Europ. J. Agronomy 35 (2011) 237–246

Contents lists available at ScienceDirect

European Journal of Agronomy

journal homepage: www.elsevier.com/locate/eja

Effect of leaf rust (*Puccinia triticina*) on photosynthesis and related processes of leaves in wheat crops grown at two contrasting sites and with different nitrogen levels

Ramiro Carretero[a,c,*], Marie Odile Bancal[d], Daniel Julio Miralles[a,b,c]

[a] *Cátedra de Cerealicultura, Universidad de Buenos Aires, Av. San Martín 4453, C1417DSE Buenos Aires, Argentina*
[b] *IFEVA, Universidad de Buenos Aires, Av. San Martín 4453, C1417DSE Buenos Aires, Argentina*
[c] *CONICET, Argentina*
[d] *Environnement et Grandes Cultures, INRA, F-78850 Thiverval-Grignon, France*

ARTICLE INFO

Article history:
Received 6 April 2011
Received in revised form 21 June 2011
Accepted 22 June 2011

Keywords:
Conductance
Dark respiration rate
Leaf rust
Non-stomatal process
Photosynthesis rate
Wheat (*Triticum aestivum* L.)

ABSTRACT

Leaf rust is one of the main diseases affecting wheat yield production. Considering the physiological variables that determine yield, diseases could affect radiation capture and/or radiation use efficiency. Reductions in radiation use efficiency may be mediated through effects on photosynthesis rate and related variables (i.e. dark respiration rate, stomatal conductance or photosynthesis events *per se*). The aim of this study was to analyze the effects of leaf rust on wheat leaves photosynthesis rate and to understand which processes determining photosynthesis are affected by this pathogen. Gas exchange measurements were taken on flag leaves with various rust severity levels in experiments carried out on two locations which included different nitrogen fertilization rates and sowing dates. Leaf rust reduced net photosynthesis rate at light saturation through reductions in gross photosynthesis (average reduction: 6.1 μmol CO_2 m^{-2} green area s^{-1}) rather than through increases in dark respiration rate (average increase: 0.7 μmol CO_2 m^{-2} green area s^{-1}). Changes in leaf nitrogen concentration did not modify the effects of leaf rust on net photosynthesis rate. Although net photosynthesis rate at light saturation was reduced, no effects were observed at low irradiance levels. The reduction of photosynthesis was due to effects on non-stomatal processes; indeed, important reductions of SPAD units (i.e. chlorophyll) were observed on green areas of diseased leaves. SPAD values on diseased leaves were 26.4 ± 0.98 and 27.6 ± 1.05 for N_0 and N_1 treatments, respectively; while on healthy leaves, values were 32.0 ± 0.83 and 38.6 ± 0.41, respectively.

1. Introduction

Foliar diseases are among the main factors reducing wheat crop yields (*Triticum aestivum* L.) in many regions around the world (Duveiller et al., 2007; Oerke and Dehne, 1997, 2004). Leaf rust (*Puccinia triticina*) is one of the most important diseases causing around 4% mean annual yield reduction (Duveiller et al., 2007), although up to 50% reductions have been reported (Annone et al., 2001 and works cited therein). In this sense, it is important to point out that diseases intensity is associated with the environmental conditions during growing seasons and locations, cultivar susceptibility, crop rotation, sowing date, etc. (Polley and Thomas, 1991).

Damage thresholds based on empirical models that relate yield loss to diseases intensity are the most popular tools commonly used by farmers to decide the timing of fungicide applications (Carmona et al., 1999; Cortese et al., 1998; Mumford and Norton, 1984; Reis et al., 2006, 2007). However, these approaches are often inconsistent, mainly because yield losses vary greatly depending on diseases intensity, the development stage of the crop when diseases appear, the leaf area index, the architecture of the crop (light extinction coefficient) and the vertical position of the diseases into the canopy (Bancal et al., 2007; Carretero et al., 2010; Gaunt, 1995; Madden and Nutter, 1995; Serrago et al., 2009). Thus, damage thresholds do not take into account crop physiology variables that determine an important variability in the yield losses for a same value of diseases severity.

Mechanistic approaches were developed accounting for pathogen effects on the main physiological variables determining biomass production and yield (i.e. radiation interception and radiation use efficiency). They were found to predict the diseases effects across locations and years, more accurately and robustly

* Corresponding author at: Cátedra de Cerealicultura, Universidad de Buenos Aires, Av. San Martín 4453, C1417DSE Buenos Aires, Argentina.
Tel.: +54 11 4524 8053; fax: +54 11 4524 8039.
E-mail address: rcarrete@agro.uba.ar (R. Carretero).

1161-0301/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.eja.2011.06.007

than those models that considered only disease level measurements (Gaunt, 1995; Johnson, 1987; Waggoner and Berger, 1987). These studies pointed out that predicting the impact of wheat foliar diseases on yield accurately, requires understanding their effects on: (i) ecophysiological variables responsible for radiation interception (i.e. leaf area index-LAI, green leaf area index-GLAI, crop architecture and vertical distribution of diseases into the canopy) and (ii) physiological variables related to radiation use efficiency (i.e. photosynthesis rate and related process, assimilates flux and cell turgor). Wheat leaf rust effects on variables determining radiation interception were largely explored in previous works (Bancal et al., 2007; Carretero et al., 2010; Serrago et al., 2009), while leaf rust effects on variables related to radiation use efficiency were studied with less detail (Bethenod et al., 2001; Robert et al., 2005, 2004).

Pathogens can cause reductions in leaf photosynthesis rate (with respect to a healthy leaf) proportionally equal, greater or lower than the level of visual disease severity, depending on the type of pathogen and host (Erickson et al., 2003; Shtienberg, 1992). Proportionally greater reductions in photosynthetic rate suggest negative pathogen effects on the photosynthesis rate of the remaining green leaf area of diseased leaves; whereas proportionally lower reductions could be associated with an increased (compensatory) photosynthesis in the remaining green area of the diseased leaves. In this sense, previous studies showed few or imperceptible effects of leaf rust (*P. triticina*) on photosynthesis of remaining green area of diseased wheat leaves (Bastiaans, 1991; Robert et al., 2005, 2004), being radiation capture (photosynthetic active leaf area), the main process affected by leaf rust. These works considered only net photosynthesis rate at light saturation (P_{max}). However, light attenuation occurring within the canopy (Dreccer et al., 2000), as well as, large variations in incident radiation along the day, expose most of the leaves of the canopy to large variations in radiation intensities during their lifespan. Consequently, the response of photosynthetic leaf capacity to foliar diseases should be established on the range of radiation levels explored by the leaves.

Alongside with leaf light environment, foliar nitrogen content is one of the major determinants of photosynthesis rate, and more specifically P_{max} (Connor et al., 1993; Evans, 1983; Sinclair and Horie, 1989). Thus, the effects of the pathogens on photosynthesis rate could be different, depending on the nitrogen concentration of the leaf tissue. Information on the effects of foliar diseases on photosynthesis of wheat leaves with different nitrogen content is extremely limited. Robert et al. (2006, 2005) did not find interaction effects between foliar nitrogen content and diseases (when wheat plants were infected by leaf rust and *Septoria tritici* in each work, respectively) on P_{max} of remaining green area. However, in those studies, foliar nitrogen concentration did not vary sufficiently to cause any significant difference in photosynthetic rate of healthy leaves between nitrogen treatments, explaining the lack of interaction observed in such studies.

The decline in net photosynthetic rate of leaves caused by foliar pathogens can be attributed mainly to: (i) an increase of host respiration rate or pathogen respiration itself; (ii) changes on stomatal conductance; and/or (iii) effects on photochemical machinery *per se* (*e.g.* enzyme related with CO_2 fixation). To our knowledge, only one work studied the effects of wheat leaf rust on stomatal conductance and on photosynthesis events *per se* (Bethenod et al., 2001). However in that study, gas exchange measurements were carried out on autotrophic leaves of seedlings grown in pots under controlled conditions, with irradiance levels lower than those that normally occur under field conditions. Considering that in field conditions, leaf rust occurs mainly after anthesis, interactions of this pathogen with long-living leaves experiencing large range of radiation intensities and nitrogen content, need to be deepened.

