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RESEARCH PAPER

Quantification of the effects of *Septoria tritici* blotch on wheat leaf gas exchange with respect to lesion age, leaf number, and leaf nitrogen status

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Abstract

Quantification of the damaging effects of pathogens on diseased plants and inclusion of these damaging functions in crop simulation models is of great importance for a more complete understanding of yield response to diseases. In this study, the effect of *Septoria tritici* blotch (STB) on net photosynthetic and dark respiration rates of wheat flag leaves was quantified. Bastiaans' model: $Y=(1-x)^{\beta}$ was used to characterize the relationship between relative leaf photosynthesis (Y, considering Y_{net} and Y_{gross}) and STB severity (with x the proportion of the diseased area). The value of β indicates whether the effect of disease on photosynthesis is larger (β >1), lower (β <1), or equal (β =1) to the proportion of visible diseased area. In the experimental conditions used here, leaf nitrogen content (in a range from 0.18 to 0.24 mg cm^{-2}), and leaf number (flag and second leaves) did not significantly influence the effect of STB on leaf gas exchange. By contrast, damage depended strongly on the developmental stages of the STB lesions. STB lesions had no effect on inoculated leaves before visible symptoms appeared. Chlorotic symptoms had less effect on leaf net photosynthetic rate than could be accounted for by the visible diseased area ($\beta_{net}=0.81$). The effect of necrotic lesions on the leaf net photosynthetic capacity was slightly greater than that accounted for by visible symptoms ($\beta_{net}=1.35$). Our results suggest that the effect of the necrotic symptoms on the net photosynthesis expressed by $\beta_{net}>1$ is due to a combination of a decrease in the gross photosynthesis (β_{gross} still >1) and to an increase in the dark respiration rate ($\beta_{gross}<\beta_{net}$). Finally, it is discussed how the results could improve the prediction of crop loss caused by an STB epidemic in wheat fields.

Key words: Crop loss, damaging functions, disease assessment, *Mycosphaerella graminicola*, nitrogen fertilizer, pathogen damages, photosynthesis, *Septoria tritici* blotch.

Introduction

Small-grain cereals are quite systematically given two or three foliar fungicide treatments in northern Europe. Environmental concerns and changes in the cost/price ratio for wheat are, however, likely to increase the demand for more accurate identification of spraying needs. Decision systems based on crop loss rather than on the epidemic threshold could greatly improve crop protection management. Reliable estimates of crop loss depend on the ability to quantify the damage caused to the plant by the disease. This requires the identification of the main sources of variation in disease damage and the quantification of their respective effects. In the case of foliar pathogens, it is necessary to establish the quantitative relationship between the leaf physiological processes affected by the diseases and the leaf symptoms. In the present paper, the two relationships between photosynthesis of the diseased leaves and the symptoms of *Septoria tritici* blotch (STB, caused by *Mycosphaerella graminicola*) and between respiration and STB symptoms were quantified. This analysis was performed for different lesion age, leaf number, and leaf nitrogen status. One of the main issues of this work was to propose a robust model linking infected leaf photosynthesis and STB symptoms.

Foliar pathogens reduce photosynthetic activity in infected leaves by reducing green leaf area because of the

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lesions themselves and also, possibly, by stimulating senescence in infected leaves. Some pathogens (Waggoner and Berger, 1987; Van Oijen, 1990) do not disturb photosynthetic activity in the remaining green area of infected leaves. In this case, the host photosynthesis is proportional to the green leaf area. However, some foliar pathogens increase the photosynthetic rate of undamaged leaf sections, resulting in compensatory photosynthesis in the diseased plants (Scholes, 1992). By contrast, other foliar pathogens decrease the photosynthesis of the undamaged leaf sections (Rabbinge *et al.*, 1985). This decrease is due to an increase in respiration or to a decrease in the gross photosynthesis rate in the green host tissue (Bassanezi *et al.*, 2002). Quantifying the effects of disease on both net photosynthetic and dark respiration rates should allow the mechanisms of damage to be better identified. To quantify, at the leaf scale, the effect of disease in asymptomatic areas of diseased leaves, Bastiaans (1991) developed a simple model: the equation $P_x/P_0=(1-x)^{\beta}$ is fitted to empirical data to characterize the relationship between photosynthesis in a diseased leaf (P_x) relative to a healthy leaf (P_0) and the proportion of visible diseased symptoms in the leaf (x). The value of β indicates whether the effect of disease on photosynthesis is higher (β >1), lower (β <1), or equal (β=1) to that accounted for by the visible diseased area. Numerous studies have used this approach to model the net photosynthesis of diseased leaves (Garry *et al.*, 1998; Lopes and Berger, 2001; Erickson *et al.*, 2003; Robert *et al.*, 2005). Moreover, incorporating Bastiaans (1991) model (by using the parameter β) improved accuracy of the estimates of biomass loss at the canopy scale (Bastiaans, 1993; Beasse *et al.*, 2000; Bassanezi *et al.*, 2001; Robert *et al.*, 2004*a*).

Few studies have been carried out on leaf physiological processes affected by STB. Infection of wheat by *M. graminicola* has been reported to reduce net photosynthesis (Shtienberg, 1992; Zuckerman *et al.*, 1997) and leaf transpiration (Cornish *et al.*, 1990). Shtienberg (1992) found, in a field experiment, that the reduction in photosynthetic activity was slightly greater than could be accounted for by visible STB symptoms, suggesting a β-value (Bastiaans, 1991) greater than 1. Nevertheless, this parameter has not yet been precisely quantified. There is also no documentation on how this disease quantitatively affects leaf respiration. Glasshouse experiments were therefore performed where net photosynthetic rates and dark respiration rates were assessed on wheat infected leaves. Bastiaans' model was fit to the data to calculate the parameter β for both net and gross photosynthesis.

Moreover, given the importance of accurately quantifying parameter β, two sources of variation of the quantitative relationship between symptoms and leaf photosynthesis were considered in these experiments. The possible variability due to the fungus's developmental cycle was taken into account first (Scholes and Farrar, 1986; Scholes and Rolfe, 1996). Understanding how the effects on the host vary with the stages of pathogen development is of importance for modelling leaf damage (Rabbinge *et al.*, 1985; Scholes and Farrar, 1985), particularly when lesions with different ages colonize the same leaves (Bassanezi *et al.*, 2001). STB is a hemi-biotrophic fungus and the infection process is divided into different phases (Kema and Silfhout, 1996). The pathogen first becomes established in living host cells, with no apparent symptoms. The disease then causes chlorosis and finally necrosis. Sporulation only occurs on necrotic tissue. There is no documentation of how the effect of STB varies with the stage of disease development and therefore the effects of STB were quantified during the three phases of lesion development (latency, chlorotic, and necrotic symptoms). Second, some possible variability due to leaf physiology was taken into account. Considerable variation can indeed occur on leaf physiology in the canopy. It is already known that the pathogen's development is influenced by the physiological state of the infected leaves (Rapilly, 1991; Tiedemann, 1996; Snoejers *et al.*, 2000; Robert *et al.*, 2002, Robert *et al.*, 2004*b*). Concerning the influence of leaf physiology on damage expression, little information is available. Only leaf age has been shown to influence the level of damage (Grath and Pennypacker, 1990). That is why the influence of leaf nitrogen content and leaf number on the level of damage caused by STB was studied in this work.

