 compared to healthy leaves of the same seedling (Fig. 2, F=10.99, P<0.001).

Photosynthesis of leaves infected by *E. alphitoides*

Leaf mass to area ratio (LMA) did not differ between leaves with different infection levels within a seedling (F=0.98, P=0.415). Similarly, no difference could be detected between healthy leaves of infected or healthy seedlings (t=1.54, P=0.139). LMA values were in the range 30 to 35 g m^{-2}. The composition of leaves was altered by infection (Fig. 3): carbon content increased significantly with the level of infection (F=4.23, P=0.012) while the nitrogen content decreased (F=7.01, P=0.001). There was no difference in carbon content between healthy leaves of healthy seedlings compared to healthy leaves of partly infected seedlings (t=1.42, P=0.172). In contrast, the difference was significant for nitrogen content as the healthy leaves of partly infected seedlings had a higher content than the healthy leaves of entirely healthy seedlings (Fig. 3b, t=3.00, P=0.007). During these measurements, a negative impact of *E. alphitoides* was again detected on stomatal conductance of infected leaves (Fig. 3c, F=3.75, P=.0244). Dark respiration was significantly increased (Fig. 3d, F=7.60, P<0.001). The mean decrease in stomatal conductance was 22, 16 and 11% for leaves with infection levels of respectively 1, 2 and 3.

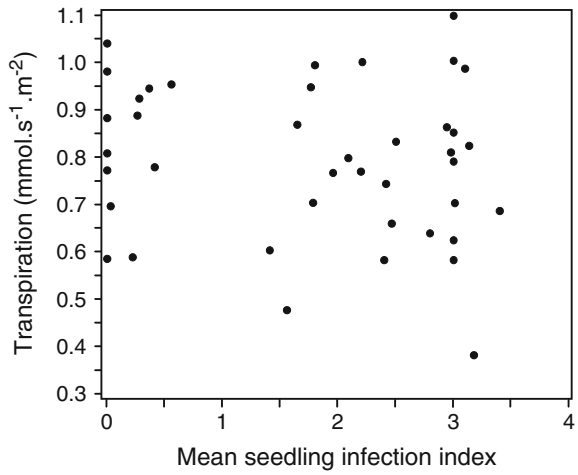

Fig. 1 Mean transpiration of whole *Q. robur* seedlings infected by oak mildew during a 5-day period in a climate chamber as a function of the mean seedling infection index (0: healthy 3: fully covered with oak mildew). Each point refers to a different seedling

Erysiphe alphitoides reduced net CO_2 assimilation of infected leaves. This was evidenced by a negative relationship between the fraction of infected leaf area and A_n, the net CO_2 assimilation rate (Fig. 4, log (A_n) = 1.96 – 0.93 × % leaf area infected, r^2=0.479, P<0.0001). While healthy leaves displayed values of 7.5±1.2 µmol m^{-2} s^{-1}, A_n was around 4.5 µmol m^{-2} s^{-1} at 50% infected leaf area, i.e., a level of net CO_2 assimilation still > 50% of the value observed in

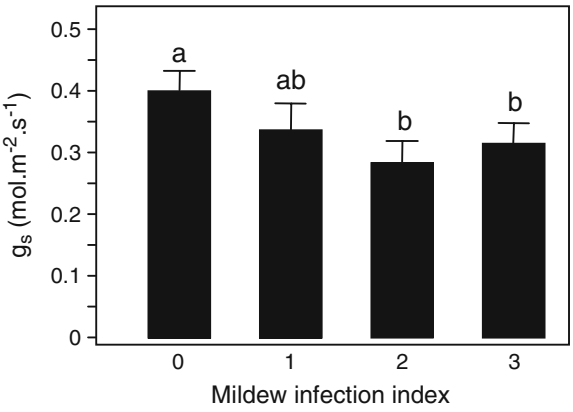

Fig. 2 Stomatal conductance (g_s) measured with a LiCor 1600 porometer on the abaxial surface of *Q. robur* leaves infected by oak mildew as a function of infection index (0: healthy 3: fully covered with oak mildew). Bar represent confidence intervals