The present study was carried out to investigate the effects of leaf rust on foliar photosynthesis parameters (i.e. P_{max}, light use efficiency and respiration) and to analyze the physiological bases of these effects (i.e. stomatal conductance and photosynthesis events *per se*) in wheat plants grown under a wide range of field conditions.

2. Materials and methods

2.1. General conditions

Four field experiments were carried out during different growing seasons. Two experiments were conducted in Grignon (France, INRA-EGC station; 48°50′N; 1°57′E) during the 2004/2005 growing season and other two experiments were set in Buenos Aires (Argentina, Facultad de Agronomía, Universidad de Buenos Aires; 34°35′S; 58°29′W) during the 2005 and 2007 growing seasons. In Grignon, wheat cultivar *Soissons* susceptible to leaf rust (and one of the most commonly commercial cultivars used in France) was sown on two different dates (*2005G1 and 2005G2 experiments*). In the 2005G1 experiment, large plots 30 m long with nine rows 0.15 m apart were sown at a density of 250 plants m^{-2} on 22 October 2004, i.e. close to the optimal sowing date at the experimental site (Grignon). In the 2005G2 experiment, vernalized seedlings were planted on 15 March 2005 in plots of 1 m long with 10 rows 0.10 m apart (50 seedlings per row). To cover the vernalization requirements, seeds were germinated during 24 h in humid cotton and then sown in Jiffy peat pots, where they were kept for eight weeks with a 16 h light period (350 $\mu E\, m^{-2}\, s^{-1}$) at 8.8 °C and an 8 h dark period at 0 °C. In Buenos Aires, the cultivar *Klein Pegaso* (susceptible to leaf rust) was sown at 400 plants m^{-2} on 3 August 2005 (*2005BA experiment*) and on 24 July 2007 (*2007BA experiment*) in plots of 10 rows 0.15 m apart, 2.8 and 2.1 m long for 2005 and 2007, respectively.

The plots for the 2005G2, 2005BA and 2007BA experiments were periodically irrigated to ensure adequate water supply throughout the crop cycle. The 2005G1 experiment was carried out under rain-fed conditions, although water was applied by irrigation the evening before the date of measurements, to avoid exposing the plants to stress due to shortage of water supply. In all the experiments, herbicides and insecticides were sprayed, when necessary, to control weeds and insect damages.

Treatments were arranged as a factorial combination of two nitrogen and two disease levels. In Buenos Aires experiments (2005BA and 2007BA), treatments were arranged in a split plot design with three blocks, where the main plots represented the nitrogen treatments (N_0 and N_1) and the sub-plots corresponded to the fungicide treatments (P and UP). In Grignon treatments, each nitrogen and disease combination was randomly applied to three plots. It is important to notice that measurements were made at leaf level on randomly selected plants of the different plots. Thus, the experimental units used for the analysis were the selected leaves and not the plots.

2.2. Nitrogen treatments

Two levels of N supply, low and high (N_0 and N_1), were applied to all experiments. In the experiments carried out in Buenos Aires, N_0 corresponded to soil nitrogen content: 13 and 50 kg N ha^{-1} (on the top 0.6 m of the soil at sowing time) in 2005 and 2007, respectively; while N_1 amounted to 300 kg N ha^{-1} (soil nitrogen content on the top 0.6 m + fertilizer nitrogen) in both years. In Grignon, N_0 supply (soil + fertilizer) was 80 and 100 kg N ha^{-1} in 2005G1 and 2005G2 experiments, respectively; while N_1 treatment supply (soil + fertilizer) was 300 kg N ha^{-1} in both experiments. Soil N

content at sowing was 15 kg N ha^{-1} (on the top 0.6 m of the soil) in Grignon experiments.

2.3. Leaf rust inoculations

Two levels of diseases control were used: (i) protected, with fungicides application and (ii) unprotected, inoculated with leaf rust without fungicides application. In the unprotected treatment, plots were inoculated by spraying the plants with a suspension of leaf rust (*P. triticina*) spores with a manually operated sprayer, except for the 2005G1 experiment, in which leaves were naturally infected. In the 2005G2 experiment, inoculations were performed with spores of isolate *B9384-1C1*, with oil as a carrier, 10 days before flag leaf emergence and later repeated at heading stage. The spores were previously increased on seedlings of the susceptible cultivar Michigan. In the experiments carried out in Buenos Aires, spores were applied using water with some drops of surfactant (Tween 20®) as a carrier, at the onset and at the middle of stem elongation in the 2005 experiment and at flag leaf emergence in the 2007 experiment. Specific race of leaf rust for the cultivar *Klein Pegaso* provided by the National Institute of Agricultural Technology (INTA), Argentina, was used in Buenos Aires. All the plots (including protected ones) were covered with plastic tents in the evening after inoculation and during the following three nights to increase humidity and the duration of leaf-wetness so as to promote the infection by the pathogen. Protected (P) plots implied maintaining the crop free of diseases during the whole cycle through systematic applications (every 7–15 days) of epoxiconazole (Opus® Basf) at 1 l ha^{-1} and tebuconazole (Folicur® Bayer SA) at 0.75 l ha^{-1} in Grignon and Buenos Aires experiments, respectively.

2.4. Leaf photosynthesis measurements

Gas exchange measurements were performed with an open-path portable photosynthesis system (LI-6400; Li-Cor, Lincoln, USA) provided with red–blue LEDs light source (6400-02B, Licor) and with a 6 cm^2 chamber (3 cm × 2 cm). Measurements were made in both healthy and diseased flag leaves (on central section of the leaf) several times since symptoms appearance, i.e. from flag leaf fully expanded stage. Thus, from flag leaf expanded stage to physiological maturity, gas exchange measurements were made at light saturation, that is, 1500 μmol photon m^{-2} s^{-1} of incident photosynthetically active radiation (PAR) (400–700 nm). The CO_2 concentration incoming the chamber was set at 400 ± 2 μmol CO_2 mol^{-1} air using the system's CO_2 injector (6400-01, LI-COR). In the 2007BA experiment, gas exchange of each sampled leaf was measured over a range of irradiances that changed as follows: 800, 1000, 1800, 1500, 1000, 500, 200, 100, 50, 20 and 0 μmol photon m^{-2} s^{-1} of incident PAR. Gas exchange measurements were taken for each radiation level once CO_2 and stomatal conductance readings into the sample chamber got stabilized.

Leaf temperature was measured with a thermocouple situated at the bottom of the chamber of the photosynthesis system. Air temperature and atmospheric pressure were also measured by the system. Vapor pressure deficit (VPD) between the air and the substomatal cavity of the leaf was calculated by photosynthesis system, which considers leaf temperature, air temperature and atmospheric pressure.

2.5. Selection and grouping of leaves for gas exchange measurements

From flag leaf expanded stage to physiological maturity, healthy and diseased leaves (with different degree of severity) were randomly selected from the different plots. To ensure comparisons between leaves exposed to similar environmental conditions (i.e. VPD and temperature), leaves were grouped considering their closeness in time within each date of measurement. Hence, comparisons for gas exchange variables were made between healthy (2 or 3) and diseased (5–10) leaves measured within a time period of half an hour approximately. Fewer healthy than diseased leaves were considered, as it was expected that gas exchange measurements were more similar among healthy leaves than among diseased leaves, since the latter ones were selected to obtain different severity levels.

2.6. Disease severity assessments and remaining green area determinations

Once gas exchange measurements were taken, the central leaf sections on which measurements were performed were photographed using a digital camera. These digital images were used to determine green and diseased areas (cm^2) using images analysis software Assess (Lamari, 2002). The leaf areas covered by pustules, as well as the chlorotic (yellow) areas, were considered as diseased tissue. Thus, leaf rust severity was calculated as the ratio between diseased and total leaf area within the measured leaf section.

2.7. SPAD measurements

Chlorophyll-meter measurements (SPAD-502; Minolta Camera Co., Osaka, Japan) were taken in the 2007BA experiment as an indicator of the leaf nitrogen and chlorophyll status in the leaves. Immediately after photosynthesis assessment, three chlorophyll-meter measurements were made on green portions along the section of the leaf where photosynthesis was assessed.