Materials and methods

Overview

Two experiments were conducted in a glasshouse to quantify the effects of STB on leaf physiological processes. In experiment 1 (spring 2000), the effect of STB on leaf CO_2 exchange was quantified in plants with optimal fertilization. The net photosynthetic rate and dark respiration rate were measured at different lesion developmental stages in the flag leaf and second leaf of adult wheat plants. In experiment 2 (spring 2001), the effect of STB was quantified on leaf net photosynthetic rate in flag leaves of adult plants having three different fertilization treatments.

Plant material

Adult wheat plants of the susceptible cv. Soissons (one of the most commonly grown cultivars in France) were used in both experiments. Seeds were left to germinate for 24 h in humid cotton and then sown in Jiffy peat pots, where they were kept for 8 weeks with a 16 h light period (350 μE m^{-2} s^{-1}) at 8 °C and an 8 h dark period at 0 °C for vernalization. They were then transferred to square pots (1.1 l) filled with commercial compost (peat substrate, Gebr. Brill Substrate, Germany) and placed in the glasshouse. Daylight was complemented by sodium lamps from 08.00 h to 20.00 h. The temperature was regulated by a cooling system and plants were watered daily. They were treated against powdery mildew (Ethyrimol, 2 ml l^{-1}) three weeks before inoculation. Nitrogen fertilization can alter (i) the physical structure of the crop and thus the microclimate and (ii) the leaf nitrogen content. Both could induce changes in disease damage. In order to minimize the differences in microclimate due to the

fertilization in the experiments, plants were grown individually in separate pots and all the secondary tillers were cut before inoculation.

The day they were placed in the glasshouse, the plants were fertilized with Osmocote (10N+11P+18K). In experiment 2, three fertilizer concentrations were applied: N_0 was a low fertilizer treatment with 2 g of Osmocote per pot; treatment N_1 received a standard dose of 7 g per pot; N_2 was an over-fertilized treatment with 18 g per pot. Experiment 1 included only the N_1 fertilizer treatment. The nitrogen content of the control and inoculated flag leaves was measured the day before inoculation and at the end of the experiment. The day before inoculation, the nitrogen content assessment had to be non-destructive and an indirect method was then used. Three chlorophyll-meter measurements (SPAD-502, Minolta Camera Co., Tokyo, Japan) were made along each leaf and the average value (m) to estimate the specific leaf nitrogen content (SLN in mg N cm^{-2}) by: SLN=0.0233exp(0.0426m). This equation (R^2=0.91) was derived from data obtained on 20 flag leaves fertilized with 0–25 g of Osmocote per pot by relating their measured (Nelson and Sommers, 1980) SLN to their chlorophyll-meter measurement (m). When the diseased leaves were almost totally necrotic, (60 d after STB inoculation), the control and infected leaves were harvested and their nitrogen contents were measured directly (Nelson and Sommers, 1980). Due to an unequal number of leaves assessed for each treatment combination, the level of nitrogen was compared using a REsidual Maximum Likelihood (REML) analysis (Patterson and Thompson, 1971) which is implemented in the (GenStat^{TM} (2003) statistical package.

The outlines of the leaves were traced on transparency films the day before inoculation, and these were scanned to measure the leaf area (in cm^2) with image analysis software (Optimas, Media Cybernetics, Silver Spring, MD). For each fertilization treatment, four plants were not inoculated and were used as control plants.

Inoculation

A single pycnidiospore isolate of *M. graminicola* obtained from a naturally infected Soissons field was used for all plants. This isolate was increased on PDA medium in a growth chamber at 18 °C. Spores were collected from 3-d-old cultures in sterile distilled water. The spore suspension was adjusted with a haemocytometer to 10^7 spores ml^{-1} in experiment 1 and to 10^8 spores ml^{-1} in experiment 2. One drop of surfactant (Tween 20) was added before inoculation.

In both experiments, inoculation was done on flag leaves of plants at the heading stage because flag leaves are infected at this developmental stage in natural STB epidemics in northern Europe. Flag leaves were used because little variation was expected between flag leaves of different plants and because of the large contribution of flag leaves to grain filling and thus to yield (Paveley, 1999).

In experiment 1, the spore suspension was sprayed on 100 flag leaves using a hand-operated sprayer (Ecospray, Prolabo, France). The leaves had been previously sprayed with distilled water to facilitate the diffusion of the spore suspension on the leaf surface and the excess water formed droplets that fell on the second leaves. This inoculation method resulted in quite severe disease on the flag leaves and fewer symptoms on the second leaves. In experiment 2, the spore suspension was applied to restricted areas of 100 flag leaves with a soft paintbrush, which gave better control of the disease level. A huge range of disease severity was obtained by inoculating 10, 20, 30, 50, and 70% of the leaf surface area. The inoculated plants were placed in a walk-in growth chamber for 5 d to incubate with a 16 h light period (350 μE m^{-2} s^{-1}) at 18 °C and an 8 h dark period at 17 °C, under 100% of relative humidity. Control plants were given the same treatment with no inoculation. Plants were then placed back in the glasshouse until the end of the experiment and gas exchange measurements were carried out in the glasshouse. When chloroses were visible (between 10 d and 12 d after inoculation), 30 plants were selected in experiment 1. In experiment 2, 10 plants from treatment N_1, 14 plants from N_2, and 16 plants from N_0 were selected so as to obtain the widest possible range of disease severity.

Gas exchange measurements

Gas exchange was measured at different dates during STB development. In experiment 1, net photosynthetic rate was assessed three times in the flag leaves at 7 d (green tissue), 13 d (chlorotic symptoms), and 20 d after inoculation (dai, necrotic symptoms). The net photosynthetic rate of the second leaves was assessed at 27 and 35 dai (necrotic symptoms). The respiration rate was measured on the day after the photosynthesis assessment. In experiment 2, net photosynthesis was assessed in the flag leaves at 7 (green tissue), 19, and 28 (necrotic symptoms) dai.

At each assessment date and for each fertilization treatment, gas exchange was measured in control leaves. Variance analyses were performed to test whether net photosynthetic rate in control leaves was influenced by assessment dates and fertilization treatments. In addition two healthy leaves were 'gas-cartographied' by measuring respiration rates along the whole length of the leaf. For statistical analysis, the location on the leaf was expressed as the ratio between the distance from the base of the leaf to the total length of the leaf. Regression analyses including the factor date were performed to test whether respiration rate was influenced by location along the leaf.

Gas exchange was measured with a portable photosynthesis system (LI-6200; Li-Cor, Lincoln, USA) mounted with a red LED light source (6400-02, Li-Cor). Net photosynthetic rate was assessed at light saturation (1500 μmol photons m^{-2} s^{-1}). Leaves were adapted to darkness (0–2 μmol photons m^{-2} s^{-1}) for 20 min before measuring the dark respiration rate. The assessments were made on leaf sections of an area of 6 cm^2 (2×3 cm). When STB symptoms appeared on the leaves, the precise location of the measurements on each leaf was chosen (one or two assessments per leaf) so that a large range of disease severity was represented. The leaf sections on which the measurements were performed were tagged with a marker and all further assessments were done at the same locations.