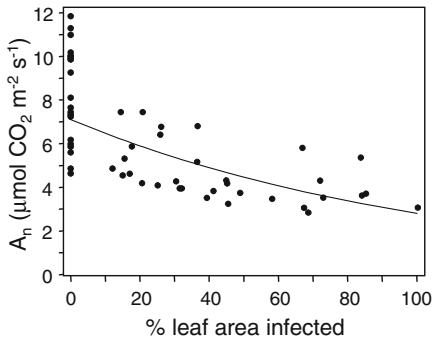

Fig. 4 Relationship between leaf infection by oak mildew and A_n, net CO_2 assimilation of leaves of *Q. robur* seedlings grown in a greenhouse. $\log(A_n) = 1.96 - 0.93 \times$ % leaf_infected, $r^2 = 0.479$, $P<0.0001$

healthy leaves. The higher infection levels (85–100%) corresponded to a decrease of A_n by about 40–50%. No difference was detected between healthy leaves of partially infected or entirely healthy seedlings for net CO_2 assimilation rates (respectively 7.6±1.4 and 9.2± 2.0 µmol m^{-2} s^{-1}, $t=1.57$, $P=0.133$).

Infection by *E. alphitoides* decreased both J_{max}, the maximum light-driven electron flux and Vc_{max}, the apparent maximum carboxylation velocity (Fig. 5). The decrease in J_{max} was significant ($F=27.93$, $P<0.001$) and was 20%, 25% and 45% for leaves with infection indices 1, 2 and 3, respectively, compared to healthy leaves on the same seedlings, while there was no difference between healthy leaves

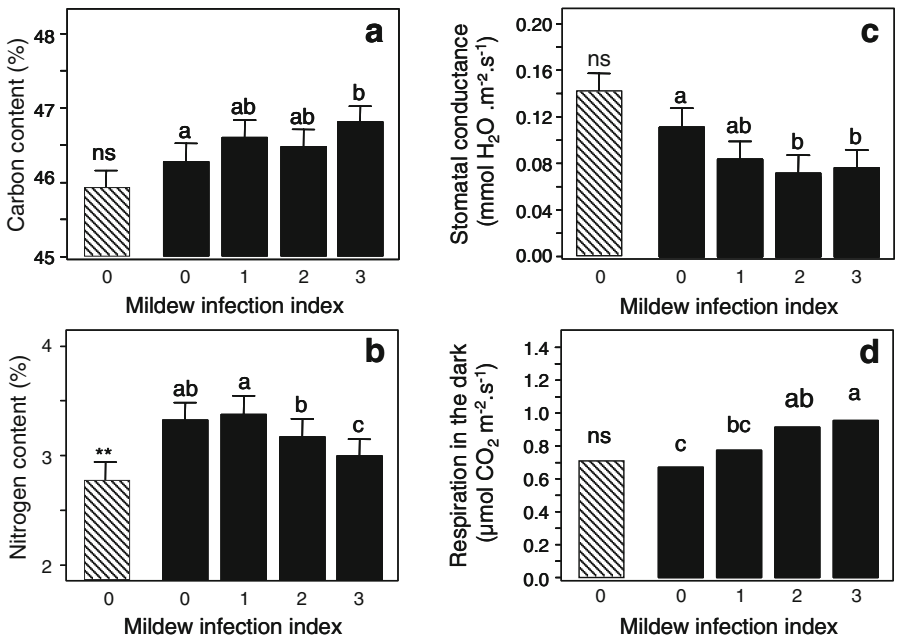

Fig. 3 Impact of oak mildew on composition and physiology of infected *Q. robur* leaves. (**a**) Carbon content; (**b**) Nitrogen content; (**c**) Stomatal conductance; (**d**) dark respiration. Stomatal conductance and dark respiration were measured with a portable photosynthesis LiCor 6400 chamber. ▨, leaves on healthy control seedlings. The sign above the bar indicates whether these leaves are significantly different from healthy leaves of partly infected seedlings. ■, leaves on seedlings partly infected by oak mildew. Infection index: 0, healthy 3: fully covered with oak mildew. Bar represent confidence intervals