2.8. Data analysis and calculations

The net photosynthesis rate (P_n) (μmol CO_2 m^{-2} green area s^{-1}), light-saturated net photosynthesis rate (P_{max}) (μmol CO_2 m^{-2} green area s^{-1}), stomatal conductance (g_s) (mol H_2O m^{-2} green area s^{-1}) and internal CO_2 concentration (C_i) (μmol CO_2 mol^{-1} air) were calculated taking into account the remaining green area (i.e. area visually healthy) of the leaf section inside the sample chamber and according to von Caemmerer and Farquhar (1981). The g_s and C_i values were calculated for light-saturated conditions (1500 μmol photon m^{-2} s^{-1}).

Besides, light-saturated net photosynthesis rate (P_{max}) from diseased and healthy leaves, considering the total leaf area of the measured section (green + diseased), was compared according to Bastiaans' (1991) model as follows:

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

which relates the relative net photosynthesis (P_x/P_0) to leaf rust severity (x). Each P_x/P_0 was calculated as the ratio of P_{max} (considering total leaf area) of a diseased leaf with severity x (P_x) to the average P_{max} of 2–3 healthy leaves (P_0), all of which were measured close in time (see Section 2.5). According to Bastiaans (1991), β represents the ratio between the leaf area occupied by virtual and visual lesion. Virtual lesion is the area in which photosynthesis is impaired and assumed to be zero. Thus β indicates if the net photosynthesis rate of the remaining green tissue of a diseased leaf is affected negatively ($\beta > 1$), positively ($\beta < 1$) or not affected ($\beta = 1$) by the pathogen.

Net photosynthesis (P_n)–light response curves were analyzed by fitting the data to a negative exponential function (Goudriaan and van Keulen, 1979) as follows:

$$P_n = [P_{max} + R][1 - \exp(-\varepsilon I/(P_{max} + R))] - R \quad (2)$$

where P_{max} is light-saturated net photosynthesis rate (μmol CO_2 m^{-2} s^{-1}), R is dark respiration rate (μmol CO_2 m^{-2} s^{-1}), I is the incident PAR (μmol photon m^{-2} s^{-1}) and ε is the apparent quantum efficiency of photosynthesis at low irradiance (μmol CO_2 μmol $photon^{-1}$), i.e. the intercepted radiation use efficiency. Gross photosynthesis rate was considered to be net photosynthesis rate plus dark respiration rate. The calculations were made in two manners: considering only green leaf area on the one hand and the total area (green + diseased) on the other hand.

Comparisons were made between diseased and healthy leaves and between N treatments within each location. *F*-test and confidence interval (95%) were used for comparisons of parameters of fitted models; while *t*-test was used for mean values comparisons. The statistical analysis used is indicated next to each comparison within the text of the results section.

3. Results

3.1. Atmospheric conditions in leaf enclosures

Considering the environments explored throughout the experiments (including sites, sowing dates and nitrogen treatments), important ranges of VPD, i.e. from 0.35 to 1.78 kPa, and of leaf temperature, i.e. from 18.5 to 31.0 °C, were explored. Vapor pressure deficit and leaf temperature are both known to affect leaf gas exchange rates through stomatal conductance and enzyme activity, respectively. Despite the wide range explored for both variables, healthy and diseased leaves submitted to gas exchange variables comparison, were exposed to similar VPD and temperature values (Fig. 1). The regression lines between healthy and diseased leaves either for leaf temperature or for VPD, were highly significant (i.e. different from 0) ($p < 0.01$), their coefficient of correlation being 0.97 and 0.68, respectively. The slopes of the regression were not significantly different from one (according to 95% confidence interval comparisons) and the *Y*-intercept not significantly different from zero (according to 95% confidence interval comparisons) for both variables.

3.2. Photosynthesis of healthy and diseased leaves under light saturated conditions

In healthy leaves, P_{max} ranged from 11.0 to 32.3 μmol CO_2 m^{-2} s^{-1}, showing that experiments explored a wide range of environmental conditions due to the different sites, sowing dates and nitrogen treatments, including variations in the age of the leaves. Except for the *2005G2* experiment, P_{max} of N_1 treatment was always significantly higher than P_{max} of N_0 treatment (according to 95% confidence interval comparisons) (Table 1). At leaf level, leaf rust severity, characterized as the proportion of area covered with pustules plus the chlorotic (yellow) area, explored a wide range, reaching maximum values close to 0.50 for N_1 treatment in almost all the experiments. On the other hand, severity values for N_0 treatment did not exceed 0.40 except for one leaf of the *2005G2* experiment, which reached 0.50 (Fig. 2).

When analyzing the effect of leaf rust on P_{max} by fitting the data to Bastiaans' (1991) model, coefficient of determination ranged between 0.41 and 0.88 (Table 1) and the scatter of points was wider in Grignon than in Buenos Aires (Fig. 2). The estimated values of β ranged between 1.92 ± 0.49 (N_0 treatment in *2005G1*) and 3.51 ± 0.49 (N_1 treatment in *2007BA*). Although β values were higher in the N_1 than in the N_0 treatment in three out of four experiments (Table 1 and Fig. 2), no significant differences were observed in this attribute (according to 95% confidence interval comparisons). In all cases, estimated β values were significantly higher than 1 ($p < 0.001$, for all *F*-tests) indicating a negative effect of leaf rust on P_{max} of the remaining green area of diseased leaves.

Table 1
Mean values of P_{max} of healthy leaves (±half confidence interval-95%) and β parameter estimations (±half confidence interval-95%) for different experiments (years, sites and sowing dates) for low and high nitrogen availability (N_0 and N_1) and for the pool data set within each experiment (all). Number of measurements (n) on diseased leaves and R^2 for the fit of data to Bastiaans' model are shown for each situation.

Exp.	N	P_{max} healthy	β	R^2	n
2005G1	N_0	15.0 ± 1.35	1.92 ± 0.49	0.42	57
	N_1	19.0 ± 1.55	2.56 ± 0.41	0.71	60
	All		2.35 ± 0.31	0.66	117
2005G2	N_0	16.7 ± 1.85	2.17 ± 0.67	0.41	37
	N_1	17.0 ± 1.91	2.64 ± 0.73	0.43	41
	All		2.31 ± 0.49	0.44	78
2005BA	N_0	21.0 ± 0.96	2.32 ± 0.29	0.62	138
	N_1	24.6 ± 0.69	2.12 ± 0.20	0.72	169
	All		2.18 ± 0.16	0.69	307
2007BA	N_0	17.7 ± 1.73	3.15 ± 0.69	0.69	35
	N_1	20.9 ± 1.82	3.51 ± 0.49	0.88	36
	All		3.37 ± 0.40	0.83	71

3.3. Photosynthesis–light response curve parameters

The main effect of leaf rust on net photosynthesis rate was mediated through reduction of gross photosynthesis rate (P_{gross}) and due to increases in dark respiration rate (R), with no effect of the pathogen on the apparent quantum efficiency (ε) (Fig. 3). The estimated β for maximum gross photosynthesis ($\beta_{gross} = 2.86 \pm 0.19$, considering both nitrogen treatments from *2007BA* experiment) was significantly lower than the β value estimated for maximum net photosynthesis ($\beta_{net} = 3.56 \pm 0.22$) ($p < 0.05$, *F*-test with dfn = 1 and dfd = 140), suggesting that R was increased by leaf rust infection, thus increasing the β_{net}. However, β_{gross} value was again significantly higher than 1.0 ($p < 0.001$, *F*-test with dfn = 1 and dfd = 70) demonstrating that P_{gross} was reduced for the remaining green area of diseased leaves. Indeed, when considering only green leaf area, P_{gross} of diseased leaves (14.8 ± 0.46 μmol CO_2 m^{-2} green area s^{-1}, averaging all data set) was significantly lower than P_{gross} of healthy leaves (20.9 ± 0.69 μmol CO_2 m^{-2} green area s^{-1}) ($p < 0.001$, *t*-test with df = 69).