Disease severity

At each assessment date, the diseased area on the leaf segment assessed for gas exchange was traced onto a transparency film and disease severity was calculated as the proportion of leaf area covered by STB symptoms (chlorotic or necrotic areas). Necrotic symptoms included sporulating and non-sporulating necroses.

Damage modelling

Before symptoms had appeared on the inoculated leaves, the effect of the latent disease (leaf inoculated or non-inoculated) and of the fertilization treatment (N_0, N_1, and N_2) on the leaf net photosynthetic rate (7 dai) and dark respiration rate (8 dai) were evaluated by an ANOVA.

After the symptom outbreak on the inoculated leaves, the rates of net photosynthesis and dark respiration were related to the symptoms of STB. The gross photosynthesis rate was evaluated from the net photosynthetic and dark respiration rates data. Relative net photosynthetic rate was related to the proportion of diseased area by a non-linear regression analysis (Weisberg, 1985; Seefeldt *et al.*, 1995), using Bastiaan's model (1991):

$$P_x/P_0 = (1 - x)^{\beta} \quad (1)$$

Relative dark respiration rate was related to the proportion of diseased area using a quadratic model, in which R_x is the dark

respiration rate of a diseased leaf with disease severity x and R_0 is the net dark respiration rate of a healthy leaf:

$$R_x/R_0 = ax^2 + bx + c \quad (2)$$

The gross photosynthesis rate was calculated as the difference between the net photosynthetic and the dark respiration rates. The gross photosynthetic rate was then related to the proportion of diseased area (x) according to equation 1 and the parameter β_g was estimated as for net photosynthesis. The procedure PROC NLIN of the SAS software was used for the three analyses (SAS institute, Cary, NC; release 6.12).

Models were evaluated with regard to each treatment and the nested model method was used for specific comparisons. Two models were compared: $Y=(1-x)^{\beta+\alpha i}$ (model 1) and $Y=(1-x)^{\beta}$ (model 2), for n treatments (i=[1,n]) using the lack-of-fit F-test (Weisberg, 1985). The effect of STB on gas exchange was first compared according to the type of symptoms: chlorotic or necrotic (experiment 1, flag leaf, day 13 and day 20, n=2). Then, the effect of necrotic symptoms on gas exchange was compared according to the leaf number (experiment 1, flag leaf and second leaf, n=2), fertilization treatment (experiment 2, three fertilization treatments N_0, N_1, and N_2, n=3), and lesion age (experiment 1, dates 20, 28, and 35; experiment 2, dates 19 and 27).

Results

Leaf nitrogen content

The day before inoculation, the average specific leaf nitrogen (*SLN*) of the future inoculated leaves in experiment 1 was 0.21 mg N cm^{-2} (σ=0.03) (Fig. 1). In experiment 2, it was 0.18 mg cm^{-2} (σ=0.02) in treatment N_0, 0.21 mg N cm^{-2} (σ=0.02) in treatment N_1, and 0.24 mg N cm^{-2} (σ=0.02) in treatment N_2. At this date, the average *SLN* was not significantly different for control and inoculated leaves in experiment 1 (P=0.37) nor in experiment 2 (P=0.24). The *SLN* in treatment N_1 of experiments 1 and 2 were not significantly different (P=0.60). In experiment 2, there was a significant effect of the nitrogen treatment (P <0.001). *SLN* was significantly different (P <0.05, t-test) in treatments N_0, N_1, and N_2 with N_2>N_1>N_0.

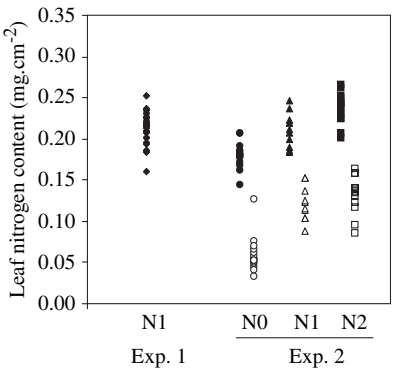

Fig. 1. Specific nitrogen content of the inoculated leaves (in mg N cm^{-2} of leaf). Plants are grown under three fertilization treatments: N_0 is a low fertilization, N_1 is a standard fertilization, and N_2 is a high fertilization. Data are from experiment 1: treatment N_1 (diamonds) and experiment 2: treatment N_0 (circle), treatment N_1 (triangles), and treatment N_2 (squares). Solid symbols are for the day before inoculation (estimation from the chlorophyll-meter measurements according to the equation: N=0.0233×exp (0.0426×m) shown in the Materials and methods) and open symbols are at 60 d after inoculation (direct measure). The x-axis represents the different treatments.

60 d after inoculation, the average *SLN* of the inoculated leaves was 0.06 mg N cm^{-2} (σ=0.02) in treatment N_0, 0.12 mg N cm^{-2} (σ= 0.02) in treatment N_1, and 0.13 mg N cm^{-2} (σ=0.02) in treatment N_2 (Fig. 1). The average *SLN* was significantly higher in the control leaves than in the inoculated leaves (P <0.001) and there was also a significant effect of the fertilization treatment (P <0.001), but no interaction between inoculation and fertilization treatment was found (P=0.27). *SLN* was significantly lower (P <0.05, t-test) in treatment N_0, relative to N_1 and N_2 but there was no longer any significant difference between N_1 and N_2.

STB lesion development

The disease developed in the same way in all leaves: the diseased tissue was green during the latent period; then light chlorotic symptoms appeared and rapidly turned yellow. The first chlorotic symptoms in the inoculated flag leaves were observed at 12 (220 degree-days, basis 0 °C) and 10 (190 degree-days) dai in experiments 1 and 2, respectively. In second leaves (experiment 2) first symptoms appeared 15 dai (230 degree-days). Lesions were then limited to chlorotic surfaces without necrosis. Necrosis started to develop 2 d after emergence of the chlorotic symptoms within the lesions, with the presence of numerous pycnidia. Chlorotic tissues were gradually replaced by necrosis (sporulating and non-sporulating areas).

Effect of STB on net photosynthesis

The net photosynthetic rate in control leaves varied from 17.2 to 29.7 μmol CO_2 m^{-2} s^{-1}. It was independent of fertilization treatment (P=0.24) and no interaction between assessment date and fertilization treatment was found (P=0.11), but there was a significant effect of the date (P=0.007).

Before any symptoms appeared in the inoculated leaves (7 dai), the net photosynthetic rate (Fig. 2) ranged from 18.2 to 29.1 μmol CO_2 m^{-2} s^{-1} in diseased leaves and from 19.3 to 29.7 μmol CO_2 m^{-2} s^{-1} in the control leaves. Net photosynthetic rate was statistically independent of the presence of latent STB (P=0.175) and there was no significant interaction between the fertilization treatment and the presence of latent STB (P=0.997). It was concluded that latent STB had no significant effect on net photosynthetic rate.