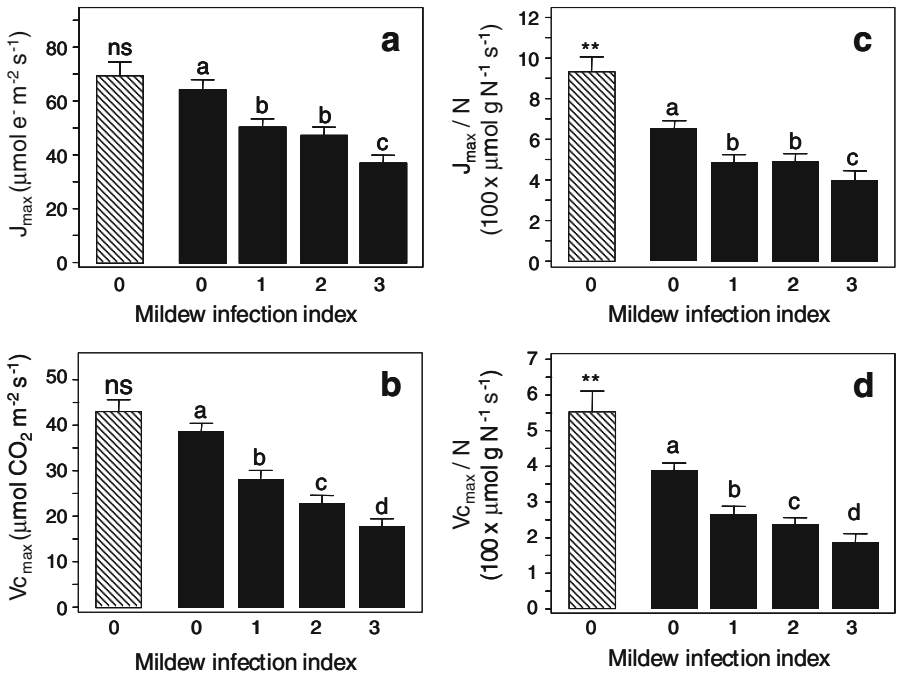

Fig. 5 Impact of oak mildew on photosynthetic capacity of infected leaves. (**a**) Maximum light driven electron flux (J_{max}); (**b**) Maximum carboxylation velocity of rubisco (V_{Cmax}); (**c**) J_{max}/N; (**d**) VC_{max}/N. ▨, leaves on healthy control seedlings. The sign above the bar indicates whether these leaves are significantly different from healthy leaves of partly infected seedlings. ■, leaves on seedlings partly infected by oak mildew. Bar represent confidence intervals

of healthy or infected seedlings (t=0.88, P=0.392). The decrease in Vc_{max} was significant (F=50.48, P<0,001) and proportional to the degree of leaf infection, with 25%, 40% and 50% lower values in leaves with infection indices of 1, 2 and 3 compared to healthy leaves of the same seedlings. As for $J_{max,}$ there were no differences between healthy leaves of the control or partly infected seedlings (t=1.18, P=0.252). To test for an impact of *E. alphitoides* on nitrogen allocation to biochemical (carboxylation by rubisco) and photochemical processes, we computed the ratios of Vc_{max} and of J_{max} to nitrogen content. The value of the two ratios was significantly different for healthy leaves of partly infected seedlings or for healthy leaves of entirely healthy seedlings (t=2.92, P=0.009 for J_{max}/N and t=2.88, P=0.001 for Vc_{max}/N, Fig. 5c and d). For infected seedlings, the two ratios decreased in parallel when infection level increased. The ratio J_{max}/Vc_{max} increased from 1.7 in healthy leaves to about 2 in the most severely infected leaves (F=15.61, P<0.001, Fig. 6) reflecting a stronger impact of infection on carboxylation capacity than on electron transport (Pearson correlation between % infected leaf area and Vc_{max}, Vc_{max}/N, J_{max} and J_{max}/N respectively of −0.781, −0.790, −0.707 and −0.770, P<0.0001).