Dark respiration rate increased with N content and disease severity (Fig. 3A). When only green leaf area was considered for calculations, diseased leaves (on average) had significantly higher R (2.1 ± 0.13 μmol CO_2 m^{-2} green area s^{-1}) than healthy leaves (1.4 ± 0.08 μmol CO_2 m^{-2} green area s^{-1}) ($p < 0.001$, *t*-test with df = 69). However, when comparing the effects of leaf rust on P_{gorss} and R (i.e. the components determining net photosynthesis rate), the absolute increases of R (on average 0.7 μmol CO_2 m^{-2} green area s^{-1}) were one order of magnitude lower than the absolute reductions on P_{gross} (on average 6.1 μmol CO_2 m^{-2} green area s^{-1}). This suggests that the main effect of leaf rust was on the photosynthetic machinery rather than on the enhancement of dark respiration. Finally, as shown in Fig. 3C, no effects of leaf rust were observed on apparent quantum efficiency of the remaining green area of diseased leaves; it decreased proportionally to green leaf area. Thus, no significant differences were found between diseased and healthy leaves when ε was estimated taking into account the remaining green area ($p > 0.1$, *t*-test with df = 69), showing values of 0.0564 ± 0.00130

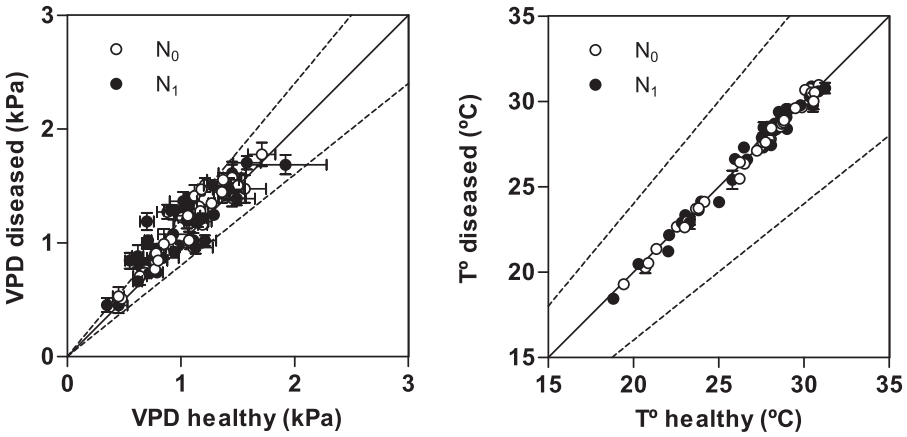

Fig. 1. Relationship between vapor pressure deficit (VPD) and temperature (T°) of diseased and healthy leaves for low (open symbols) and high (closed symbols) nitrogen levels. Each point corresponds to the average of 2–3 healthy leaves (*X* axis) and the average of 5–10 diseased leaves (*Y* axis), all of them measured close in time as explained in Section 2.5. Bold line indicates the 1:1 relationship and dotted lines indicates 20% upper and lower limits respect to the 1:1 line. Horizontal and vertical bars indicate standard errors on *X* axis and *Y* axis, respectively. Data from all the experiment were included.

and $0.0538 \pm 0.00069\ \mu mol\ CO_2\ \mu mol\ photon^{-1}$ for healthy and diseased leaves, respectively (considering both nitrogen treatments).

3.4. Disease effects on stomatal and non-stomatal processes determining photosynthesis

The wide range of environmental conditions explored due to different sites, years and dates of sampling determined a wide range of g_s being observed (Fig. 4A). In this sense, lower levels of g_s were observed in Grignon (France) with respect to Buenos Aires (Argentina) and a broader range of g_s was observed in Buenos Aires experiments than in Grignon ones. Leaf rust reduced g_s of green leaf area in those leaves exploring environmental conditions that allow reaching g_s values higher than $1\ mol\ H_2O\ m^{-2}$ green area s^{-1} under both nitrogen conditions (Fig. 4B) ($p < 0.05$, *t*-test), while no difference between diseased and healthy leaves was observed

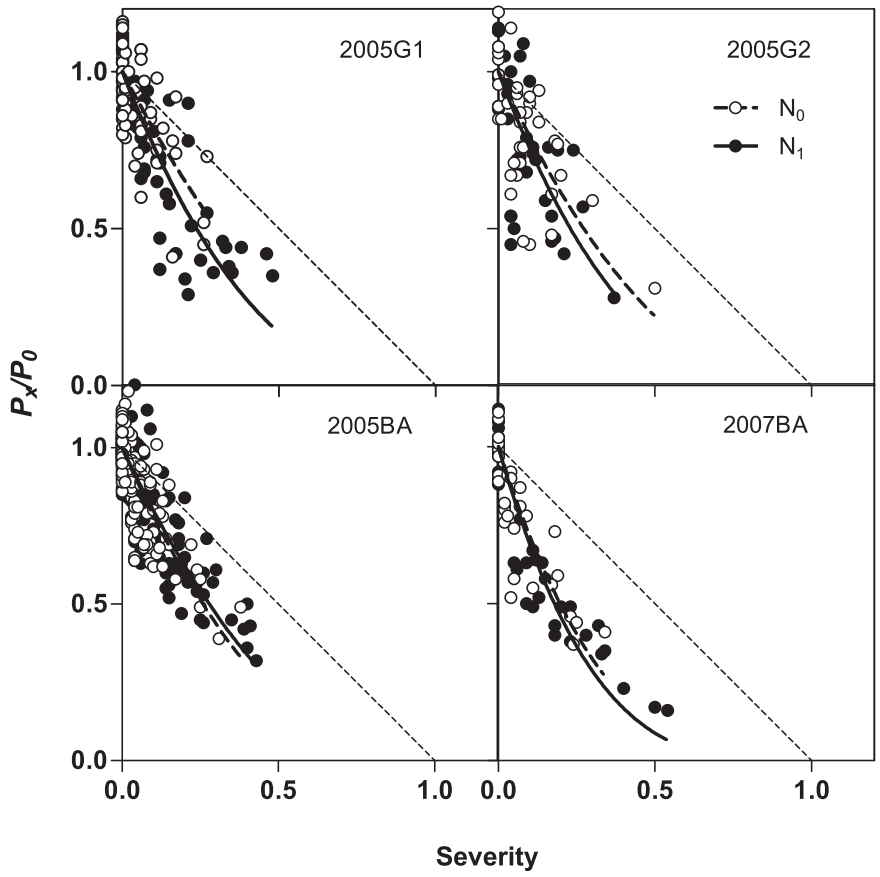

Fig. 2. Relative light-saturated net photosynthesis rate (P_x/P_0) related to leaf rust severity (proportion) for the different experiment (years, sites and sowing dates) and for both nitrogen treatments: N_0 (open symbols) and N_1 (closed symbols). Bastiaans' model fit (Eq. (1)) for N_0 and N_1 data are represented by dotted and continue curves, respectively. Diagonal dotted line showed 1:1 relationship. For parameters estimations and analysis of the models see Table 1.