Chlorotic and necrotic symptoms induced a decrease in leaf photosynthesis (Figs 2, 3). Equation 1 was fitted to the data for each combination of assessment dates (after symptoms had appeared in the inoculated leaves), fertilization treatment (N_1, N_2, and N_0), and leaf number (flag or second leaf). Coefficients of determination varied from 0.77 to

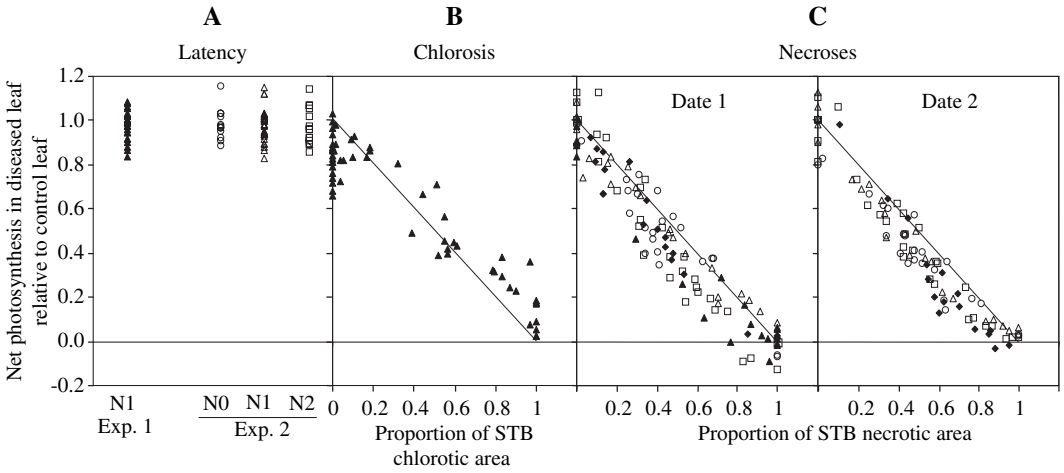

Fig. 2. Net photosynthetic rate in diseased leaf (P_x) relative to control leaf (P_0) during the development of STB. (A) 7 dai (days after inoculation), green latent tissue, measurements on flag leaves. Data are from experiment 1 with standard fertilization level (diamonds) and from experiment 2 on plants with low fertilization treatment N_0 (circles), standard fertilization treatment N_1 (triangles), and high fertilization treatment N_2 (squares). No symptoms were visible and the *x*-axis represents the different treatments. (B) 13 dai, chlorotic symptoms. Data are from experiment 1: measurements on flag leaves, standard fertilization level. Line indicates $y=1-x$. (C) Necrotic symptoms and two assessment dates: date 1 is 20 dai and 19 dai for experiments 1 and 2, respectively; date 2 is 27 dai and 28 dai for experiments 1 (solid symbols) and 2 (open symbols), respectively. STB necrotic symptoms were assessed as the total necrotic area (including sporulating and non-sporulating necrosis). Data are from experiment 1: flag leaves (triangles) and second leaves (diamonds) and from experiment 2 for the three fertilization treatments: low fertilization treatment N_0 (circles), standard fertilization treatment N_1 (triangles), and high fertilization treatment N_2 (squares).

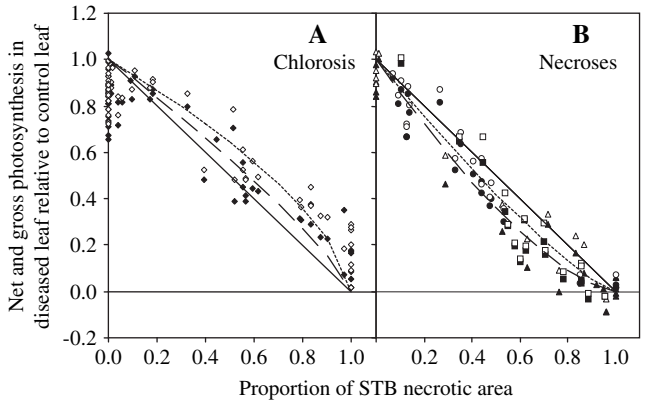

Fig. 3. Relative net (solid symbols) and gross (open symbols) photosynthesis in diseased leaf (P_x) relative to control leaf (P_0) as a function of the proportion of leaf area covered by STB symptoms. Relative gross photosynthesis has been calculated using net photosynthesis and dark respiration data (see Materials and methods). Line indicates $y=1-x$, small dashed line indicates $y=(1-x)^{\beta gross}$ and big dashed line indicate $y=(1-x)^{\beta net}$. Data are from experiment 1. (A) 13 dai, chlorotic symptoms, flag leaves. $\beta_{net}=0.81$ and $\beta_{gross}=0.64$. (B) Necrotic symptoms, 20 dai (flag leaf, triangle), 27 dai (second leaf, circles), and 35 dai (second leaf, squares). $\beta_{net}=1.47$ and $\beta_{gross}=1.25$. STB necrotic symptoms were assessed as the total necrotic area (including sporulating and non-sporulating necrosis).

0.97 for the different treatments. For chlorotic symptoms (13 dai), parameter β for net photosynthesis (equation 1) was estimated to be 0.81 and it was significantly lower than 1 (Table 1). Nested models showed a significant difference (experiment 1, $F=13.6$, $\upsilon 1=77$ and $\upsilon 2=1$) in the β-values on day 13 (chlorotic symptoms) and on day 20 (necrotic symptoms). For necrotic symptoms, the estimated values of β (equation 1) were 1.12 to 1.77 (Fig. 3), and they were significantly higher than 1 (Table 1). The β-values for flag and second leaves did not differ significantly (experiment 1, $F=2.37$, $\nu 1=56$ and $\nu 2=1$) and there was also no significant differences in the β-values for the three fertilization treatments (data from experiment 2, $F=2.08$, $\nu 1=149$ and $\upsilon 2=2$). Lastly, the measurement date had no significant effect on β-value in either experiment 1 or experiment 2 (dates 20, 27, and 35 in experiment 1: $F=1.18$, $\upsilon 1=56$ and $\upsilon 2=1$; dates 19 and 28 in experiment 2: $F=0.18$, $\upsilon 1=149$ and $\upsilon 2=1$). The values of parameter β for necrotic lesions was $\beta=1.47\pm0.07$, when estimated from the pooled data of experiment 1, and $\beta=1.32\pm0.04$ when estimated from pooled data of experiment 2. These values were significantly higher than 1 and were not affected by leaf number, nitrogen status or necrotic lesion age. Pooled data from both experiments gave $\beta=1.35\pm0.03$ ($R^2=0.93$, $n=207$).

These results suggest that equation 1 could be used to estimate relative net photosynthetic rate in diseased leaves using $\beta=1$ during the latency period (assuming $x=0$), $\beta=0.81$ for chlorotic symptoms, and $\beta=1.35$ for necrotic symptoms due to STB.

Effect of STB on dark respiration

Data from experiment 1 were used to estimate the effect of STB on dark respiration rate. The dark respiration rate of healthy leaves ranged from -0.72 to -1.93 μmol CO_2 m^{-2} s^{-1}. It was non-linearly related to the location on the leaf (Fig. 4, $P=0.048$) as well as to the assessment

Table 1. *Estimates of parameter β (equation 1) for net (β_n) and gross photosynthesis (β_g) according to STB lesion age (in days after inoculation), fertilization treatment (N_1, N_2, and N_0) and leaf number (F1, F2)*

Relative gross photosynthesis has been calculated using net photosynthesis and dark respiration data (see Materials and methods). Confidence intervals at 95% (CI) are shown for the β-values. Standard deviations (SD) are shown for control leaf photosynthesis. Number of measurements (*n*) is shown for each treatment.