Shedding of infected leaves under natural conditions

The climatic conditions during the experiment were cool and moist with average maximal and minimal

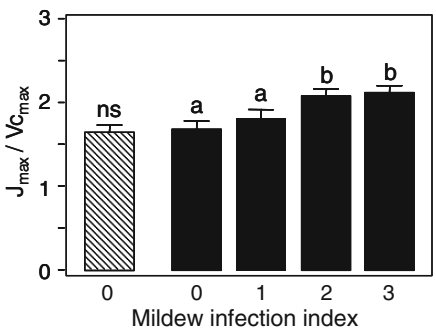

Fig. 6 Impact of oak mildew on the ratio J_{max}/V_{Cmax} in infected leaves ▨, leaves on healthy control seedlings. The sign above the bar indicates whether these leaves were significantly different from healthy leaves of partly infected seedlings. ■, leaves on seedlings partly infected by oak mildew. Bar represent confidence intervals

daily temperatures of respectively 23.3 and 13.3°C. The duration before leaf shedding greatly depended on infection level (Fig. 7). While the leaves with infection ratings of 1 or 2 and healthy leaves did not differ (P=0.89 and 0.98, respectively), leaves with infection ratings of 3 and 4 showed much shorter life-spans (P=0.004 and P<0.0001, respectively; see Fig. 7). The median time before shedding was estimated to be 20 days for leaves rated 4 (confidence interval of 10–31 days) while it was greater than the duration of the experiment for all other infection levels.

Discussion

Our results show that infection with oak powdery mildew had a moderate impact on leaf physiology, slightly reducing stomatal conductance, net CO_2 assimilation rates (A_n), and photosynthetic capacity, with a decrease in both maximal light-driven electron flux (J_{max}) and apparent maximal velocity of RuBP carboxylation (Vc_{max}). The decrease of A_n in infected leaves was not compensated for by an increase in the healthy leaves of the same seedling. However, heavy infection led to a severe decline in leaf life-span.

The seedlings infected by *E. alphitoides* displayed greater nitrogen content in the leaves than healthy controls. This could be the result of a dilution effect in the faster growing controls, as hypothesised by Bauer et al. (2000) for rust-infected spruce needles. Unfortunately, as we did not measure seedling growth, we have no data to support this hypothesis. Oak mildew-infected leaves also displayed a larger carbon content, reflecting the decrease of N, and possibly effects of the fungal biomass.

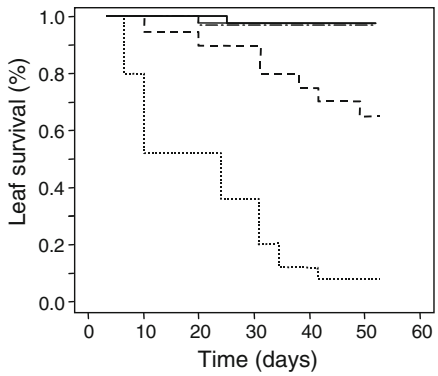

Fig. 7 Time-course of the fraction of surviving leaves of *Q. robur* seedlings naturally infected by oak mildew (July to September) in leaf cohorts with different infection indices: 0 (——), 1 (——), 2 (— - —), 3 (— · —) or 4 (·····). Start date (Time=0) was 15 July

Infection by *E. alphitoides* only moderately affected transpiration whether at the leaf or entire seedling level. The lack of any detectable transpiration on the adaxial surface of the infected leaves while a large transpiration flux was detected on their abaxial surface, where the stomata are located (Experiment 2) indicated that, unlike in observations reported by Hewitt and Ayres (1975), water loss by the epiphytic mildew mycelium on adaxial leaf surfaces was small and undetectable with our techniques. The discrepancy with our results might possibly be explained by the age of the studied leaves, as Hewitt and Ayres (1975) used very young leaves. *Erysiphe alphitoides* decreased the stomatal conductance of infected leaves by about 15–30% (Experiment 2). Altered behaviour of stomata, with incomplete opening under high light, has already been reported for mildew-infected barley leaves (Ayres and Zadoks 1979). This impact of mildew infection appears to be a very early response, occurring as soon as the epidermal cells are penetrated by the mycelium in susceptible barley cultivars (Prats et al. 2006). A decrease in stomatal conductance linked to infection has also been reported for other leaf pathogens, in particular necrotrophs (Shtienberg 1992; Roloff et al. 2004; Pinkard and Mohammed 2006). The smaller stomatal conductance of mildew-infected leaves did not result in a decrease of whole plant transpiration in severely infected saplings (Experiment 1).