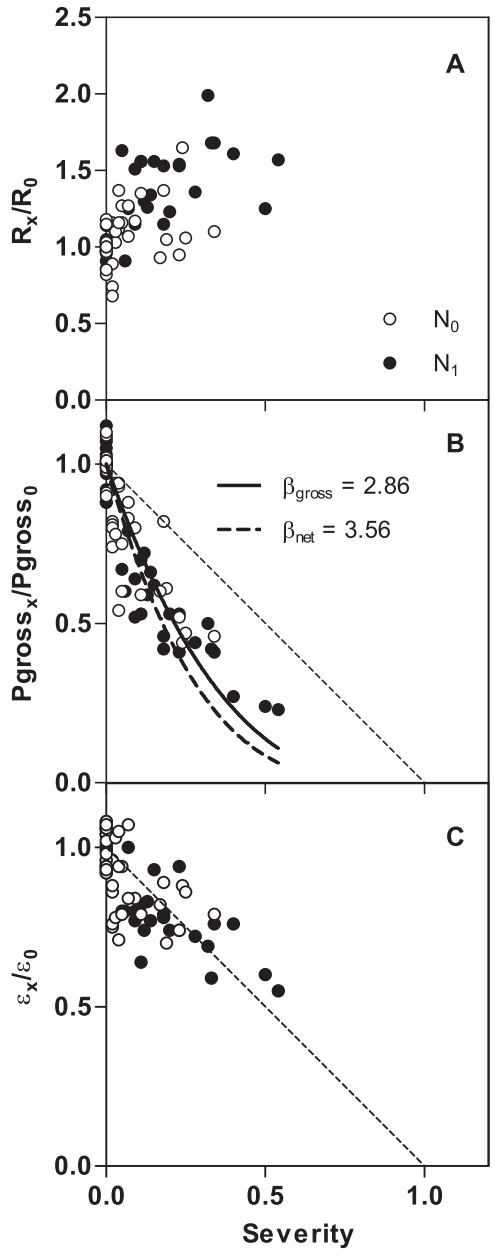

Fig. 3. (A) Relative respiration rate (R_x/R_0); (B) relative maximum gross photosynthesis rate (P_{gross_x}/P_{gross_0}) and (C) relative apparent quantum efficiency ($\varepsilon_x/\varepsilon_0$) against leaf rust severity for 2007BA experiment. High and low nitrogen treatments are represented by closed and open symbols, respectively. Bastiaans' model (Eq. (1)) was fitted for relative maximum net photosynthesis rate (dotted curve) and for maximum relative gross photosynthesis rate (continue curve). Diagonal dotted lines represent 1:1 relationship. Shown variables came from Eq. (2) fitted to 2007BA data.

for low g_s values (i.e. g_s of healthy leaves lower than 1 mol H_2O m^{-2} green area s^{-1}).

The internal CO_2 concentration (C_i), considering only the remaining green leaf area, was related to stomatal conductance (g_s) for healthy leaves and for leaves with different severity levels (Fig. 5). These data were fitted to an exponential equation with asymptote ($y = a - b \times \exp^{(-c \times x)}$) where a denotes the maximum C_i value that could be reached under no stomatal conductance limitation. The coefficients of determination for the fitted curves ranged between 0.35 and 0.89.

In both nitrogen treatments, the increases in severity levels promoted higher a (i.e. maximum C_i) values than those from healthy leaves ($p < 0.05$, according to F-test in six of the eight situations), denoting higher levels of C_i when g_s is not limiting the CO_2 diffusion. Moreover, most of the points of the relationship between C_i and g_s for the diseased leaves stayed above the fit curve for healthy leaves, being this tendency more evident as severity levels were increased. Thus, when compared for the same level of g_s, diseased leaves showed higher C_i values than healthy leaves. This kind of behavior suggests that part of the reduction in the photosynthesis rate induced by disease is mediated through a non-stomatal process.

A strong relationship between P_{max} and SPAD readings in the green portions of leaves was found, with determination coefficients of 0.63 and 0.71 for N_0 and N_1 treatment, respectively; with no significant differences ($p = 0.71$, F-test with dfn = 1 and dfd = 86) in the slope of the regression between nitrogen treatments (Fig. 6). SPAD readings of the diseased leaves were 26.4 ± 0.98 for N_0 treatment and 27.6 ± 1.05 for N_1 treatment, being significantly lower ($p < 0.001$, t-test with df = 38 and df = 48 for N_0 and N_1 treatment, respectively) than those measured in healthy leaves. Additionally, SPAD values of healthy leaves from N_1 treatment (38.6 ± 0.41) were significantly higher than those from N_0 treatment (32.0 ± 0.83) ($p < 0.001$, t-test with df = 36). These results emphasize the idea exposed before that leaf rust could be reducing P_{max} through processes other than stomatal conductance, probably chlorophyll reductions.

4. Discussion

It is important to highlight that the results shown in this paper were obtained from plants grown under field conditions at different fertilization rates, sites, sowing dates and ages of the leaves. Thus, this study allowed to test the effects of leaf rust on photosynthesis and related variables under a very wide range of environmental and soil conditions (fertility and sites). The lack of significant differences for temperature and VPD between healthy and diseased leaves (for which gas exchange variables were compared) and the wide range of explored conditions for both variables, allowed to determine the genuine effect of leaf rust on photosynthesis rate and related variables. The values of P_{max} of healthy leaves found in the present study (11.0–32.3 μmol CO_2 m^{-2} s^{-1}) were within the range of values shown in the literature for wheat leaves (Evans, 1983; Robert et al., 2006, 2005).

According to Bastiaans' (1991) approximation, β relates the virtual lesion to visual lesion, thereby, the photosynthesis of the green leaf area of diseased leaves becomes reduced whenever β is higher than 1. The results obtained in the present study showed that leaf rust reduced P_{max} of green leaf area of wheat diseased leaves since the estimated β values were significantly higher than 1. Similar β values were shown for rust (*Uromyces appendiculatus*) affecting leaves of bean (*Phaseolus vulgaris*) with a β value of 2.2 (Bassanezi et al., 2001); while β values between 0.88 and 1.54 were found by Lopes and Berger (2001) for the same pathosystem. Similarly, soybean rust (*Phakopsora pachyrhizi*) was shown to reduce P_{max} of remaining green area of diseased leaves (Kumudini et al., 2008). Other sets of evidence reported β values for biotrophic pathogens of wheat of 8.74 for powdery mildew (*Erysiphe graminis*) and 1.26 for leaf rust (*P. triticina*), being the last one no significantly different from 1 (Bastiaans, 1991).

The magnitude of the diseases effects on photosynthesis could vary with the state of development of the diseases (Robert et al., 2005; Scholes and Farrar, 1986; Scholes and Rolfe, 1996) and/or with the limits that are considered as diseased tissue (Robert et al., 2005). In relation to the limits assumed to determine diseased tissues, Robert et al. (2005) found that β values for wheat leaves affected by leaf rust varied during the sporulation period from 2 to 11, 1.4 to 2, and 0.8 to 1, when considering: (i) only the