Experiment	N[a]	Leaf[b]	dai[c]	Net photosynthesis					Gross photosynthesis				
				β_n	CI	*n*	P_0	R^2	β_g	CI	*n*	P_0	R^2
1	N_1	F1	13	0.81	0.17	56	25.4 (2.1)	0.77	0.64	0.14	53	27.3 (2.1)	0.76
			20	1.77	0.48	20	22.3 (2.1)	0.95	1.19	0.28	13	23.4 (2.1)	0.95
	N_1	F2	27	1.41	0.23	21	21.3 (2.9)	0.93	1.19	0.22	19	22.0 (2.9)	0.90
			35	1.44	0.21	16	23.1 (2.5)	0.91	1.29	0.23	16	23.9 (2.5)	0.87
2	N_0	F1	19	1.20	0.19	21	22.4 (1.0)	0.81	–	–	–	–	–
			28	1.36	0.15	23	26.7 (0.7)	0.86	–	–	–	–	–
	N_1	F1	19	1.12	0.15	23	26.4 (0.7)	0.94	–	–	–	–	–
			28	1.35	0.14	27	23.7 (1.3)	0.97	–	–	–	–	–
	N_2	F1	19	1.51	0.23	27	21.3 (1.6)	0.92	–	–	–	–	–
			28	1.30	0.14	27	19.9 (2.0)	0.95	–	–	–	–	–

[a] Fertilization treatments: N_0=low treatment, N_1=standard treatment, N_2=high treatment.
[b] Leaf assessed: F1=flag leaf, F2=second leaf.
[c] Days after inoculation.

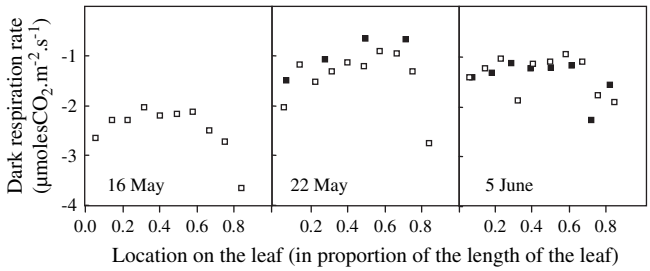

Fig. 4. Healthy flag leaf dark respiration rates (µmol CO_2 m^{-2} s^{-1}) measured at different locations on the leaf (expressed as a proportion of the length of the leaf from the leaf base). Data are from two healthy leaves, solid symbols refer to leaf 1 and open symbols to leaf 2. Respiration measurements were taken on three dates.

date (P <0.001), but with no interaction between location and date (P=0.25).

The dark respiration rate of diseased leaves during STB latency, before any symptoms were visible on the inoculated leaves (Fig. 5A), ranged from −0.81 to −2.48 (mean= −1.67) µmol CO_2 m^{-2} s^{-1}. Although this was slightly higher than the average dark respiration rate of control leaves (−1.41 µmol CO_2 m^{-2} s^{-1}), the difference was not significant (P=0.19). The parameters of the polynomial model (equation 2) for day 14 (chlorotic symptoms) and day 21 (necrosis) were significantly different (F=16.5, υ1=68 and υ2=2). When only chlorotic symptoms where visible (Fig. 5B, day 14), the polynomial model (equation 2) did not fit the data at all (R^2=0.09). A simpler model was proposed instead and the R_x/R_0 ratio was considered to be constant: R_x/R_0=1.42. For the necrotic symptoms (Fig. 5C), the model fitted the data with R^2=0.12 and equation 2: R_x/R_0= $-1.88\ x^2+1.06x$ +1.84. When considering the R_x/R_0 ratio to be constant, R_x/R_0=1.74 was found for necrotic symptoms.

Effect of STB on gross photosynthesis

The gross rate of photosynthesis was estimated from the dark respiration rate and the net photosynthetic rate, with the data of experiment 1 (Fig. 3). Bastiaans' model (equation 1) fitted the data from all treatments (leaf number and assessment date) (P <0.0001) with R^2 values of 0.76 to 0.95 (Table 1).

The parameter β_g was 0.64±0.07 (R^2=0.76) for chlorotic symptoms and was significantly lower than 1. The estimated values of β_g for necrotic lesions were from 1.16 to 1.29. The pooled data for necrotic symptoms (20, 27, and 35 dai) from experiment 1, gave β=1.25±0.06, which was significantly greater than 1.

Discussion

In this study there was no influence of leaf physiology on STB damage: it was found that leaf nitrogen status (in a range from 0.18–0.24 mg cm^{-2}) and leaf number (flag or second leaf) did not influence the effect of STB on net leaf photosynthesis. By contrast, the effect of the disease did depend on the lesion developmental stage: no effect of disease was found before symptoms appeared on the inoculated leaves, while for chlorotic and necrotic symptoms β-values of 0.8 and 1.35 (equation 1) were found, respectively.

Gas exchanges in control leaves

The quantification of the relative effect of STB on net photosynthetic (P_X/P_0 in equation 1) and dark respiration rates (R_X/R_0 in equation 2) of the diseased leaves requires the precise estimation of gas exchange in healthy leaves (P_0 and R_0). This is why for each combination of assessment

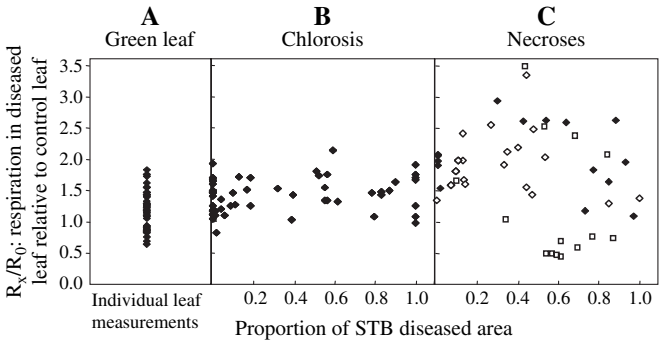

Fig. 5. Dark respiration in diseased leaf (R_x) relative to control leaf (R_0) as a function of the proportion of leaf area covered by STB symptoms during the development of disease (in dai). Data are from experiment 2: (A) day 8, green latent tissue; (B) day 14, chlorotic symptoms; (C) necrosis, 20 dai (flag leaf, solid diamond), 27 dai (second leaf, open diamond), and 35 dai (second leaf, squares). STB necrotic symptoms were assessed as the total necrotic area (including sporulating and non-sporulating necrosis).

date, fertilization treatment, and leaf number (flag or second leaf) P_0 and R_0 were estimated. However, the location of the measurement on the leaf was ignored when estimating the net photosynthesis (P_0) and dark respiration (R_0) rate on control leaves. Elsewhere we have suggested that the location of the measurements can be ignored when estimating the net photosynthesis rate on control leaves in these experimental conditions (Robert *et al.*, 2005).