Infection by *E. alphitoides* had a negative impact on net CO_2 assimilation (Experiment 3). The decrease of A_n as a function of the fraction of infected leaf area was exponential as already reported for other host/pathogen interactions (Shtienberg 1992; Roloff et al. 2004). Bastiaan (1991) proposed that the infection-induced reduction in A_n could be modelled as follows : $A_x/A_0 = (1 - X/100)^{\beta}$, with A_x/A_0, relative decrease in net CO_2 assimilation in diseased (A_x) compared to healthy leaves (A_0) and X the percent infected leaf area. This model is commonly used in plant pathology because the coefficient β provides a good measure of pathogen impact on leaf physiology (Lopes and Berger 2001; Erickson et al. 2003; Robert et al. 2004; 2006). We could not apply Bastiaan's model to our data as the most severely infected leaves, with the entire leaf area affected by the pathogen, still

displayed substantial levels of A_n, of about 50% of the value observed in healthy leaves, a result in agreement with values reported by Hewitt and Ayres (1975). This was mainly linked to the absence of necroses in infected leaves, at least under our experimental conditions. Thus, the model of Bastiaan seemed poorly adapted to oak powdery mildew and probably all powdery mildews (Mignucci and Boyer 1979; Lakso et al. 1982), although it does fit well in the case of other biotrophic pathogens such as rusts (Robert et al. 2004, 2006). Nevertheless, the impact of *E. alphitoides* on CO_2 assimilation was clearly less than what was expected from the fraction of infected leaf area. Compensation for reduced photosynthesis in infected leaves by an increase in healthy leaves did not occur in partially infected seedlings, unlike what has been reported in other host/pathogen interactions (Mayr et al. 2001).

A small fraction of the decrease in A_n was due to an increase in respiration (0.2 μmol CO_2 m^{-2} s^{-1}). Increased respiration is a common occurrence in infected leaves (Hewitt and Ayres 1975; Sabri et al. 1997; Bauer et al. 2000). It may be attributed either to an increase in leaf respiration due to infection or to the respiration of the pathogen itself. The decrease in stomatal conductance was also not likely to have caused the decrease in A_n as intercellular CO_2 did not appear to be limiting in the strongly infected leaves. By contrast, infection by *E. alphitoides* clearly impacted on the photosynthetic capacity of leaves, as they displayed reduced apparent maximal carboxylation rate by Rubisco (Vc_{max}) and maximal light-driven electron transfer rates (J_{max}). An impact of infection on leaf Vc_{max} has already been evidenced in other host/pathogen interactions (Niederleitner and Knoppik 1997; Mayr et al. 2001). The gradual increase of the J_{max}/Vc_{max} ratio with the percent affected leaf area shows that *E. alphitoides* had a stronger impact on the photosynthetic processes linked to photosynthetic carbon reduction (or to CO_2 transfer in the leaf tissues) than to those linked to photochemistry. Also, the larger nitrogen content of infected seedlings did not translate into an increased investment in photosynthesis, as Vc_{max}/N and J_{max}/N decreased in healthy leaves of infected seedlings compared to those of healthy seedlings.

Leaf life-span was significantly reduced by infection (Experiment 4) for leaves with infection levels 4 and 3. Thus, despite the fact that infection had only moderate impacts on transpiration and CO_2 assimilation of leaves with an infection level of 3, such infection by the pathogen does indeed induce some defoliation. Moreover, the studied seedlings grew under the cover of mother trees, i.e., in the shade, and the climate during the experiment was cool and wet. The impact of *E. alphitoides* on leaf survival might have been even higher under full sun during a hot summer. This impact of mildew infection on leaf life-span might thus be very important as, even under favourable conditions, only 50% of the most severely infected leaves were still alive after about 20 days. A severe impact on carbon assimilation and carbon budget may be expected from this decline in leaf life-span, which could result in an impaired survival of the seedlings.

The relatively low impact of *E. alphitoides* on photosynthesis probably explains why the disease has generally moderate consequences for tree health despite heavy infections. The most significant impact of the disease was on the life-span of severely infected leaves. Such generalised severe infections on trees occur only occasionally when massive infection occurs during leaf flushing, which is uncommon and happens in particular when oak trees refoliate after insect defoliation (Thomas et al. 2002). In such a case, the impact of oak powdery mildew is expected to be very deleterious. The relationship between leaf infection level and reduction in net photosynthesis that we identified will be very useful for assessing the impact of *E. alphitoides* on whole-tree carbon balance.

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