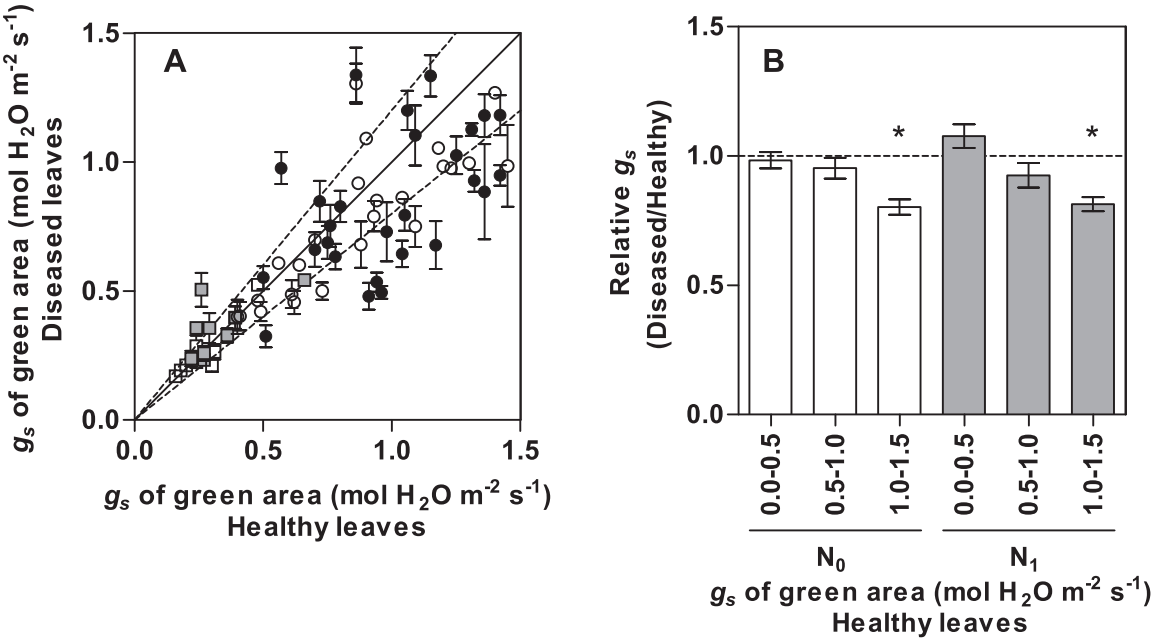

Fig. 4. (A) Stomatal conductance (g_s) of green area of diseased leaves in relation to healthy leaves for all the experiment (2005G1and 2005G2: squares; 2005BA and 2007BA: circles) for low (open symbols) and high (closed symbols) nitrogen levels. Each point corresponds to the average of 2–3 healthy leaves (*X* axis) and the average of 5–10 diseased leaves (*Y* axis), all of them measured close in time as explained in Section 2.5. Bold line indicates the 1:1 relationship and dotted lines indicates 20% upper and lower limits respect to the 1:1 line. Vertical bars indicate standard errors. (B) Relative conductance (i.e. g_s of green area of diseased leaves/g_s of healthy leaves, both measured close in time) for different g_s levels ranges of healthy leaves and for low (open bars) and high (grey bars) nitrogen availability. Vertical bars indicate standard errors. Asterisks indicate significant difference respect to 1 ($p < 0.05$).

proportion of sporulating area, (ii) the sporulating plus necrotic area and (iii) the total diseased area into the leaf, respectively. The β estimations obtained in the present study were slightly higher than those reported by Robert et al. (2005) when they considered sporulating plus necrotic area, probably due to the fact that no necrotic lesions (which are expected to determine β values near 1) were observed in any of the diseased leaves measured in the present study, being the lesions formed by sporulating plus chlorotic tissue. Thus, depending on the criteria taken to delimit the rust damage in the leaves (diseased area), β estimations could be substantially different, explaining the diverse results found in the literature.

As it is further known, leaf nitrogen concentration modifies net photosynthesis rate (Connor et al., 1993; Evans, 1983; Sinclair and Horie, 1989). In this sense, and differing from what was found by Robert et al. (2005), P_{max} of healthy leaves were significantly higher in high with respect to low nitrogen treatment. However, similar to findings by Robert et al. (2005), no significant differences were observed in β estimations for leaf rust between nitrogen treatments, when considering each experiment separately. Although differences in β were non-significant in statistical terms, they were consistently higher in the N_1 than in the N_0 treatment in three out of four experiments. As was reported by Robert et al. (2005), the reduction in P_{max} of remaining green area was higher when considering only sporulating area than when chlorotic area was also considered. In this sense, in the present study sporulating area in the N_0 treatment represented $30.5 \pm 1.2\%$ while in the N_1 treatment, it represented $47.7 \pm 1.3\%$ of the total diseased area, being this difference significant in statistical terms ($p < 0.001$). Thus, it is possible to hypothesize that the higher β values observed in N_1 with respect to N_0 treatment were probably due to the different proportions of sporulating and chlorotic area promoted by the disease.

Foliar diseases can reduce net assimilation of CO_2 through gross photosynthesis rate reductions or through dark respiration rate increase. The results of the present study showed that although leaf rust increased dark respiration rate, the reductions of net photosynthesis rate were mainly due to impaired gross photosynthesis rate at light saturation. The higher respiration rates detected on infected leaves in the present study could be due to increased host respiration (Bassanezi et al., 2002; Kumudini et al., 2008; Lopes and Berger, 2001; Scholes and Farrar, 1986) or they could be caused by pathogen respiration itself (Owera et al., 1981).

The absence of effect of leaf rust on apparent quantum efficiency suggests that leaf photosynthesis rate was not affected by leaf rust at low irradiance level, which would have important implications at crop level. Since light attenuation occurs within the canopy (Dreccer et al., 2000) and solar angle changes throughout the day, the leaves of a crop are not continuously exposed to light saturation levels (Horton, 2000). Thus, radiation use efficiency at crop level would be less affected than the P_{max} at leaf level which is in agreement with the lack of effect of leaf rust on radiation use efficiency observed in previous works (Bancal et al., 2007; Carretero et al., 2010; Serrago et al., 2009). These results also agree with Rabbinge et al. (1985) simulated data, which showed that daily gross assimilation rate of a barley crop was less strongly reduced by mildew than was P_{max} (i.e. photosynthesis at light saturation) due to absence of effect of the pathogen on the apparent quantum efficiency.

Diseases may reduce the gross photosynthesis rate by: (i) effects on CO_2 diffusivity into the leaf (reduction of stomatal conductance) or (ii) by effects on biochemical processes (non-stomatal processes), such as energy capture by photosystems (reductions of chlorophyll concentration), electron transport rate and carboxylation activity. The present study showed that stomatal conductance (considering only green area) was lower in diseased than in healthy leaves, when considering stomatal conductance of healthy leaves higher than $1\,mol\,H_2O\,m^{-2}\,s^{-1}$. When comparisons were made under the same level of conductance (whichever), higher internal CO_2 concentrations were observed in diseased leaves with respect to healthy ones. This behavior suggests that: (*a*) the reduced conductance in diseased leaves was probably in response to higher CO_2 concentration in the sub-stomatal cavity causing stomatal closure (Goudriaan and Van Laar, 1978), even more so when these