It was found that the dark-respiration rate varied non-linearly along the leaf. Due to this variation, ignoring the location of the measurement when estimating the dark respiration on control leaves (R_0) may have reduced the accuracy in quantifying the effect of the disease on respiration. Moreover the magnitude of the respiration flux is small and hence more difficult to assess precisely. These two factors may partly explain the lack of a clear pattern in Fig. 5.

Leaf nitrogen content and STB damage

Nitrogen fertilization and diseases may interact in several ways. First, nitrogen fertilization can alter the physical structure of the crop and so change the microclimate and the distance between the plant organs which may affect disease development (Leitch and Jenkins, 1995; Savary *et al.*, 1995; Neumann *et al.*, 2004) or disease damage (Erickson *et al.*, 2003). In this experiment, this source of variation was eliminated by growing plants individually in separate pots, cutting the tillers, and using the flag leaves. Second, the leaf nitrogen content can interact with pathogen development (Robert *et al.*, 2002, 2004*b*), and also with leaf damage caused by the disease (Snoeijers *et al.*, 2000). In the present work, no significant difference was found between the β-values obtained for the three fertilization treatments. This suggests that the effect of STB on the photosynthetic competence of leaves is not influenced by variation of the leaf nitrogen content in the range of 0.18–0.24 mg cm^{-2}. In a previous study the same conclusion was reached for the effect of leaf rust (*Puccinia triticina*) on wheat leaf photosynthetic rate (Robert *et al.*, 2005).

It is well known that the rate of leaf photosynthesis correlates with the leaf N content. For maize, rice, and soybean, Sinclair and Horie (1989) used a logistic equation to describe the strong correlation between leaf photosynthetic rate and leaf N content at light saturation. They observed that photosynthesis first increases with increased leaf N content and then reaches a plateau where leaf photosynthesis is independent of N content. Also for wheat plants there is a considerable correlation between leaf photosynthetic rate and N content (Hunt, 1984) and the photosynthetic rate was shown to be positively correlated with the N content per unit of surface area. In our experiment, however, photosynthesis in control leaves was not significantly different in the plants of the three fertilization treatments. This suggests that, in the range of leaf nitrogen content which was obtained in this experiment (0.18, 0.21, and 0.24 mg cm^{-2} at heading), leaf nitrogen was not limiting leaf photosynthesis; analogous to the plateau phase found by Sinclair and Horie (1989).

The quite small variation obtained in the leaf nitrogen content despite the large differences in the amount of nitrogen applied to the plants in the three treatments was because the plants given little fertilizer produced fewer tillers and their leaf surface area was much smaller. Gregory *et al*. (1981) also observed that wheat leaf photosynthesis was little affected by difference in leaf nitrogen induced by nutrient regime. In the field, under an early nitrogen deficiency, plants react first to nitrogen starvation by changing their structure and then by decreasing the leaf nitrogen content. The leaf nitrogen contents of the glasshouse-grown plants were quite close to those obtained in plants grown under standard field conditions in northern Europe (Girard, 1997; Neumann *et al.*, 2004).

STB developmental stages and damages

As long as no symptoms were apparent, STB had no significant effect on net photosynthetic rate of inoculated leaves. Similar results have been obtained for bean anthracnose (*Colletotrichum lindemuthianum*) that also develops a biotrophic phase followed by a necrotrophic phase, associated with a reduction in leaf photosynthesis activity (Bassanezi *et al.*, 2001). Cornish *et al.* (1990) found that infection by *M. graminicola* had no effect on plant water use before symptoms became visible. They suggested that leaf physiology is only slightly disturbed during this early period of the pathogen cycle. The mycelium growth during the latent period is slow and increases only when pycnidia start to develop (Jorgensen and Smedegaard-Peterson, 1999). It was found that respiration was slightly enhanced by STB before symptoms became visible, but this increase was not statistically significant. The diseased parts of the

leaves were not visible at that time, however, which could have resulted in an underestimation of the expected effect.

With the expression of symptoms, the effect of disease on leaf photosynthesis and respiration became significant and increased with the transition from chlorotic to necrotic symptoms. Chlorotic symptoms had a significant influence on net photosynthetic and dark respiration rates, but had a lower effect on net photosynthetic rate than could be accounted for by the diseased area ($\beta < 1$ in equation 1), suggesting that photosynthesis persisted in chlorosis. This would be in agreement with previous studies that have shown that the chlorotic regions surrounding sporulating areas are still photosynthetically active for rust diseases (Scholes and Rolfe, 1996).

Necrotic lesions reduced net photosynthesis according to $\beta=1.35$ which suggests that the effect of necrotic lesions on the leaf net photosynthetic capacity was slightly greater than that accounted for by visible symptoms. This result is consistent with the findings of a previous field study (Shtienberg, 1992) which showed that the impact of the disease on the photosynthetic activity of the crop was slightly greater than expected from the visible diseased area. It was found that parameter β remained constant for all the necrotrophic period, as for other pathogens (Bassanezi *et al.*, 2001). It is therefore reasonable to assume that β remains constant when necrotic lesions of different ages coexist on the same leaves. This simplification is not valid for all pathogens: for example, the β-value for wheat leaf rust lesions changes during the sporulation period (Scholes and Rolfe, 1996; Robert *et al.*, 2005).

Necrotic symptoms increased leaf respiration up to 3-fold in diseased leaves. Enhanced respiration could result from stimulation of the host defence mechanisms or from fungus respiration (Farrar and Lewis, 1987). An attempt was made to use a polynomial model to describe the increase in leaf respiration caused by STB (equation 2). Bell-shaped curves have been suggested for modelling diseased leaf respiration in other pathosystems, including necrotrophic fungi (Smedegaard-Petersen, 1984), because the relative respiration rate R_x/R_0 is close to 1 for values of small disease severity, increases during fungal development, and finally decreases when the leaf is totally necrotic. In this experiment, however, a clear pattern could not be found between the proportion of necrotic STB symptoms and the increase in respiration (Fig. 5C). It has already been mentioned that ignoring the location of the measurement when estimating dark respiration on control leaves (R_0 in equation 2) and the small magnitude of the respiration flux may have partly explained the lack of a clear pattern in Fig. 5C. Moreover, in this experiment, STB necrotic symptoms were assessed as the total necrotic area (including sporulating and non-sporulating necrosis) and so the relationship between relative dark respiration rate of diseased leaves (R_x/R_0) and proportion of total necrotic area was drawn (Fig. 5C). It can then be hypothesize that it is necessary to assess symptoms only as the sporulating necrotic area in order to obtain a robust relationship between STB severity and relative dark respiration rate of diseased leaves (R_x/R_0).

This suggests that in order to obtain a robust quantitative relationship between the dark respiration rate and STB symptoms it would be necessary to perform a new experiment with an adapted protocol. However, because the absolute carbon flows of photosynthesis and respiration differ by at least one order of magnitude (25 μmol CO_2 m^{-2} s^{-1} compared with -1.5 μmol CO_2 m^{-2} s^{-1}), it could be hypothesized that, for estimating crop loss caused by STB epidemics, the impact of the disease on leaf respiration is negligible compared with the decrease in net photosynthesis. Before performing a new experiment, the relative importance of changes in photosynthesis and respiration in the crop loss could be assessed theoretically by sensitivity analysis of a crop loss model.