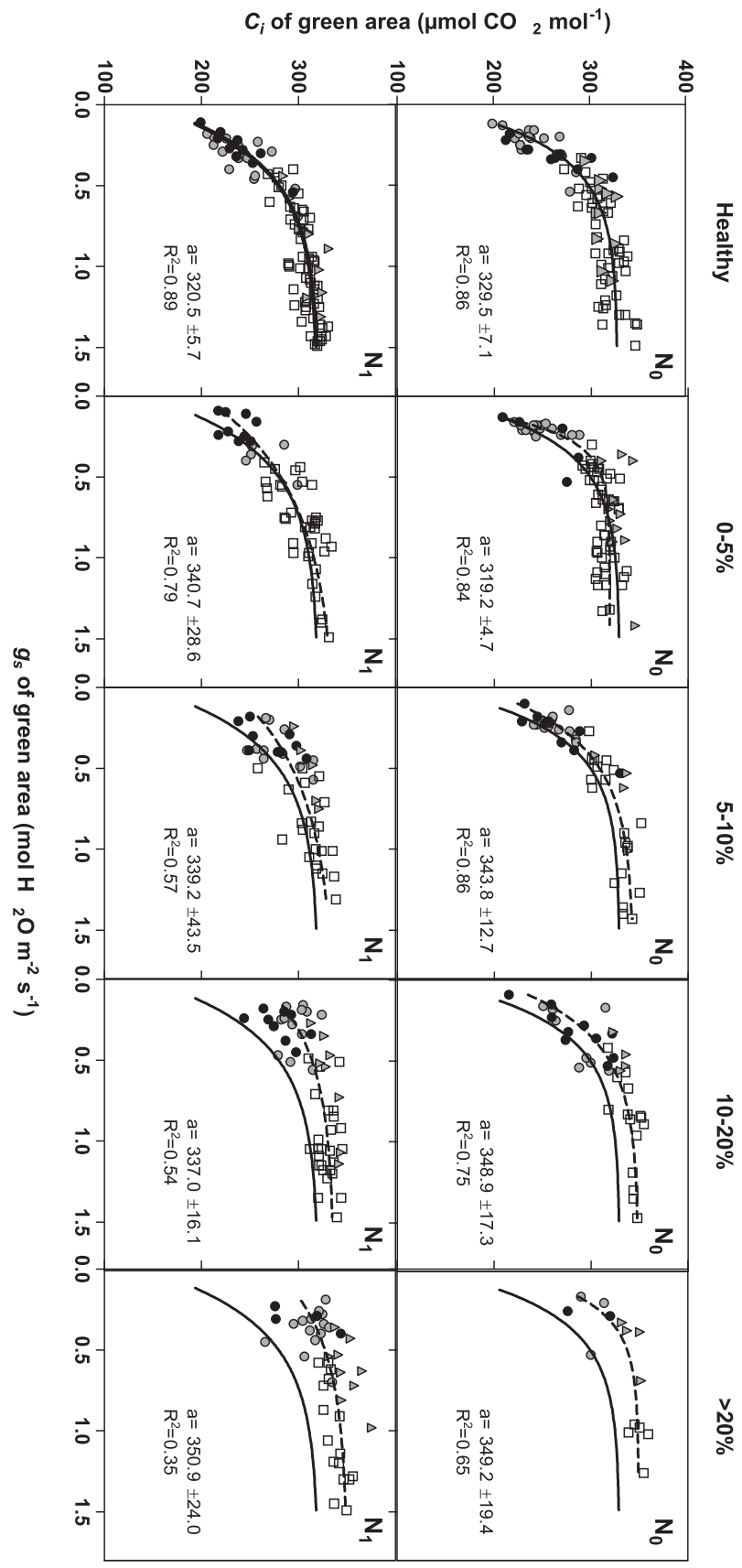

Fig. 5. Internal CO_2 concentration (C_i) of green area related to stomatal conductance (g_s) of green area for all the experiment (2005G1: grey circles; 2005G2: black circles; 2005BA: empty squares; 2007BA: grey triangles) together and for each nitrogen treatment (N_0 and N_1) separately. Data from diseased leaves were grouped according to four severity ranges (0–5%; 5–10%; 10–20% and higher than 20%) and fit to the model $y = a - b \times \exp^{(-c \times x)}$ (dotted curves). Data from healthy leaves were fit to the same model (continue curves). Curves from healthy leaves are shown in all the plots for each nitrogen treatment. Parameter a (±half confidence interval, 95%), which represent maximum C_i value that could be reached under no stomatal conductance limitation estimation is shown in each plot.

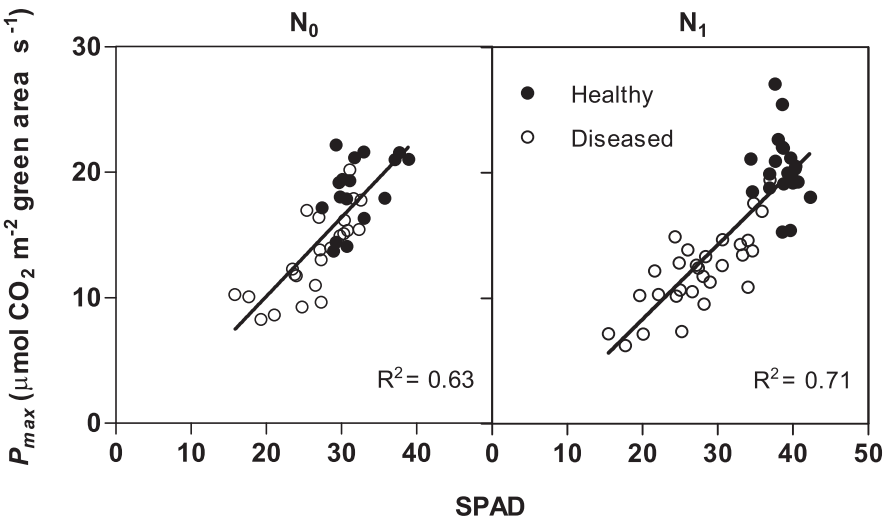

Fig. 6. Light-saturated net photosynthesis rate of green area (P_{max}) related to SPAD readings for N_0 and N_1 treatments of 2007BA experiment. Healthy and diseased leaves are represented by closed and open symbols, respectively. Lineal fit is shown for the data sets.

reductions were observed in situations of high (non-limiting) levels of conductance and (*b*) the reduction in photosynthesis rate caused by the pathogen was mainly mediated through non-stomatal events as: (i) reduced chlorophyll (reduced light harvesting); (ii) affected electron transport rate; and (iii) decreased amount or efficiency of active Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygenase) or CO_2 fixation related enzymes. The impairment of some of these processes would originate an increase of CO_2 in the sub-stomatal cavity.

Regarding photosynthetic events *per se* (non-stomatal events), carboxylation efficiency, which in turn depends on the amount and activity of Rubisco, was reduced by powdery mildew (*E. graminis*) in wheat (Rabbinge et al., 1985) and barley leaves (Walters and Ayres, 1984). In the same way, P_{max} of diseased leaves of seedlings grown in pots under controlled conditions was reduced due to effects on non-stomatal processes, being the effect on stomatal conductance more erratic (Bethenod et al., 2001). Furthermore, photosynthesis reduction in soybean leaves infected with rust (*P. pachyrhizi*) was highly associated with electron transport rate reductions of photosystem II (Kumudini et al., 2008). Other sets of evidence from the literature showed that the loss of chlorophyll was the main factor that determined photosynthesis declining in barley leaves infected with brown rust (*P. hordei*) (Scholes and Farrar, 1986). Chlorophyll reductions were also observed in wheat leaves infected with *S. tritici* blotch (Zuckerman et al., 1997). Indeed, in the present study, important SPAD reductions were observed in visually healthy tissue of diseased leaves when comparing with healthy leaves, suggesting that leaf rust might reduce photosynthesis rate through chlorophyll reductions. Further studies should be carried out to precisely determine which of the mentioned non-stomatal photosynthesis related processes are affected by leaf rust in wheat leaves.

5. Conclusions

The present study demonstrated that leaf rust reductions caused on net photosynthesis rate at light saturation was mediated by a diminished gross photosynthesis rate (average reduction: 6.1 μmol CO_2 m^{-2} green area s^{-1}) rather than through changes in dark respiration rate (average increase: 0.7 μmol CO_2 m^{-2} green area s^{-1}); both components determining net photosynthesis rate. Leaf nitrogen concentration did not determine a different effect of leaf rust on net photosynthesis rate. Photosynthesis reductions were due to effects on non-stomatal processes such as could be energy capture by photosystems (reductions of chlorophyll concentration), electron transport rate and carboxylation activity. In fact, important reductions of SPAD units (i.e. chlorophyll) were observed on green areas of diseased leaves when compared to those of healthy ones. Observed SPAD values on diseased leaves were 26.4 ± 0.98 and 27.6 ± 1.05 for N_0 and N_1 treatment, respectively; while on healthy leaves they were 32.0 ± 0.83 and 38.6 ± 0.41, respectively. Conductance was reduced probably as a consequence of the effect of the pathogen on photosynthesis machinery, which determined reductions in CO_2 utilization and led to a CO_2 accumulation in the sub-stomatal cavity, causing the stomata closure. Although wheat leaf rust reduced net photosynthesis rate at light saturation, no effects were observed at low irradiance levels. This has important consequences at canopy level since the leaves of a crop are not continuously exposed to radiation saturation conditions due to light attenuation occurring within the canopy and solar angle changing during the day. Thus, it could be possible to speculate that when photosynthesis rate is determined considering all leaves of the canopy (canopy photosynthesis = radiation use efficiency), that trait is not affected by leaf rust and thereby, this biotrophic pathogen affects light interception rather than radiation use efficiency at crop level.

Acknowledgements

The authors gratefully acknowledge Edmundo Ploschuck, Professor from Facultad de Agronomía, UBA, Buenos Aires, Argentina and Hervé Autret, scientific technician from INRA, Environnement et Grandes Cultures, Grignon, France for their excellent assistance in the use of portable photosynthesis system (LI-6400; Li-Cor, Lincoln, USA) utilization and Antonio Hall for his critical comments on the manuscript. The authors also acknowledge Francisco Sacco from INTA Pergamino for providing rust spores. RC held a postgraduate scholarship from the CONICET. This study was supported by ANPCyT (PICT), CONICET, UBACYT and ECOS-Sud grants.

References

Annone, J.G., García, R., Botta, G., Ivancovich, A., 2001. Pérdidas de rendimiento ocasionadas por la "Roya de la Hoja" y la "Mancha Amarilla" del trigo: estimaciones en el norte de la Provincia de Buenos Aires. Rev. Tecnol. Agropecuaria VI, 21–23.

Bancal, M.-O., Robert, C., Ney, B., 2007. Modelling wheat growth and yield losses from late epidemics of foliar diseases using loss of green leaf area per layer and pre-anthesis reserves. Ann. Bot. 100, 777–789.