Assessments of net photosynthesis and dark respiration rates allowed the calculation of gross photosynthesis of the leaves and then the estimation of a β-value for gross photosynthesis using equation 1. The estimated β-values for necrotic symptoms were 1.47 and 1.25 for net and gross photosynthesis, respectively (data from experiment 1). The difference between these two values corresponds to the effect of the lesions on dark respiration of the diseased leaf, while the deviation of the latter from one corresponds to the effect of the lesions on gross photosynthesis. This result suggests that the effect of the necrotic symptoms on the net photosynthesis expressed by $\beta_{net}>1$ is due to the combination of a decrease in the gross photosynthesis ($\beta_{gross}>1$) and to an increase in the dark respiration rate ($\beta_{snet}>\beta_{gross}$).

Disease classification based on the β-value

Shtienberg (1992) examined the effects of ten foliar diseases on the host rate of photosynthesis and concluded that the reduction in photosynthesis is not related to the systemic group of either the host or the pathogen, but probably to the kind of trophic relationship they develop. Bassanezi *et al.* (2001) suggested that pathogens might be classified according to their impact on the host functions, as indicated by a β-value (Bastiaans, 1991). They divided the plant pathogens into three groups, depending on their β-values. The first group, the rusts, have β-values around 1–2, which suggests that the effect of the disease is limited to the area surrounding the lesions. Group 2 includes some necrotrophic and hemibiotrophic pathogens that have β-values around 3–4. These pathogens produce toxic compounds that diffuse locally out from the lesions and disintegrate the plant cells under the lesions, preventing local water flow. The last group includes pathogens that have very high β-values, like bean or pea anthracnose (leaf blight) ($\beta > 8$), which affect the vascular system of the host and impair the water flow. However, in this study β remained close to 1 for STB, which is a very low value

compared with those measured for other necrotrophic pathogens.

STB assessment and damage modelling

Madden and Nutter (1995) have already stated that the difficulties in accurately measuring disease severity were mainly responsible for the absence of robust relationships between symptoms and damages. For wheat leaf rust (*Puccinia triticina*), it was found (Robert *et al.*, 2005) that, depending on the type of symptoms taken into account and the lesion age, the calculated effect of the disease on the host's photosynthetic competence can vary from β=0.7–10.

STB lesions increase tissue senescence around the sporulating necrotic lesions and Magboul *et al*. (1992) separated the STB sporulating area from the non-sporulating necrotic areas in an attempt to account for the fungus biology. By contrast, in the present study, STB symptoms were assessed as the total necrotic area (including sporulating and non-sporulating necrosis) and it was found that this type of assessment was compatible with accurate estimation of the effects of STB on leaf net photosynthesis. In addition, most pathogens induce an acceleration of the leaf's natural senescence, which could be taken into account in this study's approach by considering the necrotic tissue as a whole. Indeed, in a previous field experiment (Robert *et al.*, 2004*a*) the severity of STB was recorded as the total necrotic area on the leaves and this allowed crop growth losses due to the disease to be accurately estimated. This suggests that, in order to estimate the STB effect on leaf photosynthesis, it is not necessary to distinguish the sporulating and non-sporulating necrotic areas nor to take into account pycnidial density. This result is certainly linked to the weak effect of STB on the symptomless part of the leaf (β=1.35) as it can be assumed that simple necrosis gives a β-value of 1.0. Separating the STB sporulating area from the non-sporulating necrotic areas could be necessary for describing fungal development and maybe for estimating disease-increased respiration, but it does not seem to be required for estimating damage in net leaf photosynthesis.

This idea is consistent with previous results obtained with biotrophic fungi. For leaf rust (Robert *et al.*, 2005) and bean rust (Bassanezi *et al.*, 2001), it has been demonstrated that for estimating net photosynthesis loss caused by the disease, the key variable is the total visible diseased area, but that the sporulating area is the key variable for an estimation of spore production (Robert *et al.*, 2002, 2004*b*).

In conclusion, this study, by quantifying the relationships between STB symptoms and net leaf photosynthesis, contributes to modelling wheat crop loss due to STB. It was shown, in this study's experimental range, that the effect of STB on leaf photosynthesis was not influenced by the leaf nitrogen status nor by leaf number. The effect of STB depended only on the lesion developmental stage: no effect of disease was found before symptoms appeared, while for chlorotic and necrotic symptoms β-values of 0.8 and 1.35 were found, respectively. This result suggests first that neglecting chlorotic symptoms for crop damage modelling could lead to an underestimation of losses, particularly in tolerant genotypes that were shown to extend the chlorotic phase of leaf blotch (Zuckermann *et al.*, 1997). Second, it is clear that STB has a weak damaging effect during the early part of its cycle. This could be useful for orienting work in wheat cultivar selection as an increased latent period and chlorotic phase may be a good selection criterion to increase tolerance to STB. Third, this study shows that STB necroses only have a limited effect in the symptomless part of the infected leaves (β=1.35) suggesting that the reduction in green leaf area is the main damage due to the disease. In turn, this means that induced-apical senescence due to STB could be a significant damage caused by the disease and these results therefore suggest that study of STB-induced apical senescence is necessary.

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References

Bassanezi RB, Amorim L, Filho AB, Berger RD. 2002. Gas exchange and emission of chlorophyll fluorescence during the monocycle of rust, angular leaf spot and anthracnose on bean leaves as a function of their trophic characteristics. *Journal of Phytopathology* **150,** 37–47.

Bassanezi RB, Amorim L, Filho AB, Hau B, Berger RD. 2001. Accounting for photosynthetic efficiency of bean leaves with rust, angular leaf spot, and anthracnose to assess crop damage. *Plant Pathology* **50,** 443–452.

Bastiaans L. 1991. Ratio between virtual and visual lesion size as a measure to describe reduction in leaf photosynthesis of rice due to leaf blast. *Phytopathology* **81,** 611–615.

Bastiaans L. 1993. Effects of leaf blast on growth and production of a rice crop. 2. Analysis of the reduction in dry matter production, using two models with different complexity. *Netherlands Journal of Plant Pathology* **99,** 19–28.

Bastiaans L, Roumen EC. 1993. Effect on leaf photosynthesis rate by leaf blast for rice cultivars with different types and level of resistance. *Euphytica* **66,** 81–87.

Beasse C, Ney B, Tivoli B. 2000. A simple model of pea (*Pisum sativum*) growth affected by *Mycosphaerella pinodes*. *Plant Pathology* **49,** 187–200.

Cornish PS, Baker GR, Murray GM. 1990. Physiological responses of wheat (*Triticum aestivum*) to infection with *Mycosphaerella graminicola* causing *Septoria tritici* blotch. *Australian Journal of Agricultural Research* **41,** 317–327.

Erickson JE, Stanosz GR, Kruger EL. 2003. Photosynthetic consequences of *Marssonina* leaf spot differ between two poplar hybrids. *New Phytologist* **161,** 577–583.

Farrar JF, Lewis DH. 1987. Nutrient relations in biotrophic infections. In: Pegg GF, Ayres PG, eds. *Fungal infection of plants*. Cambridge University Press, 92–132.