Bassanezi, R.B., Amorim, L., Bergamin Filho, A., Berger, R.D., 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. J. Phytopathol. 150, 37–47.
Bassanezi, R.B., Amorim, L., Bergamin Filho, A., Hau, B., Berger, R.D., 2001. Accounting for photosynthetic efficiency of bean leaves with rust, angular leaf spot and anthracnose to assess crop damage. Plant Pathol. 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.
Bethenod, O., Huber, L., Slimi, H., 2001. Photosynthetic response of wheat to stress induced by *Puccinia recondita* and post-infection drought. Photosynthetica 39, 581–590.
Carmona, M., Cortese, P., Moschini, R.C., Pioli, R., Ferrazzini, M., Reis, E.M., 1999. Economical damage threshold for fungicide control of leaf blotch and tan spot of wheat in Argentina. In: XIVth International Plant Protection Congress , Jerusalem, Israel, p. 119.
Carretero, R., Serrago, R.A., Bancal, M.O., Perelló, A.E., Miralles, D.J., 2010. Absorbed radiation and radiation use efficiency as affected by foliar diseases in relation to their vertical position into the canopy in wheat. Field Crops Res. 116, 184–195.
Connor, D.J., Hall, A.J., Sadras, V.O., 1993. Effect of nitrogen content on the photosynthetic characteristics of sunflower leaves. Aust. J. Plant Physiol. 20, 251–263.
Cortese, P., Carmona, M., Pioli, R., Moschini, R.C., Reis, E.M., 1998. Nuevo enfoque para el control químico de la mancha de la hoja (*Septoria tritici*): determinación de umbrales de daño económico y de acción. In: IV Congreso Nacional de Trigo y II Simposio Nacional de Cereales de siembra Otoño-Invernal , Mar del Plata, Argentina, p. (IV) 04.
Dreccer, M.F., Van Oijen, M., Schapendonk, A.H.C.M., Pot, C.S., Rabbinge, R., 2000. Dynamics of vertical leaf nitrogen distribution in a vegetative wheat canopy. Impact on canopy photosynthesis. Ann. Bot. 86, 821–831.
Duveiller, E., Singh, R., Nicol, J., 2007. The challenges of maintaining wheat productivity: pests, diseases, and potential epidemics. Euphytica 157, 417–430.
Erickson, J.E., Stanosz, G.R., Kruger, E.L., 2003. Photosynthetic consequences of Marssonina leaf spot differ between two poplar hybrids. New Phytol. 161, 577–583.
Evans, J.R., 1983. Nitrogen and photosynthesis in the flag leaf of wheat (*Triticum aestivum* L.). Plant Physiol. 72, 297–302.
Gaunt, R.E., 1995. The relationship between plant disease severity and yield. Annu. Rev. Phytopathol. 33, 119–144.
Goudriaan, J., van Keulen, H., 1979. The direct and indirect effects of nitrogen shortage on photosynthesis and transpiration in maize and sunflower. Neth. J. Agric. Sci. 27, 227–234.
Goudriaan, J., Van Laar, H.H., 1978. Calculation of daily totals of the gross CO_2-assimilation of leaf canopies. Neth. J. Agric. Sci. 26, 373–383.
Horton, P., 2000. Prospects for crop improvement through the genetic manipulation of photosynthesis: morphological and biochemical aspects of light capture. J. Exp. Bot. 51, 475–485.
Johnson, K.B., 1987. Defoliation, disease, and growth: a reply. Phytopathology 77, 1495–1497.
Kumudini, S., Godoy, C.V., Board, J.E., Omielan, J., Tollenaar, M., 2008. Impact of *Phakopsora pachyrhizi* infection on soybean leaf photosynthesis and radiation absorption. Crop Sci. 48, 2343–2350.
Lamari, L., 2002. ASSESS: Image analysis software for plant disease quantification. APS, University of Manitoba, Winnipeg, Canada.
Lopes, D.B., Berger, R.D., 2001. The effects of rust and anthracnose on the photosynthetic competence of diseased bean leaves. Phytopathology 91, 212–220.
Madden, L.V., Nutter, F.W.J., 1995. Modeling crop losses at field scale. Can. J. Plant Pathol. 17, 124–137.
Mumford, J.D., Norton, G.A., 1984. Economics of decision making in pest management. Annu. Rev. Entomol. 29, 157–174.
Oerke, E.-C., Dehne, H.-W., 1997. Global crop production and the efficacy of crop protection—current situation and future trends. Eur. J. Plant Pathol. 103, 203–215.
Oerke, E.-C., Dehne, H.-W., 2004. Safeguarding production-losses in major crops and the role of crop protection. Crop Prot. 23, 275–285.
Owera, S.A.P., Farrar, J.F., Whitbread, R., 1981. Growth and photosynthesis in barley infected with brown rust. Physiol. Plant Pathol. 18, 79–90.
Polley, R.W., Thomas, M.R., 1991. Surveys of diseases of winter wheat in England and Wales, 1976–1988. Ann. Appl. Biol. 119, 1–20.
Rabbinge, R., Jorritsma, I.T.M., Schans, J., 1985. Damage components of powdery mildew in winter wheat. Neth. J. Plant Pathol. 91, 235–247.
Reis, E.M., Leites, A., Forcelini, C.A., 2006. Relações entre intensidade da doença, refletância da radiação solar e rendimento de grãos no patossistema ferrugem da folha de trigo Embrapa 16. Fitopatol. Bras. 31, 447–454.
Reis, E.M., Santos, J.A.P., Blum, M.M.C., 2007. Critical-point yield model to estimate yield damage caused by *Cercospora zea-maydis* in corn. Fitopatol. Bras. 32, 110–113.
Robert, C., Bancal, M.-O., Lannou, C., Ney, B., 2006. Quantification of the effects of *Septoria tritic*i blotch on wheat leaf gas exchange with respect to lesion age, leaf number, and leaf nitrogen status. J. Exp. Bot. 57, 225–234.
Robert, C., Bancal, M.-O., Ney, B., Lannou, C., 2005. Wheat leaf photosynthesis loss due to leaf rust, with respect to lesion development and leaf nitrogen status. New Phytol. 165, 227–241.
Robert, C., Bancal, M.-O., Nicolas, P., Lannou, C., Ney, B., 2004. Analysis and modelling of effects of leaf rust and *Septoria tritici* blotch on wheat growth. J. Exp. Bot. 55, 1079–1094.
Scholes, J.D., Farrar, J.F., 1986. Increased rates of photosynthesis in localized regions of a barley leaf infected with brown rust. New Phytol. 104, 601–612.
Scholes, J.D., Rolfe, S.A., 1996. Photosynthesis in localised regions of oat leaves infected with crown rust (*Puccinia coronata*): quantitative imaging of chlorophyll fluorescence. Planta 199, 573–582.
Serrago, R.A., Carretero, R., Bancal, M.O., Miralles, D.J., 2009. Foliar diseases affect the eco-physiological attributes linked with yield and biomass in wheat (*Triticum aestivum* L.). Eur. J. Agron. 31, 195–203.
Shtienberg, D., 1992. Effects of foliar diseases on gas exchange processes: a comparative study. Phytopathology 82, 760–765.
Sinclair, T.R., Horie, T., 1989. Leaf nitrogen, photosynthesis, and crop radiation use efficiency: a review. Crop Sci. 29, 90–98.
von Caemmerer, S., Farquhar, G.D., 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153, 376–387.
Waggoner, P.E., Berger, R.D., 1987. Defoliation, disease, and growth. Phytopathology 77, 393–398.
Walters, D.R., Ayres, P.G., 1984. Ribulose bisphosphate carboxylase protein and enzymes of CO_2 assimilation in barley infected by powdery mildew (*Erysiphe graminis hordei*). J. Phytopathol. 109, 208–218.
Zuckerman, E., Eshel, A., Eyal, Z., 1997. Physiological aspects related to tolerance of spring wheat cultivars to *Septoria tritici* blotch. Phytopathology 87, 60–65.