Garry G, Jeuffroy MH, Ney B, Tivoli B. 1998. Effects of *Ascochyta* blight on the decrease in photosynthetizing leaf area and the reduction of photosynthetic efficiency by green leaf area of dried-pea. *Plant Pathology* **47,** 473–479.

GenStat^{TM}. 2003. *Genstat*, 7th edn, *GenStat procedure library release PL15*. Rothamsted, Harpenden, UK: Lawes Agricultural Trust.

Girard ML. 1997. *Modélization de l'accumulation de biomasse et d'azote dans les grains de blé tendre d'hiver; simulation de leur teneur en protéînes à la récolte* (Triticum aestivum). INA-PG, INRA.

Grath MT, Pennypacker SP. 1990. Alteration of physiological processes in wheat flag leaves caused by stem rust and leaf rust. *Phytopathology* **80,** 677–685.

Gregory PJ, Marshall B, Biscoe PV. 1981. Nutrient relation of winter wheat. *Journal of Agricultural Science* **96,** 539.

Hunt LA. 1984. Relationships between photosynthesis, transpiration, and nitrogen in the flag leaf and penultimate leaves of wheat. In: Day W, Atkin RK, eds. *Wheat growth and modelling*. New York: Plenum Press, 149–156.

Jorgensen HJL, Smedegaard-Peterson V. 1999. Host–pathogen interactions in the *Septoria*-disease complex. In: Lucas JA, Bowyer P, Anderson HM, eds. *Septoria on cereals: a study of pathosystems*. Wallingford, UK: CABI Publishers, 131–160.

Kema GHJ, Silfhout CH. 1996. Histology of the pathogenesis of *Mycosphaerella graminicola* in wheat. *Phytopathology* **86,** 777–786.

Leitch MH, Jenkins PD. 1995. Influence of nitrogen on the development of *Septoria* epidemics in winter wheat. *Journal of Agricultural Science* **124,** 361–368.

Lopes DB, Berger RD. 2001. The effects of rust and anthracnose on the photosynthetic competence of diseased bean leaves. *Phytopathology* **91,** 212–220.

Madden LV, Nutter FW. 1995. Modeling crop losses at the field scale. *Canadian Journal of Plant Pathology* **17,** 124–137.

Magboul AM, Geng S, Gilchrist DG, Jackson LF. 1992. Environmental influence on the infection of wheat by *Mycosphaerella graminicola*. *Phytopathology* **82,** 1407–1413.

Nelson DW, Sommers LE. 1980. Total nitrogen analysis for soil and plant tissues. *Journal of the Association of Analytical Chemists* **63,** 770–778.

Neumann S, Paveley ND, Beed FD, Sylvester-Bradley R. 2004. Nitrogen per unit leaf area affects the upper asymptote of *Puccinia striiformis* f. sp. *tritici* epidemics. *Plant Pathology* **53,** 725–732.

Patterson HD, Thompson R. 1971. Recovery of inter-block information when block-sizes are unequal. *Biometrika* **58,** 545–554.

Paveley ND. 1999. Integrating septoria risk variables. In: Lucas JA, Bowyer P, Anderson HM, eds. *Septoria on cereals: a study of pathosystems*. Wallingford, UK: CABI Publishers, 230–250.

Rabbinge R, Jorritsma ITM, Schans J. 1985. Damage components of powdery mildew in winter wheat. *Netherlands Journal of Plant Pathology* **91,** 235–247.

Rapilly F. 1991. L′épidémiologie en pathologie végétale: mycoses aériennes. In: *Epidémiologie en pathologie végétale*. Paris: INRA, 240-261.

Robert C, Bancal MO, Lannou C. 2002. Wheat leaf rust uredospore production and carbon and nitrogen export in relation to lesion size and density. *Phytopathology* **92,** 762–768.

Robert C, Bancal MO, Nicolas P, Lannou C, Ney B. 2004*a*. Analysis and modelling of effects of leaf rust and *Septoria tritici* blotch on wheat growth. *Journal of Experimental Botany* **55,** 1079–1094.

Robert C, Bancal MO, Lannou C. 2004*b*. Wheat leaf rust uredospore production and carbon and nitrogen export in relation to lesion size and density. *Phytopathology* **94,** 712–721.

Robert C, Bancal MO, Ney B, Lannou C. 2005. Wheat leaf photosynthesis loss due to leaf rust, with respect to lesion development and leaf nitrogen status. *New Phytologist* **165,** 227–241.

Savary S, Castilla NP, Elazegui FA, McLaren CG, Ynalvez MA, Teng PS. 1995. Direct and indirect effects of nitrogen supply and disease source structure on rice sheath blight spread. *Phytopathology* **85,** 959–965.

Scholes JD. 1992. Photosynthesis: cellular and tissue aspects in diseased leaves. In: Ayres PG, ed. *Pests and pathogens—plant responses to foliar attack.* Oxford, UK: Bios Scientific Publishers, 85–101.

Scholes JD, Farrar JF. 1985. Photosynthesis and chloroplast functioning within individual pustules of *Uromyces-muscari* on bluebell leaves. *Physiological Plant Pathology* **27,** 387–400.

Scholes JD, Farrar JF. 1986. Increased rates of photosynthetis in localised regions of a barley leaf infected with brown rust. *New Phytologist* **104,** 601–612.

Scholes JD, Rolfe SA. 1996. Photosynthesis in localised regions of oat leaves infected with crown rust (*Puccinia coronata*): quantitative imaging of chlorophyll fluorescence. *Planta* **199,** 573–582.

Sinclair TR, Horie T. 1989. Leaf nitrogen, photosynthesis, and crop radiation use efficiency: a review. *Crop Science* **29,** 90–98.

Seefeldt SS, Jensen JE, Fuerst EP. 1995. Log-logistic analysis of herbicide dose–response relationships. *Weed Technology* **9,** 218–227.

Shtienberg D. 1992. Effects of foliar diseases on gas exchange processes: a comparative study. *Phytopathology* **82,** 760–765.

Smedegaard-Petersen V. 1984. The role of respiration and energy generation in diseased and disease-resistant plants. In: Wood RKS, Jellis GJ, eds. *Plant diseases: infection, damage and loss.* Cambridge University Press.

Snoeijers S, Pérez-Garcia A, Joosten M, Wit PD. 2000. The effect of nitrogen on disease development and gene expression in bacterial and fungal plant pathogens. *European Journal of Plant Pathology* **106,** 493–506.

Tiedemann AV. 1996. Single and combined effects of nitrogen fertilization and ozone on fungal leaf diseases on wheat. *Journal of Plant Diseases and Protection* **103,** 409–419.

Van Oijen M. 1990. Photosynthesis is not impaired in healthy tissue of blighted potato plants. *Netherlands Journal of Plant Pathology* **96,** 55–63.

Waggoner P, Berger R. 1987. Defoliation, disease and growth. *Phytopathology* **77,** 393–398.

Weisberg S. 1985. *Applied linear regression*. New York: John Wiley and Sons Ltd, 96–97.

Zuckerman E, Eshel A, Eyal Z. 1997. Physiological aspects related to tolerance of spring wheat cultivars to *Septoria tritici* blotch. *Phytopathology* **87,** 60–65.